Electrophysiology and plasticity in isolated postsynaptic densities

Ursula Wyneken[a,*], Juan José Marengo[b], Fernando Orrego[a]

[a] *Laboratorio de Neurociencias, Facultad de Medicina, Universidad de los Andes, San Carlos de Apoquindo 2200, Las Condes, Santiago 6782468, Chile*
[b] *Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile and Institute of Neurosurgery Prof. Asenjo, Chile*

Abstract

The organization and regulation of excitatory synapses in the mammalian CNS entails complex molecular and cellular processes. In the postsynaptic membrane, scaffolding proteins bring together glutamate receptors with multiple regulatory proteins involved in signal transduction. This gives rise to an elaborate postsynaptic structure known as the postsynaptic density (PSD). This protein network plays a critical role in the regulation of glutamate receptor function and thus in synaptic plasticity. To study this regulation, we have developed a system in which ionotropic glutamate receptors (iGluRs) can be recorded, in the steady state, by the patch clamp technique in isolated PSDs incorporated into giant liposomes. In this preparation, ionotropic glutamate receptors maintain their characteristic physiological and pharmacological properties. The recordings reflect the presence of channel clusters, as multiple conductance and subconductance states are observed. Each of the receptor subtypes is activated by a specific set of kinases that are activated differentially by Ca^{2+}: the "kainate receptor kinases" are active even in the presence of EGTA, i.e. they are not calcium-dependent; the "*N*-methyl-D-aspartate receptor (NMDAR) channel kinases" are active in the presence of submicromolar calcium concentrations, whereas the "α-amino-3- hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor kinases" need μM calcium for activation. The NMDA receptor showed its characteristic voltage-dependent Mg^{2+} blockade, and activation by phosphorylation was in part a consequence of a relief of Mg^{2+} blockade. These results allow us to propose a model in which phosphorylation of NMDA receptors can contribute to a long-lasting and self-maintained change in synaptic function.

The experimental approach we present will allow us to test the functional consequence of activation of the multiple signal transduction pathways thought to regulate excitatory neurotransmission in the adult CNS.

Theme: Excitable membranes and synaptic transmission
Topic: Postsynaptic mechanisms

Keywords: Glutamate receptors; Postsynaptic density; NMDA; Phosphorylation; Plasticity

Contents

E-mail address: uwyneken@uandes.cl (U. Wyneken).

1. Introduction

In this review, we show the advantages of studying glutamate receptor function in the postsynaptic density (PSD), the structure where they are specifically anchored in the CNS.

Rapid excitatory neurotransmission is mediated mainly by ionotropic glutamate receptors (iGluRs) which play important roles in synaptic plasticity, neuronal development and neurological disorders [9,24,25,77,106]. Pharmacological and molecular techniques have been used to identify three categories of iGluRs according to their agonist selectivity and sequence homology: *N*-methyl-D-aspartate (NMDA)-type receptors, α-amino-3- hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type receptors and kainate receptors [38,85]. iGluR subunits have been cloned and their cDNAs expressed [107,121]. To date, three major classes of NMDA receptor subunits (NR1, NR2A-D and NR3A-B), four AMPA receptor subunits (GluR1-4), and five kainate receptor subunits (GluR5-7 and KA1-2) have been reported [38]. All three families of glutamate receptors can co-assemble within families, possibly as tetramers [103,76], to produce many receptor combinations [4,135]. Alternative splicing and RNA editing generate further variability contributing to a great molecular and functional diversity [24,76].

The subunit composition of NMDARs determines many aspects of their physiology [121]. Conventional heteromeric NMDARs, which contain NR1 and NR2 subunits, require dual agonists for activation: glutamate that binds to NR2 subunits and glycine or D-serine that bind to NR1 subunits [46,87]. Remarkably, coexpression of NR3 with NR1 and NR2 subunits suppresses glutamate-induced currents. NR3A or NR3B co-assemble with NR1 when co-expressed in Xenopus oocytes to form excitatory glycine receptors that are unaffected by glutamate or NMDA and inhibited by D-serine [15]. In addition, NMDARs display sensitivity to different endogenous modulators: they are stimulated by reducing agents [1,53] inhibited by protons, and regulated by Zn^{2+} and polyamines [83].

AMPA and kainate receptors, that assemble as homo- or hetero-oligomers, mediate rapid synaptic transmission at the postsynaptic level. The contribution of kainate receptors to this phenomenon has been more difficult to assess [60,62,96,97]. In addition to the well-established role of AMPARs in synaptic plasticity [74,78], kainate receptors can regulate neurotransmitter release at excitatory and inhibitory synapses and therefore have a role in plasticity, too [41,60].

Regulation of postsynaptic glutamate receptors is one of the main mechanisms for altering synaptic efficacy in the central nervous system. One of the most important mechanisms involved is phosphorylation and dephosphorylation [123]. The regulation of GluR channels by kinases and phosphatases plays an essential role in changes of synaptic strength, such as long-term potentiation (LTP) and long-term depression (LTD) [63,77,115]. The fundamental role of NMDARs in the induction of LTP is well established. NMDARs are highly permeable to Ca^{2+} and exhibit voltage-dependent inhibition by extracellular Mg^{2+} [81,92]. At depolarizing membrane potentials, when Mg^{2+} block is relieved, Ca^{2+} enters the cell and triggers intracellular biochemical cascades that produce long-lasting changes in neuronal function. A simplified model is that Ca^{2+} entry ultimately leads to an increase in AMPAR-mediated current and thus results in a potentiation of synaptic efficacy. One of these biochemical events is activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), that is essential in LTP and behavioural memory [73,114]. However, also important roles have been assigned to the combined activation of the src family of tyrosine kinases [3], protein kinase A (PKA) [5] and protein kinase C (PKC) [75]. The consequence of GluR phosphorylation is an enhancement of glutamate-activated currents [115]. In addition, crosstalk between these and other complex biochemical pathways exists, and the depotentiation of synaptic currents has been shown to be mediated by protein phosphatases that can dephosphorylate iGluRs [59,66,145].

1.1. Regulation by phosphorylation of iGluRs

The study of the glutamate-gated ion channels has been dominated by the molecular identification of the receptor isoforms and their biophysical characterization [21]. Phosphorylation/dephosphorylation studies have mostly been done by expression in heterologous systems in vitro or in neurones in culture, and varying or even contradictory results have been found. One of the most illustrative examples are the studies regarding NMDAR regulation by PKC, which are summarized in Table 1. NMDARs are potentiated by PKC in trigeminal neurons which reduces its voltage-dependent Mg^{2+}-block [16]. PKC phosphorylates primarily but not exclusively the C1 cassette of the

Table 1
Reported effects of PKC on NMDAR function

Biological system	Subunit	Comment	Reference
(I) Stimulatory effect			
Oocytes	NR1 splice variants	degree depending on splice variant	[27]
Oocytes	NR1 splice variants NR2A	potentiated by Ca^{2+} influx through NMDAR	[149]
Oocytes	NR1 splice variants NR2A	not mediated by NMDAR phosphorylation	[151]
Oocytes	NR1 splice variants NR2A and NR2B	dependent on IC Ca^{2+} in homomeric NR1 receptors	[70]
Oocytes	$NR1_{100}$/NR2A	potentiated by intracellular Ca^{2+}	[111]
Oocytes	$NR1_{100}$/NR2A	potentiates channel gating	[57]
Oocytes	NR1/NR2B	no effect when coexpressed with PSD-95	[143]
Oocytes	NR1-011/NR2B	mediated by the NR2B tail phosphorylation	[65]
Oocytes	NR1/NR2A to D (mouse homologs)	no effect on Mg^{2+} block	[132]
HEK 293	NR1/NR2A	inhibits rundown	[141]
HEK 293	NR1/NR2B	no effect on rundown	[141]
Trigeminal isolated neurons	nd	reduces Mg^{2+} block	[16]
Hippocampal isolated neurons and slices (neonatal/ 10–24d)	nd	via CAKβ and src	[40]
Hippocampal isolated neurons neonatal/ 12–24 d)	nd	via src	[71]
Hippocampal isolated neurons (neonatal/ 12–20 d)	nd	of peak current via AKβ/src	[72]
Hippocampal cultured neurons (fetal)	nd	no effect on Mg block Ca^{2+} independent	[141]
(II) Inhibitory effect			
Cerebellar granular isolated neurons (neonatal/ 6–7 d)	nd	of NMDA-induced increases in intracellular $[Ca^{2+}]$	[20,112]
Hippocampal isolated neurons (neonatal/ 12–20 d)	nd	of steady-state current by enhancement of Ca^{2+}- dependent inactivation	[72]
(III) Changes in distribution			
Isolated Hippocampal neurions (embryonic)	nd	membrane insertion	[57]
Hippocampal culture neurons (neonatal/ 18 d)	nd	dispersal	[29]
Hippocampal slices (adult)	nd	membrane insertion, via src	[34]
Oocytes	$NR1_{100}$/NR2A	membrane insertion	[57]
Fibroblasts	NR1	dispersal mediated by NR1 phosphorylation	[126]

CAKβ: cell-adhesion kinase β/proline-rich kinase 2 (Pyk2), d = days, nd = not detected. NR1 splice variant nomenclature (Durand, 1993): The eight possible splice variants, seven of which are expressed in the CNS, arise from splicing in or out three exons in the N-terminal domain (N1), and in the C-terminal domain (C1 and C2). Splicing out of the second C-terminal domain deletes a stop codon and results in an additional open reading frame before arriving at a new stop codon. NR1 variants are denoted by the presence (1) or absence (0) of the three alternative spliced exons, for example the splice variant $NR1_{011}$ corresponds to the following exon arrangement: N1 absent, C1 present, C2 present.

NMDA subunit NR1, both in neurones in culture and in heterologous expression systems [125]. However, recombinant receptors lacking the C1 cassette exhibit greater phorbol-ester induced potentiation than do those containing this cassette, when studied in oocytes expressing different splice variants of the NR1 subunit [27]. The effect of PKC activation is NMDAR inhibition when studied in cerebellar granular cells in culture [20,112]. Also, in CA1 pyramidal neurons in culture, the steady-state NMDAR-mediated current is depressed in a calcium-dependent manner, whereas the peak current is enhanced [72]. However, when expressed in oocytes, NMDARs are activated by PKC in a calcium-dependent manner [111,149,151]. This is probably mediated by a PKC-induced membrane insertion and activation of NMDARs that is also seen in embryonic hippocampal neurons in culture [57]. A PKC- and src-dependent membrane insertion of NMDARs in hippocampal slices of adult brain has been reported [34]. Recently, it has been shown that PKC induces dispersal of NMDARs in an heterologous expression system [126] and in rat hippocampal cultures, where they move from a synaptic to an extrasynaptic localization [29]. The conflicting results may

be in part due to the fact that Fong et al [29] studied synaptic versus extrasynaptic receptors, whereas the other authors made no distinctions between them. Supporting the idea that synaptic receptors might behave differently relative to extrasynaptic ones, Yamada et al. [143] found that the PKC potentiation of NMDARs expressed in oocytes disappeared when coexpressed with PSD-95, the synaptic NMDAR scaffolding protein. The different biological systems used in the PKC studies may contribute to different results, whereas the consequences of PKC activation on exclusively synaptic NMDAR functioning have not been addressed. It is well known that kinases can phosphorylate many proteins in various systems, although only some of them are their real physiological substrates.

The role of tyrosine kinases and of other serine–threonine kinases has been less controversial. For example, the NR2B subunit of NMDARs is phosphorylated on tyrosine residues in isolated postsynaptic densities [86], in hippocampal neurons [58] and in heterologous expression systems [124,146]. Tyrosine phosphorylation, mediated by the src family of kinases, produces NMDAR upregulation in cultured neurons [3,147]. Additionally, the upregulation of NMDARs in hippocampal neurons by PKC or metabotropic receptors is mediated by src tyrosine kinases [71]. One of the current models for NMDAR upregulation by src family of kinases has become even more complex: PKC activates the cell-adhesion kinase β (CAKβ) that in turn binds to and activates src [40]. At least one paradox remains to be resolved: src-induced potentiation of recombinant NR1/NR2A receptors in HEK293 cells is due to a reduction of channel inhibition by extracellular Zn^{2+} [150] while this mechanism does not operate in neurons [142]. The precise role and fine regulation of phosphotyrosine content in NR2B at the synapse is not yet known, although there is a general agreement that src upregulation of NMDARs may be important in many physiological processes, like LTP [84,102,104].

A CaMKII phosphorylation site has been identified in the NR2B subunit, but no regulatory function has been reported [93]. Although CaMKII associates with NMDARs [32,68], it phosphorylates primarily AMPAR GluR1 subunits [6,79], causing an increase in single channel conductance when expressed in an heterologous system [22]. GluR1 phosphorylation by PKA controls the AMPAR open probability in HEK 293 cells [5] and LTP in hippocampal slices is associated with increased phosphorylation of CaMKII and PKA consensus sites of GluR1, whereas their dephosphorylation correlates with LTD [59], suggesting a role for PKA in the physiological regulation of AMPARs. GluR4 is phosphorylated by PKA, PKC and CaMKII in vitro [12,13,18]. The incorporation of AMPAR subunits into synapses is regulated by PKA phosphorylation of GluR4, whereas GluR1 incorporation requires activation of both PKA and CaMKII [28].

Accumulating evidence demonstrates that the GluR phosphorylation status is dynamically controlled in vivo by phosphorylation/dephosphorylation cycles, and that inhibition of protein phosphatases potentiates GluR function [8,54,59,66,113,134,145].

1.2. Synaptic iGluRs are anchored to PSDs

Excitatory synapses in the mammalian brain occur mostly on dendritic spines, where receptors and downstream signalling enzymes are clustered in the PSD. The PSD is a cytoskeletal web beneath the plasma membrane that contains five classes of proteins: neurotransmitter receptors, cell-adhesion proteins, adaptor molecules, signalling enzymes and cytoskeletal proteins [47]. Many of them have recently been identified by mass spectrometry [42,133]. In this complex network, specific signalling modules are brought together by scaffolding proteins that contain PDZ domains [127]. The prototypical PDZ protein, PSD-95/SAP90, belongs to the family of the membrane-associated guanylate kinases (MAGUKs), comprised of four closely related proteins. MAGUK proteins contain five domains involved in protein–protein interactions: three amino-terminal PDZ domains followed by an SH3 domain and a GuK domain homologous to yeast guanilate kinase but lacking enzymatic activity. The first and second PDZ domains bind tightly to the carboxy-terminal tails of the NR2 subunits of the NMDAR [55,90]. The third PDZ domain interacts with CRIPT, one of the probable mechanisms that link the NMDAR to the cytoskeleton [91,95]. Other proteins that interact with PSD-95 are the neuronal nitric oxide synthase [10], the kainate receptor subunits KA2 and GluR6 [31], the neuronal Rho-GEF Kalirin-7 [98], neuroligins [43], SynGAP, a synaptic ras GTPase-activating protein [51], GKAP that interacts with the GK domain [49], Shaker-type potassium channels [48], a Ca^{2+}-ATPase isoform [50] and ephrins and their receptors [129].

In addition, a lattice of scaffold proteins can link metabotropic GluRs to the NMDAR complex [130], whereas AMPARs form part of a different signalling complex. While immature AMPARs associate with SAP97, a member of the MAGUK family [105], mature AMPARs are anchored to the PSD by another type of PDZ domain-containing proteins called glutamate-receptor-interacting protein (GRIP) [26], ABP [118] and Protein interacting with C kinase (PICK1), which targets PKCα to AMPARs [139].

The C-termini of GluRs also interact with non-scaffold proteins. For example, AMPARs associate with the *N*-ethylmaleimide-sensitive factor (NSF) [117], whereas the NR2 subunit of NMDARs binds calmodulin and alpha-actinin [138], as well as CaMKII, the most abundant signalling protein in the PSD. The NR1 subunit of the NMDAR can be complexed with protein phosphatase-1 and cAMP-dependent protein kinase (PKA) by means of the scaffold protein Yotiao, while the protein phosphatase 2A associates with the NR3A subunit [14]. The presence of protein phosphatases 1 and 2A in PSDs may be involved in the temporally and spatially precise control of the phosphorylation state of its proteins [119,152].

As shown by electron microscopy of immunogold-labeled synapses, the NMDAR signalling complex forms the core of the PSD, whereas AMPARs are distributed more uniformly and metabotropic glutamate receptors are concentrated in the outer rim of the PSD [47,116]. This ordered PSD architecture is a consequence of association of iGluRs to specific proteins and hence distinct signalling systems, within the PSD. For example, synaptic NMDARs have anti-apoptotic activity mediated by CREB phosphorylation, whereas stimulation of extrasynaptic NMDARs triggers cell death [37]. Interestingly, extrasynaptic NMDARs can mediate inhibition by coupling to Ca^{2+}-activated K^{+} channels [44].

1.3. Functional studies of iGluRs in PSDs

Although it seems that the molecular composition of synaptic iGluRs does not vary from extrasynaptic ones [2], many studies suggest that the functional properties of GluRs are modified when they are associated with the PSD or with their scaffold proteins. For example, clustering of kainate receptors by PSD-95 reduces its desensitization [31]. The association of NMDARs to PSD-95 decreases the sensitivity of the channel to L-glutamate and inhibits the PKC potentiation of the channels when expressed in oocytes [143], while coexpression of NMDARs and PSD-95 with GKAP potentiates channel activity [144]. Liao [64] confirmed that PKC potentiation in oocytes was eliminated by PSD-95 coexpression, while src potentiation was promoted by PSD-95 coexpression in heterologous cells [64,124]. Phosphorylation on specific sites of GluR subunits depends on their PSD localization [122]. For example, although the AMPAR subunit GluR1 can be phosphorylated by PKA on Ser-845 in heterologous cells and in neurons [101], this site is mostly occluded in PSD-anchored receptors [131], so, it is difficult to assign a physiological role to it. Similar results were found regarding potential phosphorylation sites for src in PSD-anchored NMDARs [17].

Some attempts have been made to study iGluR function in isolated PSDs: NMDAR activity and its regulation by kinases was inferred studying the binding of the open-channel blocker MK 801 to them [52]. The regulation of iGluR sensitivity to agonists and antagonists has also been studied in isolated PSDs by measuring the binding of selective substances to each receptor subtype [7,108,128]. However, these attempts do not clarify many of the complex mechanisms thought to participate in iGluR regulation.

Based on these evidences, we propose that the function of iGluRs is specifically modulated by their anchoring in the PSD that, in turn, depends on the influence of other neighboring regulatory proteins, that are probably not present in extrasynaptic glutamate receptors, which are the ones usually subject to electrophysiological recordings. For this reason, we have developed a system, in which isolated PSDs can be recorded by the patch clamp method following their incorporation into giant liposomes. This has allowed us to record specific ionotropic glutamate receptor subtypes present in them, as well as their regulation by different modifications induced on the "intracellular" side of the PSD, where kinases and phosphatases associate in the vicinity of their targets.

2. Methods

2.1. Subcellular fractionation

For PSD isolation, the method of Carlin et al. [11] was slightly modified [137].

2.2. Electrophysiological recordings

Giant liposomes containing PDSs were prepared as described earlier [99]. Reconstituted glutamate receptors present in the PSD were activated by injection of 0.5 to 1 μl of the 10-fold concentrated agonist or antagonist into the recording pipette through a plastic catheter. The agonist concentrations in the injection solutions were: 50 μM NMDA (RBI) and 5 μM glycine for NMDARs, 5 μM glutamate-free quisqualate (Tocris) for AMPARs or 10 μM kainate for kainate receptors. The antagonists used were D-2-amino-5-phosphonovaleric acid (APV) for NMDARs and 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) for non-NMDARs. A simplified scheme of the PSD isolation followed by incorporation of them into giant liposomes and their subsequent recording is shown in Fig. 1. Once the excised patch containing a PSD was obtained, a control recording was performed at the holding potential before injecting the agonist, in this case NMDA and glycine, which evoked a current specifically related to the injection. Patch clamp recordings were obtained using an EPC-9 patch amplifier (HEKA Electronics) at a gain of 50 mV/pA. Data analysis, like mean current values and current amplitude histograms of 1-min current traces, was performed using the Microcal Origin software. All measurements were performed at room temperature. All reagents except those specified were from Sigma.

3. Results

3.1. Presence of functional glutamate receptors in reconstituted PSDs

Membrane patches in which currents were present in the absence of glutamate or other glutamate receptor agonists in the patch pipette were discarded [99]. In silent seals, when glutamate was added to the "intracellular" face (i.e. to the bath) of a reconstituted PSD, no currents were evoked. However, when glutamate, or one of the agonists selective for each of the glutamate receptor subtypes, was injected into the patch pipette, i.e. close to the "extracellular" side of

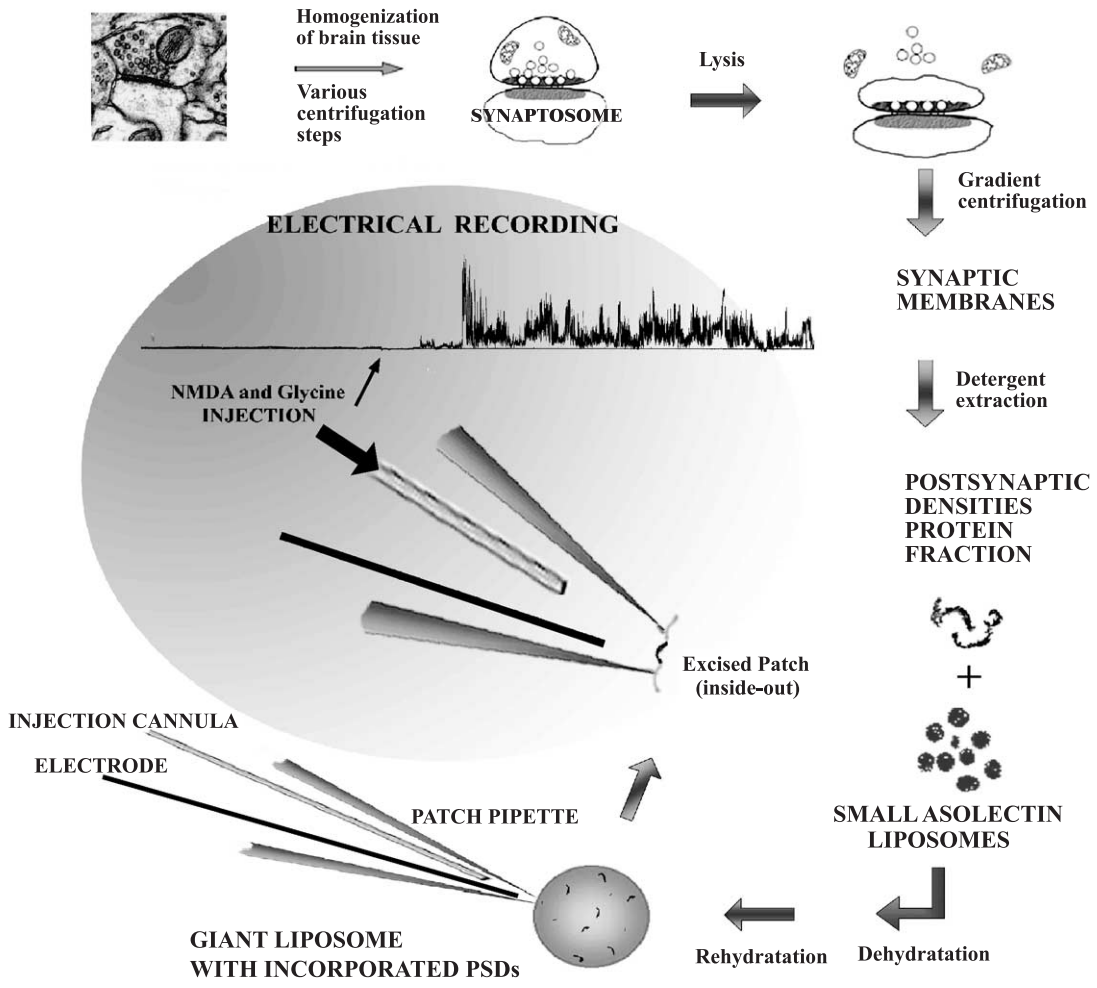

Fig. 1. Scheme of PSD isolation and incorporation into giant liposomes and procedure to measure glutamate-gated currents in them. PSDs were isolated from rat telencephalon by a modification of the method of Carlin [11]. Following tissue homogenization and various centrifugation steps, synaptosomes, synaptic membranes and finally PSDs were obtained. These were incubated with small asolectin liposomes which, following a dehydration and rehydration step, became incorporated into giant liposomes. The liposomes were plated and an excised membrane patch was obtained on the tip of the patch pipette. A plastic cannula (0.4 mm of diameter) in the interior of the patch pipette allowed the injection of the glutamate receptor agonists NMDA and glycine, in this case. In a previously silent seal, this induced a current related to the injection procedure.

the PSD, robust currents of a complex type were induced following a latency of 10–20 s, interpreted as due to diffusion of the agonist from the tip of the injection cannula to the PSD.

Kainate, at a 0.2–2-μM final concentration in the pipette, which is selective for activating kainate receptors, induced a complex current pattern (Fig. 2, top record), which is thought to represent the asynchronous openings and closures of multiple glutamate receptor channels clustered in the PSD. Similar patterns were seen when 1 μM quisqualate, an agonist of the AMPAR was injected together with 2 μM APV, an NMDA-receptor antagonist. These currents were blocked by the AMPA-kainate receptor antagonist, CNQX (Fig. 2, lower trace).

Activation of NMDARs by injection of 10 μM NMDA plus 1 μM glycine, in the presence of 2 mM "extracellular" magnesium $(Mg^{2+})_o$, led also to very complex evoked currents (Fig. 3A) that were of much greater magnitude at positive "intracellular" potentials relative to those seen at negative ones (Fig. 3B). This indicated that the NMDARs present in this preparation also showed the well-established voltage-dependent Mg^{2+} block. Due to the rapid flickering of the receptors, the current amplitude histograms did not allow the identification of the multiple conductances that could be recognized visually (Fig. 3C), nor the calculation of the open probability or single conductance levels as activity index. For this reason, to assess channel activity, we have calculated the mean current flow in 1-min recordings. The fractional current flow at positive and negative potentials was calculated at each membrane potential by dividing the mean 1-min current at negative potentials by the mean 1-min current at positive potentials. In this case, the fractional current flow was of 0.246.

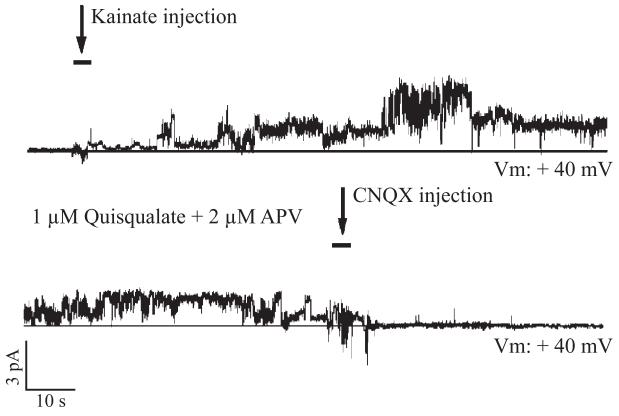

Fig. 2. GluR-mediated currents were activated or inhibited by the injection of specific agonists or antagonists into the patch pipette. Upper trace: injection of kainic acid to a final concentration of about 1 μM induced a current on a previously silent seal. Lower trace: currents recorded in the presence of 1 μM quisqualate plus 2 mM APV were inhibited by the injection of CNQX into the patch pipette. Both injections were done at a membrane potential (V_m) of +40 mV.

3.2. Effects of glutamate receptor phosphorylation

Addition of 2 mM ATP–Mg^{2+} to the "intracellular" face of the PSDs, a procedure which is known to phosphorylate a large number of PSD proteins by means of endogenous protein kinases present in the PSD, led to increased currents in kainate, AMPA and NMDA receptor activity (Figs. 4, 6 and 7). In the latter case, the results are of particular interest because phosphorylation, in the presence of 2 mM $(Mg^{2+})_o$, resulted in large increases in currents seen both at negative and positive potentials (Fig. 4). In this set of experiments, the increases were of 153 ± 8% ($n = 8$, $p < 0.01$), and 103 ± 12% ($n = 8$, $p < 0.01$), respectively. The voltage-dependent current block seen in the presence of $(Mg^{2+})_o$ was markedly attenuated by phosphorylation, and the extent of this unblocking depended on the magnitude of the negative potential. A Boltzmann fit [19] of the relative currents I_-/I_+ seen before and after the addition of ATP–Mg^{2+} (Fig. 5) showed that phosphorylation shifted the potential that gave a 50% current block, from − 53.9 mV in control PSDs, to − 74.8 mV, indicating that voltage-dependent Mg^{2+} block was greatly attenuated. Also, the parameter δ, that represents a measure of the electrical field distance that is crossed by Mg^{2+} to its blocking site, changed from 0.27 to 0.51, suggesting that the parameters that describe Mg^{2+} block change after phosphorylation, possibly because of a conformational change of the receptor. As in Fig. 5, the current ratio between positive and negative potentials is given; it could be argued that this ratio is enhanced at more negative potentials, because "intracellular"Mg (Mg_i^{2+}) could inhibit NMDA receptor currents at the corresponding symmetric positive potential [69]. We think this possibility is unlikely because phosphorylation increases the absolute magnitude of the current seen at negative potentials (cf. Fig. 4), and also because in an intracellular solution that contains 2 mM ATP, 2 mM Mg^{2+} and 0.1 mM EDTA at pH 7.4, the estimated concentration of free Mg ion is only 0.3 mM, a concentration that has no significant blocking effect on NMDA receptor currents at positive potentials [69].

3.3. Selective effect of different protein kinases

Phosphorylation of PSDs, in the presence of 0.1 mM EGTA (pCa 9), enhanced in them the current induced by 1 μM kainate by 157 ± % (Fig. 6). No further activation was found following the addition of 10 μM Ca^{2+} and 12.5 μg/ml of calmodulin, thus showing that the kinase responsible for this effect was calcium-independent.

In the case of AMPARs, the effect of phosphorylation was abolished when it was performed at pCa 9, and was maximal when 10 μM Ca^{2+} and 12.5 μg/ml of calmodulin were present (Fig. 6), as is to be expected due to the well-established functional association between CaMKinase II and this receptor [67,68].

NMDARs, on the other hand, showed a more complex pattern. Thus, 0.1 mM EGTA inhibited significantly the induced receptor currents, but the effect of ATP–Mg was near maximal in the absence of added Ca^{2+} and calmodulin (only contaminating calcium was present, which gave a measured pCa of 7). This indicated that the protein kinase responsible for the effects on NMDARs is activated by very low calcium concentrations.

These results show that each glutamate receptor subtype is selectively associated in the PSD to a different type of protein kinase, of which CaMKinase II is the only one that could be indirectly characterized. The general PKC inhibitor, 1 μM Bisindolylmaleimide I (BisI), was incapable of changing the effects of phosphorylation on the activity of NMDARs (not shown), thus suggesting that PKC is not involved in these effects, at least under the present experimental conditions, i.e. without neuronal cytosolic enzymes present.

3.4. Functional association of protein phosphatases to the PSDs

Finally, some evidence for the presence of functional protein phosphatases in our PSD preparation is shown in Fig. 7. AMPAR currents activated by quisqualate could be enhanced by the addition of phosphorylation conditions to the recording bath, as described. The subsequent addition of the general phosphatase inhibitor, NaF (4 mM), produced a two- to three-fold increase in AMPAR currents in an experiment that lasted about 15 min. On the other hand, when the phosphorylation conditions were washed out, AMPAR activation diminished gradually, as shown in Fig. 8.

4. Conclusions

Our recordings of iGluRs in isolated patch-clamped PSDs, in which receptor function is preserved, validate this

system for the study of the intrincate molecular mechanisms thought to be involved in their physiological regulation. Regarding iGluR subtypes, kainate receptors have been difficult to record because they induce small and rapidly desensitizing currents [30,96]. In this case, we think that we have been able to record functional kainate receptors because our PSD preparation contains the kainate subunits KA2 [137] and GluR6/7 (Wyneken, unpublished results). Also, the kainate concentrations used in these studies (0.2 to 2 μM) are in the selective range for this receptor subtype [61], and finally, kainate receptors were activated by the addition of ATP–Mg^{2+} to the recording solution in the presence of EGTA, a condition that was never able to activate AMPARs.

In relation to voltage-dependent $(Mg^{2+})_o$ block of NMDA receptors, there is no agreement regarding the effect of phosphorylation on this key control mechanism. Thus, in isolated trigeminal neurons, intracellular perfusion of PKC led to a reduction of the blocking effect of low (30 μM) $(Mg^{2+})_o$ [16]. In cultured rat cortical neurons, stretch-induced injury was found to markedly reduce $(Mg^{2+})_o$ inhibition [148] and in excised patches of CA1 hippocampal neurons, it is suggested that a decreased $(Mg^{2+})_o$ inhibition may be responsible for NMDAR potentiation by the protease thrombin, that should be mediated by kinases and/or metabotropic receptors [33] On the other hand, in isolated hippocampal neurons, although PKC potentiates NMDA receptor activity, it does not affect $(Mg^{2+})_o$ block [141]. This is also seen in cloned NMDA receptors expressed in Xenopus oocytes [132], while in cerebellar granule cells PKC inhibits NMDA receptor function without changing their sensitivity to $(Mg^{2+})_o$ [112]. We have tried to solve this contradictory findings by further exploring the effect that phosphorylation of isolated, patch-clamped PSDs, by the endogenous protein kinases present in them, has on the blocking action of $(Mg^{2+})_o$ present at physiological concentration. In this preparation, it has been reported that phosphorylation is indeed capable of increasing NMDA receptor currents at negative membrane potentials even in the presence of $(Mg^{2+})_o$ [136]. The results obtained at mM, physiological concentrations of $(Mg^{2+})_o$, indicate that phosphorylation of PSD proteins by endogenous kinases is able to greatly relieve the blocking effect of this cation [92]. Such unblocking shall enhance ion flow, especially Ca^{2+}, through the NMDA receptor ion channels, even at quite negative membrane potentials. This has an important physiological significance, because NMDA receptors, unblocked by phosphorylation, become less dependent for activation on the simultaneous depolarization of non-NMDA receptors and, even at resting potential (ca. −70 mV), their stimulation by glutamate shall lead to a substantial amount of Ca^{2+} influx into dendritic spines. This self-sustaining NMDAR activity may help trigger the many plasticity mechanisms in which this receptor type is involved.

The precise nature of the protein kinases involved, as well as the proteins whose phosphorylation leads to the unblocking, remains, however, undefined and open to further research. The effects of phosphorylation observed here are not due to a decrease in the tonic inhibition by $(Zn^{2+})_o$ of NMDA receptors [82], because our recording solution contained 0.1 mM EDTA, that chelates any contaminating Zn. This result is similar to that seen with Src potentiation of hippocampal NMDARs [142]. The present study thus supports the initial findings of Chen and Huang [16], obtained in trigeminal neurons, extending them to physiological levels of $(Mg^{2+})_o$, as well as to the whole brain cortex and hippocampus, from which the PSDs were derived. The differences with other studies, in which NMDAR phosphorylation did not change voltage-dependent block by $(Mg^{2+})_o$, may possibly be explained because these negative results were obtained using patch-clamped cortical or hippocampal neurons, in which the NMDARs are present mainly in dendritic spines that are distant from the cell body, where the clamp electrode is present. This would not allow adequate voltage control them. It also seems possible that in those studies, most of the receptor currents recorded are generated at extrasynaptic NMDARs present in the soma and proximal dendrites, that lack the associated proteins that allow, when phosphorylated, the relief of $(Mg^{2+})_o$ block. On the other hand, isolated nucleus caudalis trigeminal neurons are small, i.e. 10–20 μm in diameter, and their primary dendrites relatively short (Huang, personal communication), thus allowing an adequate voltage control of the synaptic NMDARs presumably present in them. In the present work, by definition, only synaptic receptors, i.e. those present in the PSD, are recorded, and these are located at the tip of the patch pipette, thus allowing an excellent voltage clamp. The differences, regarding the effect of phosphorylation on voltage-dependent Mg^{2+} block, between synaptic (Ref. [16] and present work) and extrasynaptic or cloned NMDARs (Table 1), could also be explained if only synaptic receptors, incorporated into PSDs, were coupled to the specific kinase, we have now described, which, when activated, leads to the unblocking effect.

The fact that the general protein phosphatase inhibitor NaF enhanced greatly the AMPAR currents following PSD phosphorylation confirms that GluR function in PSDs is tightly controlled by the opposing activities of phosphatases and kinases, and also that, in the PSD, protein phosphatases are highly active.

5. Physiological implications

The results shown above, when extrapolated to in vivo conditions, have many physiological implications. Thus, as the concentration of ATP may be presumed to be nearly constant in dendritic spines, where PSDs are mainly present, the enhancement by phosphorylation of iGluR activity shall be a function of the conditions that activate

each of the protein kinases, which we have shown to be selectively associated to the different glutamate receptor subtypes. Thus, kainate receptors, that only need ATP and Mg^{2+} to be activated in the PSD, shall always be maximally activated, and not subject to further activation when $(Ca^{2+})i$ increases, or to deactivation when it decreases. Thus, synaptic transmission mediated by the postsynaptic kainate receptor subtype is not regulated by changes in $(Ca^{2+})_i$ and its efficacy is always very high. This makes these receptors ideally suited for the unfailing transmission of signals in circuits that are vital for the individual and that may be presumed to be phylogenetically more ancient.

On the other hand, AMPARs, because they are maximally dependent on intraspine calcium concentrations, have a very large dynamic range of activation, which can vary from a low level of activity at pCa 9 to a very high one at pCa 5. This makes this type of glutamate receptor most suited for plasticity. However, we have also shown that protein phosphatases functionally linked to AMPARs are highly active, and that the activation of these receptors is greatly enhanced when the phosphatases are

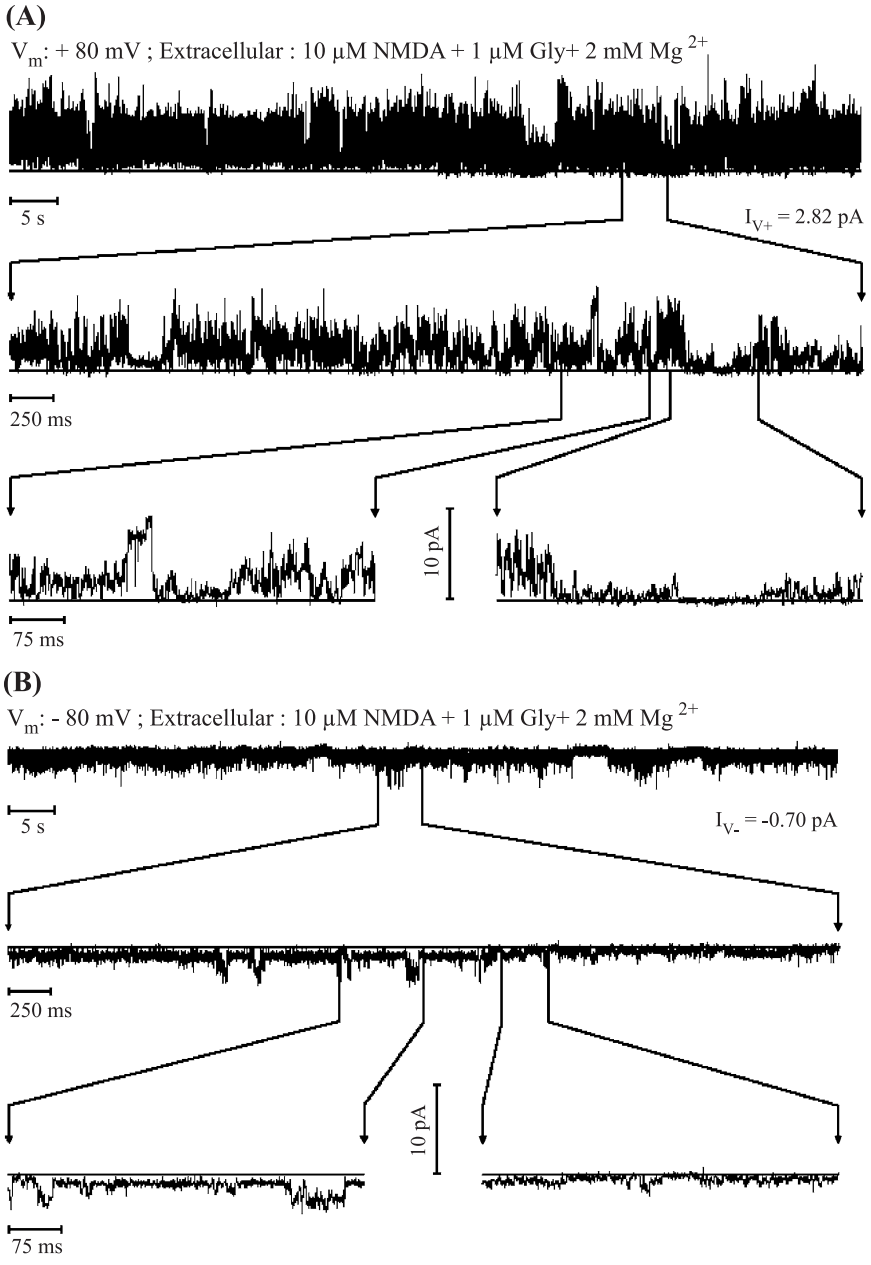

Fig. 3. NMDA plus glycine-activated currents show a complex kinetic behaviour and voltage-dependent Mg^{2+} blockade. The ionic composition of the recording solutions was symmetric except that the external recording solution contained 2 mM Mg^{2+}. Different time scales of a recording at a membrane potential (V_m) of +80 mV (A) and at −80 mV (B). One-minute current amplitude histograms for both membrane potentials are shown in (C). Note the marked current inhibition seen at −80 mV. Mean current values of 1-min recordings are given.

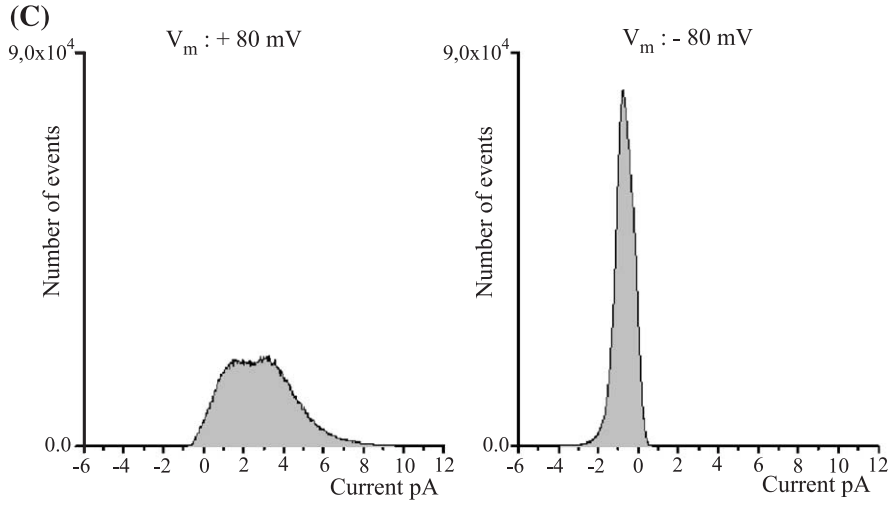

Fig. 3 (*continued*).

inhibited. This is an indication that the degree of AMPAR phosphorylation is in a steady-state, with phosphoryl groups being added and removed continuously. Also, when $(Ca^{2+})_i$ decreases, the phosphorylation of the receptor and thus, its state of activity, decreases. We have observed that, when the recording bath after phosphorylation with 2 mM ATP–Mg^{2+}, 10 μM calcium and 12.5 μg/ml calmodulin is returned to the control situation, i.e. no ATP–Mg^{2+} and the pCa is 7 (Fig. 8), AMPARs deactivate. This indicates that the phosphorylated state of AMPARs is highly labile, but, this does not deny that persistently activated CaMKII may continue to phosphorylate them, as cogently argued by Lisman et al. [68]. However, as our finding that protein phosphatases are highly active in the PSD does not fit into the current version of the CaMKII memory switch theory [68], we suggest, rather, that additional mechanisms are needed to sustain the increased efficacy during long periods of time. Recently, "upstream" mechanisms have started to be clarified [45,73,74,78,120]. These include increased synthesis and insertion into PSDs of GluRs and other synaptic proteins, which may even increase the size of PSDs, as well as the activation of several plasticity-related genes [89]. It is not clear what triggers these upstream mechanisms, although increased Ca^{2+} in the spines, cyclic AMP, and the activation of different protein kinases and signalling cascades have been shown to be involved [36,39,73]. It is not known whether genomic changes are irreversible, and the genes become permanently activated, although this, by homology to other genes, would seem unlikely, since it would need a mutation-like event. The other non-genomic upstream mechanisms, all of which involve some sort of protein modification, usually a

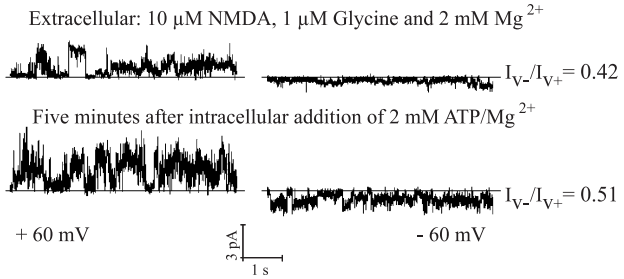

Fig. 4. Effect of PSD phosphorylation on NMDA receptor currents in the presence of 2 mM "extracellular" Mg^{2+}. Upper panel: Control recording of a membrane patch in the presence of 10 μM NMDA plus 1 μM glycine in the patch pipette. Lower panel: Recording of the same membrane patch after the addition of 2 mM ATP–Mg^{2+} to the "intracellular" side of the PSD. Current increases at both 60 and −60 mV could be observed.

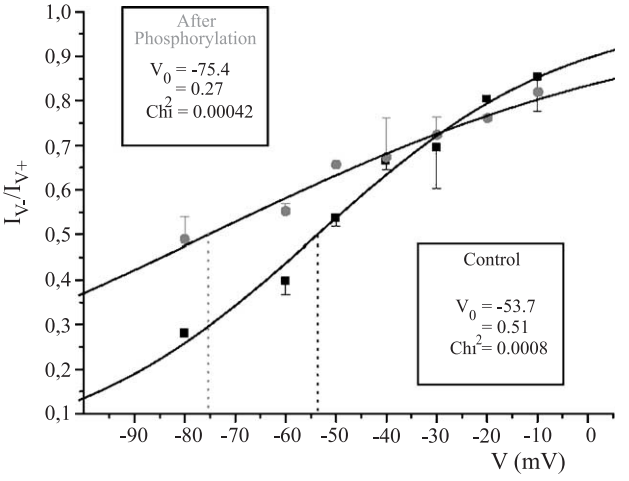

Fig. 5. PSD phosphorylation-induced changes of the $(Mg^{2+})_o$-dependent voltage block in NMDA plus glycine gated currents. Mean current values for 1-min recordings at each membrane potential were calculated. The ratio between mean currents ± S.E.M. at a negative and the same, but positive membrane potential (V_m) was plotted versus V_m. The values for both the control situation (black squares) and after PSD phosphorylation (grey circles) were fitted with a Boltzmann function [18] according to the following equation: $I_{V_{m-}}/I_{V_{m+}} = 1/(1+\exp(-zF\delta(V-V_o)/RT))$, where z is the valence, F the Faraday constant, δ the electric distance, R the gas constant, T the absolute temperature, V the membrane potential and V_o the membrane potential at which 50% channel block occurs. Phosphorylation shifted the voltage that blocked 50% of the current from −53.9 to −74.8 mV and δ changed from 0.51 to 0.27.

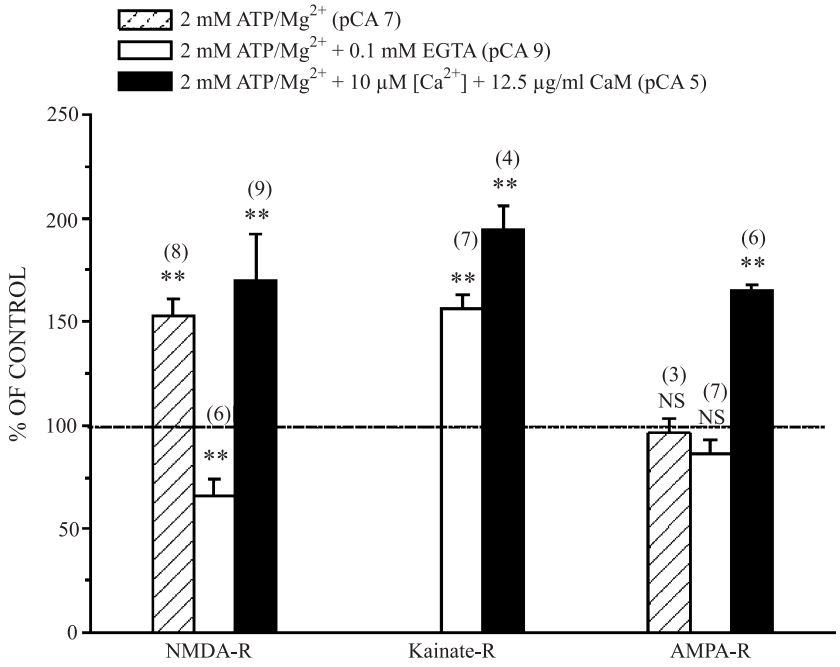

Fig. 6. Effects of PSD phosphorylation on iGluR mediated currents. Bars are the mean 1-min currents ± S.E.M. in different experiments (n shown in parenthesis) when compared to controls. The different phosphorylation conditions were: hatched bar: 2 mM ATP−Mg^{2+} in the presence of contaminating Ca^{2+} (pCa 7); white bars: 2 mM ATP−Mg^{2+} plus 0.1 mM EGTA (pCa 9); black bars: 2 mM ATP−Mg^{2+} plus 10 μM Ca^{2+} (pCa 5) plus 12.5 μg/ml of calmodulin.

phosphorylation, are certainly reversible and should revert to the initial state once the stimulus that switched them on, ceases. The half-life for the decay of these genomic and non-genomic mechanisms may be conceived as longer than the one seen in phosphorylated AMPARs, but nevertheless deactivation should occur.

In our opinion, the changes that allow a very long permanence of increased synaptic efficacy cannot exclusively be due to a static structural modification, i.e. the phosphorylation of a protein or the insertion of a greater number of GluRs into PSDs, because all these processes are subject to a turnover, and once the stimulus that led to a modified structural state ceases, turnover shall revert the changed structure to its original state. A stable change in synaptic efficacy would require that some of the initial changes, that occur during synaptic activation, give rise to

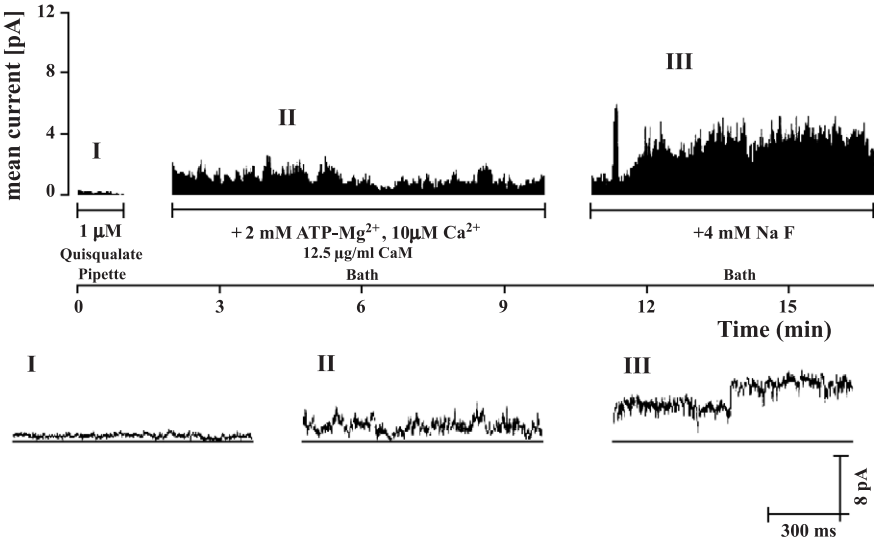

Fig. 7. Inhibition of phosphatases induces further AMPAR activation. Upper panel: the mean current of consecutive 1.024 s of quisqualate-activated receptors in a membrane patch was plotted as a function of time. Three sequential experimental conditions are shown: (I) a control situation in the presence of 1 μM quisqualate in the patch pipette; (II) after addition to the recording bath of 2 mM ATP−Mg^{2+} plus 10 μM Ca^{2+} (pCa 5) plus 12.5 μ/ml of calmodulin; (III) after the addition to the recording bath of 4 mM NaF. Lower panel: Representative recordings for each situation are shown.

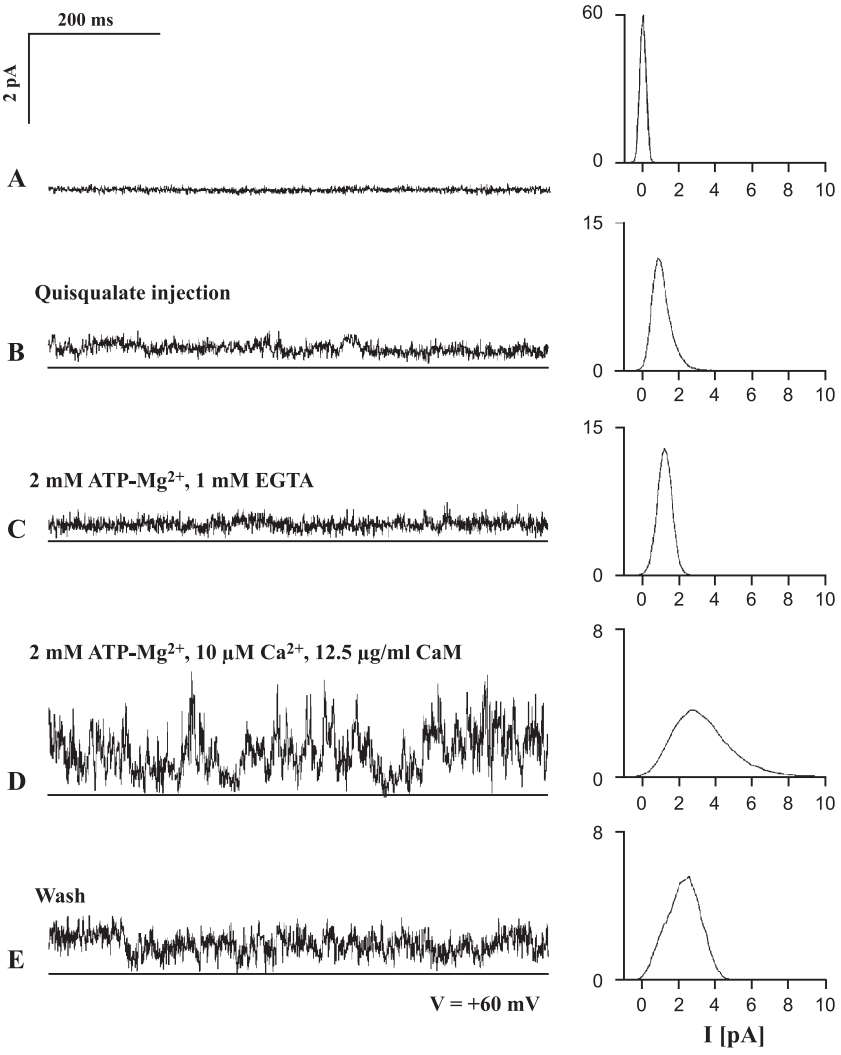

Fig. 8. Effect of PSD phosphorylation on AMPAR currents. All the recordings were obtained from the same membrane patch. At the left hand side, current records; at the right, 1-min current amplitude histograms are shown. (A) Control recording of a membrane patch at +60 mV in the absence of agonists in the patch pipette. (B) Quiqualate (0.5 µM) was injected into the patch pipette, (C) 2 mM ATP–Mg^{2+} plus 1 mM EGTA were added to the bath ("intracellular" side of the PSD). (D) 2 mM ATP–Mg^{2+} plus 10 µM Ca^{2+} (pCa 5) plus 12.5 µ/ml of calmodulin were added to the bath. (E) 10 min after, the bath fluid was replaced by one without ATP–Mg^{2+}, Ca^{2+} or calmodulin, that is, similar to the initial one. The control recording solution was 140 mM NaCl, 0.1 µM EDTA, 10 mM HepesNa, pH 7.4. Traces A, B and D are reproduced from [136].

a self-sustained dynamic state, in which the activated state is maintained by the continuous re-generation of the conditions present at the beginning of activation. One of these mechanisms has been recently discussed by Si et al. [109,110] who found prion-like properties in the synaptic protein cytoplasmic polyadenylation element binding protein (CPEB). Synaptic activation induces a conformational change that is self-perpetuating.

Our model predicts that the effect of phosphorylation on the activity of NMDARs and, especially, the dependency on low Ca^{2+} concentrations of the protein kinase selectively associated to them, is another example of a self-sustained mechanism that may keep a glutamatergic synapse in a state of enhanced efficacy for a very long period (Fig. 9). Thus, once a glutamatergic synapse is potentiated because of an intense release of glutamate, phosphorylation of NMDARs allows them to unblock from voltage-dependent Mg^{2+} inhibition at substantially smaller depolarizations (cf. Fig. 5) relative to non-potentiated NMDARs. This shall lead, during normal, low frequency operation of the synapse, to an easier opening of NMDAR channels, and to greater Ca^{2+} influx, able to repotentiate AMPARs. When excitation ceases, intraspine Ca^{2+} starts to decrease, and so AMPARs progressively deactivate. This does not exclude the operation of mechanisms like those proposed by Lisman [68], where AMPAR deactivation can be counteracted by rephosphorylation by active CaMKII. However, as the NMDAR-associated kinase is active at low $(Ca^{2+})i$ (i.e. pCa 7), it shall be much less affected by the decreasing Ca^{2+} concentration, keeping the receptors activated also for long periods. Slow frequency synaptic activation, and even quantal spontaneous glutamate release, could keep feeding

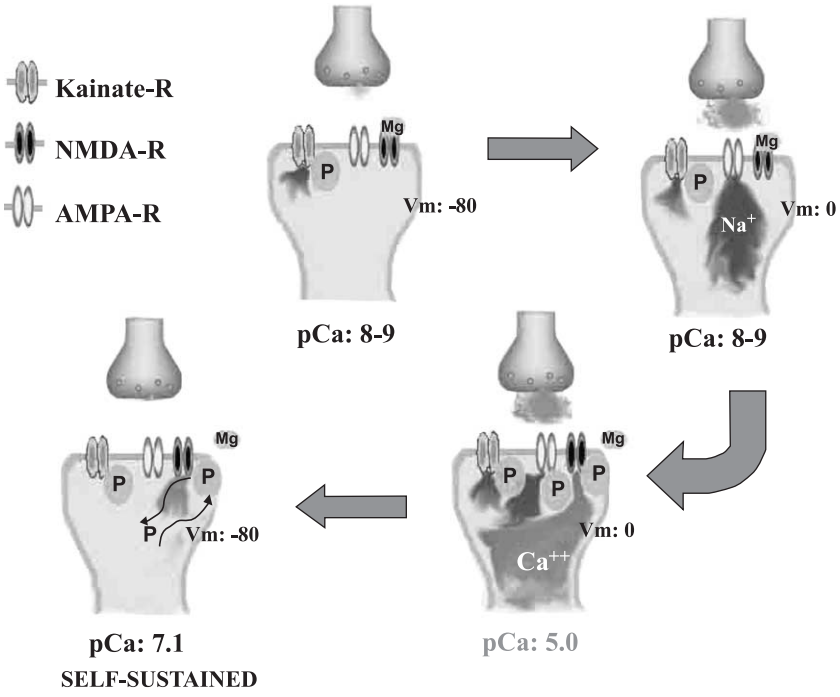

Fig. 9. The model based on the differential requirements of Ca^{2+} concentrations for GluR phosphorylation and activation. Before potentiation, only kainate receptors are phosphorylated, and thus, maximally active when low glutamate is released. During high activity periods, the intracellular Ca^{2+} concentration rises to μM levels allowing the phosphorylation of both AMPA and NMDARs. When returning to basal activity, increased Ca^{2+} influx through phosphorylated NMDARs keeps the intraspine Ca^{2+} at about pCa7, that is enough to maintain its specific kinase active, which in turn allows enough Ca^{2+} influx to become a self-sustained mechanism.

Ca^{2+} into the spines in sufficient amounts to keep its concentration at a level that stimulates the ''NMDAR kinase'', and thus, keeps the synapse potentiated. Release of Ca^{2+} from intraspine stores could also contribute to this [100]. This is supported by the finding that APV is able to reduce an established hippocampal LTP [140], and, especially by the findings that the resting Ca^{2+} concentration in resting spines can reach 50 to 79 nM (pCas of 7.3 to 7.1) [35,88], which would keep the NMDARs in a state of high responsiveness. The recent findings that subthreshold activation of NMDARs is able to elicit in them significant Ca^{2+} elevations [56], also support our hypothesis. This long-term activation of NMDARs, with its consequent maintenance of an intraspine pCa of 7.1, could also be part of the so-called ''tag'', that signals to the genomic products where an activated synapse is, and induces their localization in them [80].

This postulated self-sustaining NMDAR mechanism may be only one of a series of other self-sustaining processes that can contribute to the establishment of an increased efficacy of central glutamatergic excitatory synapses. Such mechanisms seem closely related to Descartes view on brain function, when in his Traité de l'Homme he stated: ''When the action induced by the object increases the opening of the tubes (or pores), and causes the (animal) spirits to penetrate in larger amounts than if such increase in opening had never occurred, they will have the strength to form certain passages that shall remain open even after the action of the object has ceased. In case they close, at least the small pores retain a certain disposition to be opened again'' [23] (In Descartes, the concept of ''animal spirits'' was equivalent to our nerve impulses, and we may equate his ''small pores'' to synapses or, even, to glutamate receptor ion channels.)

In the recent past, our laboratory has shown that synaptic vesicles derived from the rat CNS have a very high glutamate content, and that this amino acid is the only physiologically relevant transmitter that is able to interact with kainate, AMPA or NMDARs, thus establishing the concept of the glutamatergic central synapse (reviewed by Orrego and Villanueva [94]). With this new physiological preparation, the isolated patch-clamped PSD, we now offer a tool of great promise for future research.

Acknowledgements

This work is supported by Fondecyt Grants 198063, 1020257, by the Volkswagen Stiftung and Universidad de los Andes Projects.

References

[1] E. Aizenman, S.A. Lipton, R.H. Loring, Selective modulation of NMDA responses by reduction and oxidation, Neuron 2 (1989) 1257–1263.

[2] A. Al-Hallaq, R.P. Yasuda, B.B. Wolfe, Enrichment of NMDA NR1 splice variants and synaptic proteins in rat postsynaptic densities, J. Neurochem. 77 (2001) 110–119.
[3] D.W. Ali, M.W. Salter, NMDA receptor regulation by src kinase signalling in excitatory synaptic transmission and plasticity, Curr. Opin. Neurobiol. 11 (2001) 336–342.
[4] G. Ayalon, Y. Stern-Bach, Functional assembly of AMPA and kainate receptors is mediated by several discrete protein–protein interactions, Neuron 31 (2001) 103–113.
[5] T.G. Banke, D. Bowie, H. Lee, R.L. Huganir, A. Schousboe, S.F. Traynelis, Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase, J. Neurosci. 20 (2000) 89–102.
[6] A. Barría, V. Derkach, T.R. Soderling, Identification of the Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in the α-amino-3-hydroxy-5-methyl-4-isoxasole-propionate type glutamate receptor, J. Biol. Chem. 272 (1997) 32727–32730.
[7] J. Bernard, Ch. Chabot, J. Gagné, M. Baudry, G. Massicotte, Melittin increases AMPA receptor affinity in rat brain synaptoneurosomes, Brain Res. 671 (1995) 195–200.
[8] T. Blank, I. Nijholt, U. Teichert, H. Kugler, H. Behrsing, A. Fienberg, P. Greengard, J. Spiess, The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal *N*-methyl-D-aspartate responses, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 14859–14864.
[9] T.V. Bliss, G.L. Collingridge, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361 (1993) 31–39.
[10] J.E. Brenman, D.S. Chao, S.H. Gee, A.W. McGee, S.E. Craven, D.R. Santillano, Z. Wu, F. Huang, H. Xia, M.F. Peters, S.C. Froehner, D.S. Bredt, Interaction of nitric oxide synthase with the post-synaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains, Cell 84 (1996) 757–767.
[11] R.K. Carlin, D.J. Grab, R.S. Cohen, P. Siekevitz, Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities, J. Cell Biol. 86 (1980) 831–843.
[12] A.L. Carvalho, K. Kameyama, R.L. Huganir, Characterization of phosphorylation sites on the glutamate receptor 4 subunit of the AMPA receptors, J. Neurosci. 19 (1999) 4748–4754.
[13] A.L. Carvalho, S. Correia, C.J. Faro, C.B. Duarte, A.P. Carvalho, E.M.V. Pires, Phosphorylation of GluR4 AMPA-type glutamate receptor subunit by protein kinase C in cultured retina amacrine neurons, Eur. J. Neurosci. 15 (2002) 465–474.
[14] S.F. Chan, N.J. Sucher, An NMDA receptor signalling complex with protein phosphatase 2A, J. Neurosci. 21 (2001) 7985–7992.
[15] J.E. Chatterton, M. Awobuluyi, L.S. Premkumar, H. Takahashi, M. Talantova, Y. Shin, J. Cui, S. Tu, K.A. Sevarino, N. Nakanishi, G. Tong, S.A. Lipton, D. Zhang, Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits, Nature 415 (2002) 793–798.
[16] L. Chen, L.Y. Mae Huang, Protein Kinase C reduces Mg^{2+} block of NMDA-receptor channels as a mechanism of modulation, Nature 356 (1992) 521–523.
[17] H.H. Cheung, Tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor by exogenous and postsynaptic density-associated src-family kinases, J. Neurochem. 78 (2001) 524–534.
[18] S.S. Correira, C.B. Duarte, C.J. Faro, E.V. Pires, A.L. Carvalho, Protein kinase C gamma associates directly with the GluR4 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor subunit. Effect on receptor phosphorylation, J. Biol. Chem. 278 (2003) 6307–6313.
[19] R. Coronado, C. Miller, Voltage-dependent caesium-blockade of a cation channel from fragmented sarcoplasmic reticulum, Nature 280 (1979) 807–810.
[20] M.J. Courtney, D.G. Nicholls, Interactions between phospholipase C-coupled and *N*-methyl-D-aspartate receptors in cultured cerebellar granule cells: protein kinase C mediated inhibition of *N*-methyl-D-aspartate responses, J. Neurochem. 59 (1992) 983–992.
[21] S. Cull-Candy, S. Brickley, M. Farrant, NMDA receptor subunits: diversity, development and disease, Curr. Opin. Neurobiol. 11 (2001) 327–335.
[22] V. Derkach, A. Barría, T.R. Soderling, Ca^{2+}/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 3269–3274.
[23] R. Descartes, Traité de l'Homme, Spanish Edition based on the 1677 French version, Alianza Editorial, Madrid, 1990.
[24] R. Dingledine, K. Borges, D. Bowie, S. Traynelis, The glutamate receptor ion channels, Pharmacol. Rev. 51 (1999) 7–61.
[25] A. Doble, The role of excitotoxicity in neurodegenerative disease: implications for therapy, Pharmacol. Ther. 81 (1999) 163–221.
[26] H. Dong, R.J. O'Brien, E.T. Fung, A.A. Lanahan, P.F. Worley, R.L. Huganir, GRP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors, Nature 386 (1997) 279–284.
[27] G. Durand, M.V.L. Bennett, R.S. Zukin, Splice variants of the *N*-methyl-D-aspartate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 6731–6735.
[28] J.A. Esteban, S.H. Shi, C. Wilson, M. Nuriya, R.L. Huganir, R. Malinow, PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity, Nat. Neurosci. 6 (2003) 136–143.
[29] D.K. Fong, A. Rao, F.T. Crump, A.M. Craig, Rapid synaptic remodeling by protein kinase C: reciprocal translocation of NMDA receptors and calcium/calmodulin-dependent kinase II, J. Neurosci. 22 (2002) 2153–2164.
[30] M. Frerking, R.A. Nicoll, Synaptic kainate receptors, Curr. Opin. Neurobiol. 10 (2000) 342–351.
[31] E.P. Garcia, S. Mehta, L.A.C. Blair, D.G. Wells, J. Shang, T. Fukushima, J. Fallon, C. Garner, J. Marshall, SAP90 binds and clusters kainate receptors causing incomplete desensitization, Neuron 21 (1998) 727–739.
[32] F. Gardoni, A. Caputi, M. Cimino, L. Pastorino, F. Cattabeni, M. Di Luca, Calcium/calmodulin-dependent protein kinase II is associated with NR2A/B subunits of NMDA receptor in postsynaptic densities, J. Neurochem. 71 (1998) 1733–1741.
[33] M.B. Gingrich, C.E. Junge, P. Lyuboslavsky, S.F. Traynelis, Potentiation of NMDA receptor function by the serine protease thrombin, J. Neurosci. 20 (2000) 4582–4595.
[34] D.R. Grosshans, D.A. Clayton, S.J. Coultrap, M.D. Browning, LTP leads to rapid surface expression of NMDA but not AMPA receptors in adult rat CA1, Nat. Neurosci. 5 (2002) 27–33.
[35] P.B. Guthrie, M. Segal, S.B. Kater, Independent regulation of calcium revealed by imaging dendritic spines, Nature 354 (1991) 76–80.
[36] G.E. Hardingham, S. Chawla, F.H. Cruzalegui, H. Bading, Control of recruitment and transcription-activating function of CBP determines gene regulation by NMDA receptors and L-type calcium channels, Neuron 22 (1999) 789–798.
[37] G.E. Hardingham, Y. Fukunaga, H. Bading, Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways, Nat. Neurosci. 5 (2002) 405–414.
[38] M. Hollmann, S. Heinemann, Cloned glutamate receptors, Annu. Rev. Neurosci. 17 (1994) 31–108.
[39] S.-C. Hu, J. Chrivia, A. Ghosh, Regulation of CBP-mediated transcription by neuronal calcium signaling, Neuron 22 (1999) 799–808.
[40] Y.Q. Huang, W.Y. Lu, D.W. Ali, K.A. Pelkey, G.M. Pitcher, H. Aoto, J.C. Roder, T. Sasaki, M.W. Salter, J.F. MacDonald, CAKβ/Pyk2 kinase is a signalling link for induction of long-term potentiation in CA1 hippocampus, Neuron 29 (2001) 485–496.
[41] J.E. Huettner, Kainate receptors: knocking out plasticity, Trends Neurosci. 24 (2001) 488.
[42] H. Husi, M.A. Ward, J.S. Choudrhary, W.P. Blackstock, S.G.N. Grant, Proteomic analysis of NMDA receptor–adhesion protein signaling complexes, Nat. Neurosci. 3 (2000) 661–669.

[43] M. Irie, Y. Hata, M. Takeuchi, K. Ichtchenko, A. Toyoda, K. Hirao, Y. Takai, T.W. Rosahl, T.C. Südhof, Binding of neuroligins to PSD-95, Science 277 (1997) 1511–1515.
[44] J.S. Isaacson, G.J. Murphy, Glutamate-mediated extrasynaptic inhibition: direct coupling of NMDA receptors to Ca^{2+}-activated K^+ channels, Neuron 31 (2001) 1027–1034.
[45] C. Job, J. Eberwine, Localization and translation of mRNA in dendrites and axons, Nat. Rev., Neurosci. 2 (2001) 889–898.
[46] J.W. Johnson, P. Ascher, Glycine potentiates the NMDA response in cultured mouse brain neurones, Nature 325 (1987) 529–531.
[47] M. Kennedy, Signal-processing machines at the postsynaptic density, Science 290 (2000) 750–754.
[48] E. Kim, M. Niethammer, A. Rothschild, Y.N. Jan, M. Sheng, Clustering of Shaker-type K^+ channels by interaction with a family of membrane-associated guanylate kinases, Nature 378 (1995) 85–88.
[49] E. Kim, S. Naisbitt, Y.P. Hsueh, A. Rao, A. Rothschild, A.M. Craig, M. Sheng, GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules, J. Cell Biol. 136 (1997) 669–678.
[50] E. Kim, S.J. DeMarco, S.M. Marfatia, A.H. Chishti, M. Sheng, E.E. Strehler, Plasma membrane Ca^{2+} ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains, J. Biol. Chem. 273 (1998) 1591–1595.
[51] J.H. Kim, D. Liao, L.F. Lau, R.L. Huganir, SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family, Neuron 20 (1998) 683–691.
[52] Y. Kitamura, A. Miyazaki, Y. Yamanaka, Y. Nomura, Stimulatory effects of protein kinase C and calmodulin kinase II on *N*-methyl-D-aspartate receptor/channels in the postsynaptic density of rat brain, J. Neurochem. 61 (1993) 100–109.
[53] G. Köhr, S. Eckardt, H. Lüddens, H. Monyer, P. Seeburg, NMDA receptor channels: subunit specific potentiation by reducing agents, Neuron 12 (1994) 1031–1040.
[54] L. Köles, K. Wirkner, P. Illes, Modulation of ionotropic glutamate receptor channels, Neurochem. Res. 26 (2001) 925–932.
[55] H.C. Kornau, L.T. Schenker, M.B. Kennedy, P.H. Seeburg, Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95, Science 269 (1995) 1737–1740.
[56] Y. Kovalchuk, J. Eilers, J. Lisman, A. Konnerth, NMDA receptor-mediated subthreshold Ca^{2+} signals in spines of hippocampal neurons, J. Neurosci. 20 (2000) 1791–1799.
[57] J.Y Lan, V.A. Skeberdis, T. Jover, S.Y. Grooms, Y. Lin, R.C. Araneda, X. Zheng, M.V. Bennett, R.S. Zukin, Protein kinase C modulates NMDA receptor trafficking and gating, Nat. Neurosci. 4 (2001) 382–390.
[58] L.F. Lau, R.L. Huganir, Differential tyrosine phosphorylation of *N*-methyl-D-aspartate receptor subunits, J. Biol. Chem. 270 (1995) 20036–20041.
[59] H.-K.Lee,M.Barbarosie,K.Kameyama,M.F.Bear,R.L.Huganir,Huganir, Huganir, Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity, Nature 405 (2000) 955–959.
[60] J. Lerma, Rules and rules of kainate receptors in synaptic transmission, Nat. Rev. Neurosci. 4 (2003) 481–495.
[61] J. Lerma, A.V. Paternain, J.R. Naranjo, B. Mellström, Functional kainate-selective glutamate receptors in cultured hippocampal neurons, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 11688–11692.
[62] J. Lerma, M. Morales, M.A. Vicente, O. Herreras, Glutamate receptors of the kainate type and synaptic transmission, TINS 20 (1997) 9–14.
[63] C. Levenes, H. Daniel, F. Crepel, Long-term depression of synaptic transmission in the cerebellum: cellular and molecular mechanisms revisited, Prog. Neurobiol. 55 (1998) 79–91.
[64] G.Y. Liao, M.A. Kreitzer, B.J. Sweetman, J.P. Leonard, The postsynaptic density protein PSD-95 differentially regulates insulin- and src-mediated current modulation of mouse NMDA receptors expressed in Xenopus oocytes, J. Neurochem. 75 (2000) 282–287.
[65] G.Y. Liao, D.A. Wagner, M.H. Hsu, J.P. Leonard, Evidence for direct protein kinase-C mediated modulation of *N*-methyl-D-aspartate receptor current, Mol. Pharmacol. 59 (2001) 960–964.
[66] D.N. Liebermann, I. Mody, Regulation of NMDA receptor function by endogenous Ca dependent phosphatase, Nature 369 (1994) 235–239.
[67] J. Lisman, A.M. Zhabotinsky, A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly, Neuron 31 (2001) 191–201.
[68] J. Lisman, H. Schulman, H. Cline, The molecular basis of CaMKII function in synaptic and behavioural memory, Nat. Rev. Neurosci. 3 (2002) 175–190.
[69] Y. Li-Smerin, J.W. Johnson, Effects of intracellular Mg^{2+} on channel gating and steady-state responses of the NMDA receptor in cultured rat neurons, J. Physiol. (Lond.) 491 (1996) 137–150.
[70] S.M. Logan, F.E. Rivera, J.P. Leonard, Protein kinase C modulation of recombinant NMDA receptor currents: roles for the C-terminal C1 exon and calcium ions, J. Neurosci. 19 (1999) 974–986.
[71] W.Y. Lu, Z.G. Xiong, S. Lei, B.A. Orser, E. Dudek, M.D. Browning, J.F. MacDonald, G-protein-coupled receptors act via protein kinase C and Src to regulate NMDA receptors, Nat. Neurosci. 2 (1999) 331–338.
[72] W.-Y. Lu, M.F. Jackson, D. Bai, B.A. Orser, J.F. MacDonald, In CA1 pyramidal neurons of the hippocampus protein kinase C regulates calcium-dependent inactivation of NMDA receptors, J. Neurosci. 20 (2000) 4452–4461.
[73] W.-Y. Lu, H.-Y. Man, W. Ju, W.S. Trimble, J.F. MacDonald, Y.T. Wang, Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons, Neuron 29 (2001) 243–254.
[74] C. Lüscher, R.A. Nicoll, R.C. Malenka, D. Muller, Synaptic plasticity and dynamic modulation of the postsynaptic membrane, Nat. Neurosci. 3 (2000) 545–550.
[75] J.F. MacDonald, S.A. Kotecha, W.Y. Lu, M.F. Jackson, Convergence of PKC-dependent kinase signal cascades on NMDA receptors, Curr. Drug Targets 2 (2001) 299–312.
[76] D.R. Madden, The structure and function of glutamate receptor ion channels, Nat. Rev. Neurosci. 3 (2002) 91–101.
[77] R.C. Malenka, R.A. Nicoll, Long-term potentiation—a decade of progress, Science 285 (1999) 1870–1874.
[78] R. Malinow, Z.F. Mainen, Y. Hayashi, LTP mechanisms: from silence to four-lane traffic, Curr. Opin. Neurobiol. 10 (2000) 352–357.
[79] A.L. Mammen, K. Kameyama, K.W. Roche, R.L. Huganir, Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II, J. Biol. Chem. 272 (1997) 32528–32533.
[80] K.C. Martin, K.S. Kosik, Synaptic tagging—who's it? Nat. Rev. Neurosci. 3 (2002) 813–820.
[81] M.L. Mayer, G.L. Westbrook, P.B. Guthrie, Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones, Nature 309 (1984) 261–263.
[82] M.L. Mayer, L.J. Vicklicky, G.L. Westbrook, Modulation of excitatory amino acid receptors by group II B metal cations in cultured mouse hippocampal neurones, J. Physiol. (Lond.) 415 (1989) 329–350.
[83] C.J. Mc Bain, M.L. Mayer, *N*-methyl-D-aspartic acid receptor structure and function, Physiol. Rev. 74 (1994) 723–760.
[84] Y. Ming Lou, J.C. Roder, J. Davidow, M.W. Salter, Src activation in the induction of long-term potentiation in CA1 hippocampal neurons, Science 279 (1998) 1363–1367.
[85] D.T. Monaghan, R.J. Bridges, C.W. Cotman, The excitatory amino-acid receptors, Annu. Rev. Pharmacol. Toxicol. 29 (1989) 365–402.
[86] I.S. Moon, M.L. Apperson, M.B. Kennedy, The major tyrosine-

phosphorylated protein in the postsynaptic density fraction is *N*-methyl-D-aspartate receptor subunit 2B, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 3954–3958.
[87] J.P. Mothet, A.T. Parent, H. Wolosker, R.O. Brady Jr., D.J. Linden, C.D. Ferris, M.A. Rogawski, S.H. Snyder, D-serine is an endogenous ligand for the glycine site of the *N*-methyl-D-aspartate receptor, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4926–4931.
[88] W. Müller, J. Connor, Dendritic spines as individual neuronal compartments for synaptic Ca^{2+} responses, Nature 354 (1991) 73–76.
[89] E. Nedivi, D. Hevroni, D. Naot, D. Israeli, Y. Citri, Numerous candidate plasticity-related genes revealed by differential cDNA cloning, Nature 363 (1993) 718–722.
[90] M. Niethammer, E. Kim, M. Sheng, Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases, J. Neurosci. 16 (1996) 2157–2163.
[91] M. Niethammer, J.G. Valtschanoff, T.M. Kapoor, T.W. Allison, T.M. Weinberg, A.M. Craig, M. Sheng, CRIPT, a novel postsynaptic protein that binds to the third PDZ domain of PSD-95/SAP90, Neuron 20 (1998) 693–707.
[92] L. Novak, P. Bregestovski, P. Ascher, A. Herbet, A. Prochiantz, Magnesium gates glutamate-activated channels in mouse central neurones, Nature 307 (1984) 462–465.
[93] R.V. Omkumar, M.J. Kiely, A.J. Rosenstein, K.-T. Min, M.B. Kennedy, Identification of a phosphorylation site for Calcium/calmodulin-dependent protein kinase II in the NR2B subunit of the *N*-methyl-D-aspartate receptor, J. Biol. Chem. 271 (1996) 31670–31678.
[94] F. Orrego, S. Villanueva, The chemical nature of the main central excitatory transmitter: a critical appraisal based upon release studies and synaptic vesicle localization, Neuroscience 56 (1993) 539–555.
[95] M. Passafaro, C. Sala, M. Niethammer, M. Sheng, Microtubule binding by CRIPT and its potential role in the synaptic clustering of PSD-95, Nat. Neurosci. 2 (1999) 1063–1069.
[96] A.V. Paternain, M. Morales, J. Lerma, Selective antagonism of AMPA receptors unmasks kainate receptor mediated responses in hippocampal neurons, Neuron 14 (1995) 185–189.
[97] A.V. Paternain, M.T. Herrera, M.A. Nieto, J. Lerma, GluR5 and GluR6 kainate receptor subunits coexist in hippocampal neurons and coassemble to form functional receptors, J. Neurosci. 20 (2000) 196–205.
[98] P. Penzes, R.C. Johnson, R. Sattler, X. Zhang, R.L. Huganir, V. Kambampati, R.E. Mains, B.A. Eipper, The neuronal Rho-GEF Kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic morphogenesis, Neuron 29 (2001) 229–242.
[99] G. Riquelme, U. Wyneken, S. Villanueva, F. Orrego, Recordings of glutamate receptor channels in isolated postsynaptic densities, NeuroReport 4 (1993) 1163–1166.
[100] R. Rizzuto, Intracellular Ca^{2+} pools in neuronal signalling, Curr. Opin. Neurobiol. 11 (2001) 306–311.
[101] K.W. Roche, R.J. O'Brien, A.L. Mammen, J. Bernhardt, R.L. Huganir, Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit, Neuron 16 (1996) 1179–1188.
[102] K. Rosenblum, Y. Dudai, G. Richter-Levin, Long-term potentiation increases tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor subunit 2B in rat dentate gyrus in vivo, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 10457–10460.
[103] C. Rosenmund, Y. Stern-Bach, C.F. Stevens, The tetrameric structure of a glutamate receptor channel, Science 280 (1998) 1596–1599.
[104] J.A. Rostas, V.A. Brent, K. Voss, M.L. Errington, T.V.P. Bliss, J.W. Gurd, Enhanced tyrosine phosphorylation of the 2B subunit of the *N*-methyl-D-aspartate receptor in long-term potentiation, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 10452–10456.
[105] N. Sans, C. Racca, R.S. Petralia, Y.-X. Wang, J. Mc Callum, R.J. Wenthold, Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway, J. Neurosci. 21 (2001) 7506–7516.
[106] R. Sattler, M. Tymianski, Molecular mechanisms of calcium-dependent excitotoxicity, J. Mol. Med. 78 (2000) 3–13.
[107] P.H. Seeburg, The molecular biology of mammalian glutamate receptor channels, TIPS 14 (1993) 297–303.
[108] K. Shahi, M. Baudry, Increasing binding affinity of agonists to glutamate receptors increases synaptic responses at glutamatergic synapses, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 6881–6885.
[109] K. Si, S. Lindquist, E.R. Kandel1, A neuronal isoform of the Aplysia CPEB has prion-like properties, Cell 115 (2003) 879–891.
[110] K. Si, M. Giustetto, A. Etkin, R. Hsu, A.M. Janisiewicz, M.C. Miniaci, J.-H. Kim, H. Zhu, E.R. Kandel1, A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in *Aplysia*, Cell 115 (2003) 893–904.
[111] S.L.V.A. Skeberdis, J. Lan, T. Opitz, X. Zheng, M.V. Bennett, R.S. Zukin, mGluR1-mediated potentiation of NMDA receptors involves a rise in intracellular calcium and activation of protein kinase C, Neuropharmacology 40 (2001) 856–865.
[112] L.D. Snell, K.R. Iorio, B. Tabakoff, P.L. Hoffman, Protein Kinase C activation attenuates *N*-methyl-D-aspartate-induced increases in intracellular calcium in cerebellar granule cells, J. Neurochem. 62 (1994) 1783–1789.
[113] G.L. Snyder, A.A. Fienberg, R.L. Huganir, P. Greengard, A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor, J. Neurosci. 18 (1998) 10297–10303.
[114] T.R. Soderling, CaM kinases: modulators of synaptic plasticity, Curr. Opin. Neurobiol. 10 (2000) 375–380.
[115] T.R. Soderling, V.A. Derkach, Postsynaptic protein phosphorylation and LTP, TINS 23 (2000) 75–80.
[116] P. Somogyi, G. Tamás, R. Lujan, E.H. Buhl, Salient features of synaptic organisation in the cerebral cortex, Brain Res. Rev. 27 (1998) 113–135.
[117] I. Song, S. Kamboj, J. Xia, H. Dong, D. Liao, R.L. Huganir, Interaction of the *N*-ethylmaleimide-sensitive factor with AMPA receptors, Neuron 21 (1998) 393–400.
[118] S. Srivastava, P. Osten, F.S. Vilim, L. Khatri, G. Inman, B. States, C. Daly, S. DeSouza, R. Abagyan, J.G. Valtschanoff, R.J. Weinberg, E.B. Ziff, Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP, Neuron 21 (1998) 581–591.
[119] S. Strack, M.A. Barban, B.E. Wadzinski, R.J. Colbran, Differential inactivation of postsynaptic density-associated and soluble Ca^{2+}/calmodulin-dependent protein kinase II by protein phosphatases 1 and 2A, J. Neurochem. 68 (1997) 2119–2128.
[120] O. Steward, P.F. Worley, Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation, Neuron 30 (2001) 227–240.
[121] N.J. Sucher, M. Awobuluyi, Y.-B. Choi, S.A. Lipton, NMDA receptors: from genes to channels, TIPS 17 (1996) 348–355.
[122] P.C. Suen, K. Wu, J.L. Xu, S.Y. Lin, E.S. Levine, I.B. Black, NMDA receptor subunits in the postsynaptic density of rat brain: expression and phosphorylation by endogenous protein kinases, Brain Res. Mol. Brain Res. 59 (1998) 215–228.
[123] S.L. Swope, S.I. Moss, L.A. Raymond, R.L. Huganir, Regulation of ligand-gated ion channels by protein phosphorylation, Adv. Second Messenger Phosphoprot. Res. 33 (1999) 49–78.
[124] T. Tezuka, H. Umemori, T. Akiyama, S. Nakanishi, T. Yamamoto, PSD-95 promotes fyn-mediated tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor subunit NR2A, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 435–440.
[125] W.G. Tingley, K.W. Roche, A.K. Thompson, R.L. Huganir, Regulation of NMDA receptor phosphorylation by alternative splicing of the C-terminal domain, Nature 364 (1993) 70–73.
[126] W.G. Tingley, M.D. Ehlers, K. Kameyama, C. Doherty, J.B. Ptak, C.T. Riley, R.L. Huganir, Characterization of protein kinase A and

protein kinase C phosphorylation of the *N*-methyl-D-aspartate receptor NR1 subunits using phosphorylation site-specific antibodies, J. Biol. Chem. 272 (1997) 5157–5166.

[127] S. Tomita, R.A. Nicoll, D.S. Bredt, PDZ protein interactions regulating glutamate receptor function and plasticity, J. Cell Biol. 153 (2001) F19–F24.

[128] G. Tong, D. Sheperd, C.E. Jahr, Synaptic desensitization of NMDA receptors by calcineurin, Science 267 (1995) 1510–1512.

[129] R. Torres, B.L. Firestein, H. Dong, J. Staudinger, E.N. Olson, R.L. Huganir, D.S. Bredt, N.W. Gale, G.D. Yancopoulos, PDZ proteins bind, cluster and synaptically colocalize with Eph receptors and their ephrin ligands, Neuron 21 (1998) 1453–1463.

[130] J.C. Tu, B. Xiao, S. Naisbitt, J.P. Yuan, R.S. Petralia, P. Doan, A. Doan, V.K. Aakulu, A.A. Lanahan, M. Sheng, P.F. Worley, Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins, Neuron 23 (1999) 569–582.

[131] R.S.L. Vinade, A. Dosemeci, Regulation of the phosphorylation state of the AMPA receptor GluR1 subunit in the postsynaptic density, Cell. Mol. Neurobiol. 20 (2000) 451–463.

[132] D.A. Wagner, J.P. Leonard, Effect of protein kinase-C activation on the Mg^{2+} sensitivity of cloned NMDA receptors, Neuropharmacology 35 (1996) 29–36.

[133] R.S. Walikonis, O. Jensen, M. Mann, W. Provance, J. Mercer, M. Kennedy, Identification of proteins in the postsynaptic density fraction by mass spectrometry, J. Neurosci. 20 (2000) 4069–4080.

[134] L.-Y. Wang, M.W. Salter, J.F. MacDonald, Regulation of kainate receptors by cAMP-dependent protein kinases and phosphatases, Science 253 (1991) 1132–1135.

[135] R.J. Wenthold, N. Yokotani, K. Doi, K. Wada, Immunochemical characterization of the non-NMDA glutamate receptor using subunit-specific antibodies. Evidence for a hetero-oligomeric structure in rat brain, J. Biol. Chem. 267 (1992) 501–507.

[136] U. Wyneken, G. Riquelme, S. Villanueva, F. Orrego, Effect of glutamate receptor phosphorylation by endogenous protein kinases on electrical activity of isolated postsynaptic densities of rat cortex and hippocampus, Neurosci. Lett. 224 (1997) 131–135.

[137] U. Wyneken, K.H. Smalla, J.J. Marengo, D. Soto, A. de la Cerda, W. Tischmeyer, R. Grimm, T. Boeckers, G. Wolf, F. Orrego, E.D. Gundelfinger, Kainate-induced seizures alter protein composition and NMDA receptor function of rat forebrain postsynaptic densities, Neuroscience 102 (2001) 65–74.

[138] M. Wyszynski, J. Lin, A. Rao, E. Nigh, A.H. Beggs, A.M. Craig, M. Sheng, Competitive binding of alpha-actinin and calmodulin to the NMDA receptor, Nature 385 (1997) 439–442.

[139] J. Xia, X. Zhang, J. Staudinger, R.L. Huganir, Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1, Neuron 22 (1999) 179–187.

[140] M.-Y. Xiao, M. Karpefors, Y.-P. Niu, H. Wigström, The complementary nature of long-term depression and potentiation revealed by dual component excitatory postsynaptic potentials in hippocampal slices from young rats, Neuroscience 68 (1995) 625–635.

[141] Z.-G. Xiong, R. Raouf, W.Y. Lu, L.Y. Wang, B.A. Orser, E.M. Dudek, M.D. Browning, J.F. MacDonald, Regulation of *N*-methyl-D-aspartate receptor function by constitutively active protein kinase C, Mol. Pharmacol. 54 (1998) 1055–1063.

[142] Z.-G. Xiong, K.A. Pelkey, W.Y.Y.M. Lu, J.C. Roder, J.F., MacDonald, M.W. Salter, Src potentiation of NMDA receptors in hippocampal and spinal neurons is not mediated by reducing zinc inhibition, J. Neurosci. 19 (RC37) (1999) 1–6.

[143] Y. Yamada, Y. Chochi, K. Takamiya, K. Sobue, M. Inui, Modulation of the channel activity of the $\varepsilon 2/\zeta 1$-subtype *N*-methyl-D-aspartate receptor by PSD-95, J. Biol. Chem. 274 (1999) 6647–6652.

[144] Y. Yamada, Y. Chochi, J.A. Ko, K. Sobue, M. Inui, Activation of channel activity of the NMDA receptor–PSD-95 complex by guanylate kinase-associated protein (GKAP), FEBS Lett. 458 (1999) 295–298.

[145] Z. Yan, L. Hsieh-Wilson, J. Feng, K. Tomizawa, P.B. Allen, A.A. Fienberg, A.C. Nairn, P. Greengard, Protein phosphatase 1 modulation of neostriatal AMPA channels: regulation by DARPP-32 and spinophilin, Nat. Neurosci. 2 (1999) 13–17.

[146] M. Yang, J.P. Leonard, Identification of mouse NMDA receptor subunit NR2A C-terminal tyrosine sites phosphorylated by coexpression with v-src, J. Neurochem. 77 (2001) 580–588.

[147] X.-M. Yu, R. Askalan, G.J. Keil, M.W. Salter, NMDA channel regulation by channel-associated protein tyrosine kinase src, Science 275 (1997) 674–678.

[148] L. Zhang, B.A. Rzigalinski, E.F. Ellis, L.S. Satin, Reduction of voltage-dependent Mg^{2+} blockade of NMDA current in mechanically injured neurons, Science 274 (1996) 1921–1923.

[149] X. Zheng, L. Zhang, A.P. Wang, M.V.L. Bennett, R.S. Zukin, Ca^{2+} influx amplifies protein kinase C potentiation of recombinant receptors, J. Neurosci. 15 (1997) 8676–8686.

[150] F. Zheng, M.B. Gingrich, S.F. Traynelis, P.J. Conn, Tyrosine kinase potentiates NMDA receptor currents by reducing tonic zinc inhibition, Nat. Neurosci. 1 (1998) 185–191.

[151] X. Zheng, L. Zhang, A.P. Wang, M.V.L. Bennett, R.S. Zukin, Protein kinase C potentiation of *N*-methyl-D-aspartate receptor activity is not mediated by phosphorylation of *N*-methyl-D-aspartate receptor subunits, PNAS 96 (1999) 15262–15267.

[152] E.B. Ziff, Enlightening the postsynaptic density, Neuron 19 (1997) 1163–1174.