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Regulation of Neuronal Plasticity in the Central Nervous System by Phosphorylation and Dephosphorylation

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Abstract

Neuronal plasticity can be defined as adaptive changes in structure and function of the nervous system, an obvious example of which is the capacity to remember and learn. Long-term potentiation and long-term depression are the experimental models of memory in the central nervous system (CNS), and have been frequently utilized for the analysis of the molecular mechanisms of memory formation. Extensive studies have demonstrated that various kinases and phosphatases regulate neuronal plasticity by phosphorylating and dephosphorylating proteins essential to the basic processes of adaptive changes in the CNS. These proteins include receptors, ion channels, synaptic vesicle proteins, and nuclear proteins. Multifunctional kinases (cAMP-dependent protein kinase, Ca^{2+}/phospholipid-dependent protein kinase, and Ca^{2+}/calmodulin-dependent protein kinases) and phosphatases (calcineurin, protein phosphatases 1, and 2A) that specifically modulate the phosphorylation status of neuronal-signaling proteins have been shown to be required for neuronal plasticity. In general, kinases are involved in upregulation of the activity of target substrates, and phosphatases downregulate them. Although this rule is applicable in most of the cases studied, there are also a number of exceptions. A variety of regulation mechanisms via phosphorylation and dephosphorylation mediated by multiple kinases and phosphatases are discussed.

Index Entries: Kinases; phosphatases; phosphorylation; dephosphorylation; neuronal plasticity; central nervous system; receptors; ion channels; synaptic vesicles; neurotransmitters.

Neuronal plasticity can be defined as adaptive changes in structure and function of the nervous system during postnatal development, in learning and memory, in recovery from injury, and in aging. The immature central nervous system (CNS) is considered to be extremely plastic, but the mature or aged CNS was once assumed to have little plastic capability. However, ever since the rapid progress of biochemical techniques in late 1970s and 1980s, the nervous system is continually being understood at the molecular level. New electrophysiological techniques, such as the patch-clamp technique, have also been developed that allow

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the minute changes of current of even a single ion channel to be recorded. The properties of many individual ion channels and receptors have thereby been elucidated. By these analyses, the basic mechanisms underlying adaptive neuronal processes, such as axonal sprouting, dendritic growth, and glial changes are now being elucidated.

Neuronal plasticity has been a topic of increasing interest in 1980s and 1990s. Because the hippocampus is the part of the cerebral cortex that is essential for learning and memory, it has been most frequently used for these studies. When it is destroyed, the ability to form new memories is effectively lost.

Using slices of hippocampus, experimental models of memory, i.e., long-term potentiation (LTP) and long-term depression (LTD), have been introduced and extensively studied. A short burst of high frequency (> 25 Hz) repetitive firing causes long-term potentiation (LTP) such that subsequent single-action potentials in the presynaptic cells evoke a greatly enhanced response in the postsynaptic cells. Low frequency firing (1–5 Hz), on the other hand, causes long-term depression (LTD); the hallmark of which is a significantly suppressed response. These effects last days or weeks and are interpreted as the experimental models of learning and memory. Since LTP and LTD have been discovered in other parts of the CNS such as cerebellum and visual cortex, these phenomena are used as generalized models to explain memory processes in those tissues.

Through numerous studies on the molecular mechanisms of learning and memory, the phosphorylation status of key proteins has been known to be crucial to the regulation of many processes that induce functional or structural changes in the neuronal network. The relative balance of protein kinase and phosphatase activity determines the outcome of specific forms of synaptic plasticity. For example, low-frequency electric stimulation dominantly activates protein phosphatases to induce LTD, whereas high-frequency stimulation dominantly activates protein kinases to induce LTP. In the present review, the regulatory mechanisms of neuronal plasticity in the CNS are, therefore, described from the aspect of phosphorylation and dephosphorylation of essential target proteins: receptors, ion channels, synaptic vesicle proteins, enzymes, and gene-regulating proteins. In phosphorylation and dephosphorylation of these targets, kinases and phosphatases with broad substrate specificity are involved. Examples of these multifunctional kinases are cAMP-dependent protein kinase (PKA), Ca^{2+}/phospholipid-dependent protein kinase (PKC), and Ca^{2+}/calmodulin-dependent protein kinases (CaMKs). PKA is composed of regulatory (R) and catalytic (C) subunits, both of which exist as multiple isoforms. PKA is activated by cAMP, which is generated by adenylyl cyclase via G protein-coupled receptor activation. Intracellular Ca^{2+} increases via influx from the extracellular space and via mobilization from intracellular stores by inositoltriphosphate (IP_3) and increased Ca^{2+} activates two multifunctional kinases: PKC and CaMKs. The activated kinases are involved in the downstream signal transduction, however, they also provide positive or negative feedback control of the receptors or ion channels by phosphorylation. In addition, other serine/threonine kinases and protein-tyrosine kinases (PTKs) are also discussed. Calcineurin (CaN), a Ca^{2+}/calmodulin-dependent protein phosphatase (phosphatase 2B, PP2B), is enriched in the CNS, and has been studied for its role in neuronal plasticity. Protein phosphatase 1 (PP1) and 2A (PP2A) are also discussed in this review.

Regulation of Receptor Functions

In neuronal communications, signals are transduced by neurotransmitters and neuropeptides released from presynaptic cells, bind to membrane-bound receptors, and transduce signals to the inside of the postsynaptic cells. Functions of various receptors are known to be regulated by phosphorylation and dephosphorylation (Levitan, 1994). The effects of phosphorylation include modification of ligand-gated ion channel conductance, regulation

of receptor-desensitization rate, subunit assembly, and receptor aggregation at synapses. Phosphorylation and dephosphorylation of receptors are therefore important mechanisms in the regulation of receptor function and may play a crucial role in synaptic plasticity.

Transmitter-gated ion channels are specialized for rapidly converting extracellular chemical signals into electrical signals at chemical synapses. When transmitters bind to receptors, ion channels open transiently, thereby producing a brief flux of ions across the plasma membrane. Phosphorylation or dephosphorylation of these receptors alters channel conductance. Excitatory neurotransmitters such as acetylcholine, glutamate, and serotonin cause the opening of cation channels causing Na^+ influx that then depolarizes the postsynaptic membrane. However, inhibitory transmitters—such as gamma-aminobutyric acid (GABA) and glycine—open Cl^- channels and this causes the postsynaptic membrane to be polarized. The activity of most transmitter-gated ion channels are controlled by phosphorylation and dephosphorylation.

Glutamate Receptors (Glu-Rs)

Glutamate is the most common excitatory neurotransmitter for mammalian neuronal cells in the CNS. The glutamate signal is transduced by binding to Glu-Rs, the activity of which is regulated by various kinases and phosphatases (Malenka and Nicoll, 1993). Glu-Rs are classified as either ionotropic or metabotropic receptors. The ionotropic Glu-Rs are either Ca^{2+}-channel receptors (NMDA type) or Na^+-channel receptors (AMPA/KA type).

Non-NMDA Glu-Rs, GluR1(AMPA) and GluR6(KA), have been shown to be phosphorylated by PKA. Furthermore, intracellularly applied PKA increases the amplitude of the glutamate response (Gasic and Hollman, 1992; Raymond et al., 1993). NMDA receptors have been shown to be activated by PKC phosphorylation (Ben-Ari et al., 1992). Intracellularly applied PKC potentiates the NMDA currents by reducing the voltage-dependent Mg^{2+} block of ion channels (Chen and Huang, 1992). The CaMK II regulatory phosphorylation site is conserved in all ionotropic Glu-Rs suggesting that CaMK II activity influences this class of Glu-Rs (Yakel et al., 1995; Omkumar et al., 1996). Consistent with this suggestion, intracellular application of alpha-subunit of CaMK II enhances AMPA/KA and NMDA currents (Kolaj et al., 1994). Since all these three kinases phosphorylate serine/threonine residues, all may phosphorylate the identical sites of Glu-Rs, and may therefore have redundant functions in upregulating channel activity. Alternatively, these kinases may phosphorylate different sites and perform differential regulation on the receptors. Targeted experiments of phosphorylation sites will be required to elucidate these issues.

Tyrosine phosphorylation on NMDA receptors may also participate in regulation of the receptors (Moon et al., 1994; Wang and Salter, 1994). For example, tyrosine phosphorylation of the NR2 subunits of Glu-Rs may be important for regulating its function (Suzuki and Okumura-Noji, 1995). Tyrosine residues responsible for potentiation are continually rephosphorylated by some long-term PTK activity (Chen and Leonard, 1996), and tyrosine phosphorylation of the NMDA receptor contributes to the maintenance of LTP (*see* below) (Rostas et al., 1996). NMDA-channel activity is reduced by a protein tyrosine phosphatase (Wang et al., 1996).

Involvement of phosphatases in NMDA-receptor regulation has been reported (Yakel, 1997). Phosphatase inhibitors enhance NMDA currents, whereas injected PP1 or PP2A decreases the open probability of these channels (Wang et al, 1994). CaN inhibition experiments suggested that this phosphatase also desensitizes NMDA receptors (Tong et al., 1995). Yakel also showed that prolonged duration of single NMDA-receptor channel openings is observed by the inhibition of CaN by FK506 (10–50 n*M*) (Yakel, 1997). It is possible that the effect of CaN phosphatase activity is mediated by its inactivation of the inhibitor-1 protein. When inhibitor-1 is phosphorylated, it binds to PP1 and inactivates PP1 activity. However, when dephosphorylated by CaN, it does not bind to

PP1 and PP1 is then active (Mulkey et al., 1994). Whether CaN also acts directly by dephosphorylating NMDA receptors in vivo is not yet clear.

Metabotropic NMDA receptor has also been demonstrated to be regulated by protein kinases. Intracellular application of protein kinase A catalytic subunit or a phosphatase inhibitor mimics the ligand-mediated effect, indicating the involvement of PKA in this process (Pedarzini and Storm, 1994). Similar inhibition of this current has been observed by the application of phorbol esters that mimic diacylglycerol (DAG) in their ability to activate PKC (Kawabata et al., 1996). Phosphorylation of the threonine residue at position 840 of mGluR5a by PKC is responsible for the generation of Ca^{2+} oscillations in mGluR5a-expressing cells.

GABA Receptors (GABA-Rs)

GABA is an inhibitory neurotransmitter that participates in the suppression of synaptic transmission in the CNS. PKA and PKC have been shown to phosphorylate $GABA_A$ receptors at specific residues (Moss et al., 1992). The consequence of PKC-kinase action on GABA-R activity is somewhat controversial. Activation of PKC has been shown to cause a reduction of GABA-activated current (Leidenheimer et al., 1992; Kellenberger et al., 1992). Selective site-directed mutagenesis of PKC phosphorylation sites results in a decreased effect of phorbol ester. Mutation of serine 343 residue in the gamma-2 subunit exerts the largest effect on the GABA-activated response (Krishek et al., 1994). However, a recent report demonstrated that PKC activation enhanced recombinant alpha-1, beta-1, gamma-2L GABA-R by increasing maximal current in L929 fibroblasts without increasing the affinity of GABA for the GABA-Rs (Lin et al., 1994). It is possible that there are different mechanisms on the regulation of GABA-Rs by PKC. Further studies will be necessary to elucidate the precise regulation mechanisms by PKC phosphorylation. In addition to PKC, CaMK II phosphorylates beta-1 and gamma (2L, 2S) subunits of $GABA_A$ receptor (Machu et al., 1993; McDonald and Moss, 1994). Beta-1 subunit is also known to be phosphorylated by cGMP-dependent protein kinase (PKG) (McDonald and Moss, 1994; Leidenheimer, 1996). GABA-mediated inhibitory synaptic transmission undergoes a long-lasting rebound potentiation in cerebellar Purkinje cells, and this rebound potentiation is markedly enhanced by the intracellularly applied CaMK II (Kano et al., 1996). Tyrosine phosphorylation by vSrc of gamma-2L and beta-1 subunits of $GABA_A$-Rs enhances the whole-cell current induced by GABA (Moss et al., 1995). Thus, phosphorylation of $GABA_A$ receptors in general enhances GABA-mediated current.

Retinal $GABA_C$ receptors in bipolar cells have also been shown to be modulated by PKC (Feigenspan and Bormann, 1994). For example, GABA rho-1 receptor function can be regulated by PKC-mediated phosphorylation events (Kusama et al., 1995). Since this receptor is also modulated by G proteins, multiple regulatory mechanisms seem to play a role in retinal $GABA_C$-receptor regulation.

Involvement of phosphatases in GABA-receptor regulation has recently been suggested. Intracellular application of CaN inhibitors blocks the suppression of the $GABA_A$ response. The application of the selective inhibitor of CaN—the cyclosporin A–cyclophilin A complex—results in a faster recovery from desensitization following ligand binding (Martina et al., 1996).

Glycine Receptor

Glycine is another inhibitory neurotransmitter that causes the opening of Cl^- channels and, as a result, renders postsynaptic cells refractory to excitation. Gly-Rs are substrates for various kinases and phosphatases, and the function of Gly-Rs is regulated by phosphorylation and dephosphorylation.

cAMP selectively enhances the channel-open frequency of the glycine receptor through PKA-mediated phosphorylation (Agopyan et al., 1993; Vaello et al., 1994). Conversely, PKC

phosphorylation of the glycine receptor suppresses Gly-R-channel activity (Ruiz-Gomez et al., 1991). Glycine receptors therefore seem to be oppositely regulated by PKA and PKC.

Since the two inhibitory neurotransmitter receptors (GABA-R and Gly-R) have similar molecular structures (Devillers-Thiery et al., 1993), the stimulatory effects by PKA and the suppressive effects by PKC may be achieved by analogous sites of phosphorylative regulation. Further studies are necessary to clarify the mechanism of Gly-R together with GABA-R functions by phosphorylation and dephosphorylation.

G Protein-Coupled Receptors (G-Rs)

Ever since the discovery of rhodopsin kinase, the phosphorylation of G-Rs by analogous kinases was predicted (Kuhn, 1978). Subsequently, a number of similar kinases (G protein-coupled receptor kinases; GRK including beta-adrenergic receptor kinase) have recently been cloned (Bonovic et al., 1987, 1991; Ambrose et al., 1993; Kunapuli and Benovic, 1993; Benovic and Gomez, 1993). GRKs phosphorylate G-Rs with a strict substrate specificity and phosphorylation results in a decreased affinity of G proteins for G-Rs resulting in the desensitization of those receptors (Lefkowitz, 1993).

Whereas the regulation of G-Rs is predominantly achieved by GRKs, multifunctional kinases have also been suggested to play a role. For example, muscarinic Ach receptor (mAChRs) has been shown to be stoichiometrically phosphorylated by PKC (Haga et al., 1996). mAChRs are also phosphorylated by PKA (Rosenbaum et al., 1987). Phosphorylation by GRKs and by multifunctional kinases may have differential regulatory effects on these receptors, and the precise roles of each class of kinase need to be examined.

Regulation of Voltage-Dependent (Gated) Ion Channels

Voltage-dependent (gated) ion channels are responsible for the generation of action potentials in electrically excitable cells. In nerve cells, a stimulus that causes sufficient depolarization opens voltage-dependent Na^+ channels, inducing a transient and rapid Na^+ influx, and generates an action potential. When voltage-dependent K^+ channels open, the transient influx of Na^+ is rapidly overwhelmed by an efflux of K^+, and this drives the membrane back toward the resting potential. Since the response of these K^+ channels is slower than that of the Na^+ channels, voltage-dependent K^+ channels are often called delayed K^+ channels. Na^+ channels are not the only voltage-dependent cation channels that generate an action potential. In some muscle, egg, and endocrine cells, the action potentials predominantly depend upon voltage-dependent Ca^{2+} channels. Ca^{2+} channels also play a crucial role in the influx of Ca^{2+} into depolarized cells and cause a rapid increase in the intracellular Ca^{2+} concentration. Structural and functional diversity within each of three classes of voltage-dependent cation channels (Na^+, K^+, Ca^{2+}) exists because these channels are derived from multiple genes with frequent alternative splicing. Nonetheless, the amino acid sequences of these three channels show striking homologies, suggesting that these types of channels belong to a large superfamily of evolutionarily and structurally related proteins (Guy and Conti, 1990; Stuhmer, 1991).

Voltage-Dependent Calcium Channels

The Ca^{2+} influx from the extracellular space through voltage-dependent Ca^{2+} channels (VDCC) is one of the main mechanisms of intracellular increase of Ca^{2+} in neuronal cells. L-type VDCC has so far been the most extensively studied VDCC. VDCC consists of four subunits: alpha 1, alpha 2, beta, and gamma, and alpha 1 composes main structure of Ca^{2+} channels. Phosphorylation sites have been identified in both alpha-1 and beta subunits. The function of alpha-1 and beta subunits of the L-type VDCC has been shown to be regulated by PKA (Levitan, 1994). PKA phosphorylation enhances the Ca^{2+} current of VDCC. Hell

et al. (1993) showed differential phosphorylation of the alpha-1 subunit of neuronal VDCC. The neuronal L-type VDCC alpha-1 subunit exists as a 210–235-kDa peptide and as a 190–195-kDa peptide and the former but not the latter is phosphorylated in vitro by PKA. In contrast, both peptides are substrates for PKC, CaMK II, and PKG. Hell et al. also did similar analysis for alpha-1 subunit of N-type VDCC, both the long (250-kDa) and short (220-kDa) forms were phosphorylated by PKA, PKC, and PKG, whereas, only the short form was a substrate for CaMK II (Hell et al., 1994). Similarly, direct intracellular injection of cAMP results in an increase in activity of the macroscopic Ca^{2+} channel of P-type VDCC (Fournier et al., 1993). The increase in current amplitude is then followed by a delay in current inactivation. This delay is attributed to the activation of PKA, since it could be antagonized by a peptidic inhibitor of this enzyme.

Chad and Eckert described the dephosphorylation of VDCC by CaN in vitro (Chad and Eckert, 1986). Additionally, Lai et al. showed that PP1 and PP2A dephosphorylate both the alpha-1 and beta subunits of VDCC, whereas CaN preferentially dephosphorylates the beta subunit (Lai et al., 1993). Dephosphorylation of VDCC leads to a diminution of the Ca^{2+} current. Although most of these data are performed in vitro, these data strongly suggest that phosphorylation-dephosphorylation is likely to be one of the main regulatory mechanisms of VDCC function in vivo.

Voltage-Dependent Sodium Channels

Voltage-dependent Na^+ channels consist of alpha and beta subunits; the former is responsible for channel activity. The alpha subunit is phosphorylated by PKA (Costa et al., 1982; Smith and Goldin, 1992; Murphy et al., 1996) and by PKC (West et al., 1991), and as a consequence, Na^+ current is significantly decreased (Numann et al., 1991). Using inhibitor experiments, Chen et al. showed that PP2A and CaN dephosphorylate Na^+ channels in brain and thus may counteract the effect of cAMP-dependent phosphorylation on Na^+ channel activity (Chen et al., 1995).

Voltage-Dependent Potassium Channels

K^+ currents are crucial for the repolarization of electrically excitable membranes, and slight modifications to K^+ channel function can result in changes in synaptic efficacy. Structure/function studies have been centered around the *D. melanogaster* K^+ channel and Shaker gene products and have given important insights into the mechanisms of K^+-channel regulation (Timpe et al., 1988). The introduction of a dominant-negative PKA regulatory subunit into CHO cells in which the mouse Shaker homolog is highly expressed results in high K^+ current (Bosma et al., 1993), suggesting that the activation of PKA reduces K^+ current. Analogous studies involving PKC have shown that in contrast to the effect of PKA, activation of PKC increases K^+ current and this effect can be reversed by PKC inhibitors (Furukawa et al., 1995). Site-directed mutagenesis of *Aplysia* K^+ channel revealed that an important target for PKC-dependent regulation is Thr345 (Furukawa et al., 1995). Huang et al. also showed that the G protein-coupled m1 muscarinic-Ach receptor potently suppresses K^+ current through direct tyrosine phosphorylation of the cloned K^+ channel (Huang et al., 1993), indicating that PTKs also are involved in K^+-channel regulation.

Other Substrates of Phosphorylation and Dephosphorylation

There are a number of substrates of kinases and phosphatases in addition to the receptors and ion channels described above that have been reported to be involved in plasticity of the CNS. Several representative proteins that have been frequently linked to neuronal plasticity processes are discussed.

Synaptic Vesicle Proteins and Related Proteins

Several proteins associated with synaptic vesicles are targets for protein phosphorylation and dephosphorylation (Greengard et al., 1993). Synapsin I is a synaptic vesicle-associated phosphoprotein that is involved in the modulation of neurotransmitter release. CaMK II phosphorylates synapsin I and promotes its dissociation from vesicles, thus removing a constraint on the release of neurotransmitters (Baines and Bennett, 1986). In LTP, increased phosphorylation of synapsin I has been demonstrated (Fukunaga et al., 1995). PKA (Bahler and Greengard, 1987) and PKC (Severin et al., 1989) also phosphorylate synapsin I. Actin-bundling activity of synapsin I was reduced by the CaMK II phosphorylation (Petrucci and Morrow, 1987). CaMK I (DeRemer et al., 1992) and proline-directed protein kinases such as mitogen-activated protein (MAP) kinase and cyclin-dependent protein kinase 5 (Cdk5) (Matsubara et al., 1996), can phosphorylate synapsin I in vitro, although the physiological significance of this has yet to be determined.

Synaptotagmin is also a synaptic vesicle protein and has an essential function in neurotransmission. Synaptotagmin has the Ca^{2+}-phospholipid-binding activity and, therefore, has been implicated in the docking and fusion of synaptic vesicles with the presynaptic membrane during Ca^{2+}-induced exocytosis. Phosphorylation by CaMK II may modulate its binding activity (Papoli et al., 1993).

Rab3A, a small GTP-binding protein, and its associated proteins—such as Rabphilin-3A, a putative target protein for Rab3A—are implicated in neurotransmitter release. Rabphilin-3A interacts with rab3A in a GTP-dependent manner and binds Ca^{2+} in a phospholipid-dependent manner. Rabphilin-3A is efficiently phosphorylated at residue 234 by both PKA and CaMK II and at residue 274 by CaMK II. It is possible that these kinases may thereby regulate neurotransmitter release (Fykse et al., 1995).

GAP-43 (B-50, neuromodulin, and F1) is a presynaptic membrane-associated protein that plays a key role in guiding the growth of axons and modulating the formation of new neuronal connections (Pasinelli et al., 1995; Benowitz and Routtenberg, 1997). Transgenic mice that overexpress GAP-43 show a spontaneous formation of aberrant connections in the CNS (Aigner et al., 1995). GAP-43 appears to be involved in transducing intra- and extracellular signals to regulate cytoskeletal reorganization at the nerve ending. Phosphorylation by PKC on Ser41 is particularly significant in this regard and is linked with both nerve-terminal sprouting and LTP. Transgenic experiments demonstrate that phorphorylation of GAP-43 appears to induce a cellular state associated with growth and sprouting (Aigner et al., 1995). The PKC phosphorylation site (Ser41) has been shown to be dephosphorylated by PP1, PP2A, and CaN in vitro (Benowitz and Routtenberg, 1997).

There have been an increasing number of publications that indicate phosphorylation-dephosphorylation control of other synaptic vesicle proteins. Neurotransmitter release and subsequent neuronal plasticity are most likely modulated by phosphorylation status of those proteins as well.

Nitric Oxide Synthase

Nitric oxide (NO) has been implicated to function as a retrograde messenger in NMDA-receptor-mediated LTP (Jaffrey and Snyder, 1995). NO is formed in postsynaptic cells and acts to control glutamate release at presynaptic terminals. NO synthase (NOS) is a Ca^{2+}/calmodulin-dependent enzyme and its activity is modulated by its phosphorylation status. NOS is stoichiometrically phosphorylated by PKA, PKC, and CaMK II at independent serines (Bredt et al., 1992). Phosphorylation by PKC markedly reduces NOS enzyme activity. An increase in NOS enzyme activity is observed following its dephosphorylation by CaN and this effect can be reversed by the addition of FK506 (Dawson et al., 1993).

cAMP-Responsive Element-Binding Protein

Adaptative neuronal processes are assumed to be accompanied by the synthesis of novel proteins that are required to form new network architectures. The cAMP-responsive element binding protein (CREB) has been shown to mediate transcriptional activation of such genes in response to both cAMP and Ca^{2+} signals. CREB has also been shown to be a memory modulator in *Drosophila* (Yin et al., 1995) and phosphorylation and dephosphorylation of CREB is thought to be important in processes underlying long-term memory (Deisseroth et al., 1996).

PKA phosphorylates Ser133 of CREB and the synthesis of several proteins (e.g., somatostatin) is subsequently enhanced (Gonzalez and Montminy, 1989). Phosphorylation of this residue is essential for PKA-mediated activation of CREB (Gonzalez and Montminy, 1989). CREB is also a substrate for CaMK I, II, and IV (Sheng et al., 1991; Enslen et al., 1994). Residue Ser133 is the major site of phosphorylation by CaMKs in vitro and phosphorylation of this site is observed following membrane depolarization in vivo (Sheng et al., 1991; Sun et al., 1994). Additionally, Ser142 has also been shown to be phosphorylated by CaMK II (Sun et al., 1994). Both CREB phosphorylation sites can be dephosphorylated by PP1, PP2A, and CaN (Sun et al., 1994; Bito et al., 1996), however, nuclear PP2A appears to be the primary phosphatase antagonistic to PKA-phosphorylation of CREB (Wadzinski et al., 1993). Thus, a balance of kinase and phosphatase activity regulate phosphorylation level of CREB and subsequent CREB-dependent gene expression.

Roles of Phosphorylation and Dephosphorylation in LTP and LTD

The roles of phosphorylation and dephosphorylation in neuronal plasticity have been extensively studied in hippocampal LTP and LTD models.

Long-Term Potentiation

A rise in Ca^{2+} concentration at postsynaptic sites provides an initial step in inducing both LTP and LTD in the CA1 region of the hippocampus (Bear and Malenka, 1994; Malenka, 1994). LTP induction requires the activation of Ca^{2+}-sensitive protein kinases following the rise in Ca^{2+} through the activation of both AMPA- and NMDA-glutamate receptors. CaMK II is assumed to play a pivotal role in LTP, and it phosphorylates intrinsic substrates that include glutamate receptor/ion channels, synaptic proteins and various enzymes (Soderling, 1993). Targeted disruption of the CaMK IIα gene produces deficits in LTP and severely impairs performance in hippocampus-dependent memory tasks (Silva et al., 1992a, b). It has been proposed that autophosphorylation of CaMK II acts as a molecular switch that might be responsible for long-lasting biochemical changes (Lisman and Goldring, 1988; Fukunaga et al., 1993). Experiments using tyrosine-kinase inhibitors demonstrated that tyrosine-kinase activity might be required postsynaptically for long-term synaptic plasticity in the hippocampus (O'Dell et al., 1991). Recent studies describing mice harboring mutations in five kinase genes expressed in the hippocampus found that two of these kinases, the alpha CaMK II and the Fyn tyrosine kinase (Grant et al., 1992) are necessary for the establishment of long-term potentiation (Grant and Silva, 1994). We recently demonstrated that besides CaMK II, CaMK I and CaMK IV may also play important roles in LTP (Tokuda et al., 1997). By Western blot and immunohistochemical analyses, both CaMK I and IV were shown to increase in abundance in LTP hippocampal slices, and the translocation of these enzymes during LTP was shown to occur (Tokuda et al., 1997). Additionally, PKC has also been shown to be important in LTP formation (Muller et al., 1991). Hvalby et al. performed the experiment using inhibitors of PKC and CaMK II, and showed that both inhibitors blocked the induction of LTP. The PKC inhibitor had a much more potent effect than that of the CaMK II inhibitor on LTP

induction (Hvalby et al., 1994). Thus, at least three multifunctional kinases—CaMK II, PKC, and tyrosine kinases—have been implicated in the events responsible for LTP, and may play differential roles in induction and expression of LTP.

Inhibition of CaN with FK506 in adult rat hippocampal slices enhances synaptic transmission and the magnitude of the potentiation of LTP (Lu et al., 1996). CaN has also been shown to play a role in processes antagonizing the induction of LTP in the visual cortex (Funauchi et al., 1994). Inhibition of the synthesis of CaN A-alpha and A-beta catalytic subunits reduces the threshold of induction for commissural-CA1 LTP (Ikegami et al., 1996). On the other hand, there are reports contradictory to the idea that CaN plays an antagonistic role in LTP induction. Intracellular application of cypermethrin, an inhibitor of CaN, suppresses LTP expression in hippocampus (Wang and Stelzer, 1994). We also demonstrated that bath application of FK506 (50 μ*M*) or cyclosporin A (100 μ*M*) inhibits both the NMDA-receptor-mediated potentials and the induction of LTP, but has no effect on the depotentiation in the CA1 hippocampus (Lu et al., 1996). Difference in the age of the animals used or in the method to apply CaN inhibitors (whether both pre- and postsynaptic cells or only the latter cells are exposed to those inhibitors) may be possible reasons for this discrepancy.

There is an additional LTP induction and expression mechanism that occurs in the mossy-fiber synapses in the CA3 region of hippocampus (reviewed by Nicoll and Malenka, 1995). This LTP is independent of NMDA receptors, but requires a rise in presynaptic Ca^{2+} (Castillo et al., 1994). PKA has been shown to play an important role in the expression of this type of LTP and to modulate presynaptic Ca^{2+} channels (Weisskopf et al., 1994; Huang et al., 1994). Recently, PKC activation has also been shown to be necessary but not sufficient for induction of LTP of the mossy fibers (Son and Carpenter, 1996). PKC-dependent protein phosphorylation of GAP-43 in vitro was reported to be associated with the induction, but not the maintenance phase of LTP (Son et al., 1997).

Although NMDA-receptor-dependent LTP is observed throughout the brain, mossy-fiber LTP seems to be specific to CA3 region of the hippocampus. One might speculate that there may be other mechanisms that cause LTP in other parts of the brain.

LTP is generated by presynaptic modifications and postsynaptic modifications. Other factors such as involvement of glial-cell uptake of neurotransmitters, regulation by retrograde messengers, and the morphological changes of activated synapses are also important, but will not be discussed in this review.

Presynaptic modifications cause a regulated release of neurotoransmitters. Increased Ca^{2+} levels in the presynapses lead to an increase in neurotransmitter in synaptosomes (Lynch and Voss, 1991). Synaptic vesicle proteins are phosphorylated by several kinases during LTP. For example, presynaptic PKC activated by arachidonic acid, a retrograde messenger, phosphorylates GAP-43, and results in an increase in free CaM (Gianotti et al., 1992). CaM-dependent enzymes are then activated. CaMK II, for example, phosphorylates synapsin I, leading to modulation of vesicle fusion and neurotransmitter release as described earlier.

In postsynaptic modifications, many kinases and phosphatases have been implicated in LTP. As mentioned earlier, AMPA receptor is phosphorylated by PKA leading to an increase in sensitivity following the induction of LTP (Malenka and Nicoll, 1993; Gasic and Hollman, 1992; Raymond et al., 1993). Activation of NMDA-receptor function by PKC phosphorylation is also involved in the postsynaptic modification (Ben-Ari et al., 1992; Chen and Huang, 1992). Phosphorylation or dephosphorylation of other components such as Ca^{2+} channels, nitric oxide synthase, and CREB following LTP also contributes to the postsynaptic modification (summarized in Table 1).

Figure 1 summarizes the involvement of the kinases and phosphatases discussed in this review.

Table 1
Effects of Phosphorylation and Dephosphorylation on Various Substrates

Receptors	Role	References
Glutamate receptor		
Ionotropic-glutamate receptors		
Non-NMDA		
PKA	Activation	Gasic et al., 1992; Raymond et al., 1993
NMDA		
PKC	Activation	Ben-Ari et al., 1992
CaMKII	Activation	Chen and Huang, 1992; Kolaj et al., 1994; Yakel et al., 1995; Omkumar et al., 1996
Tyrosine kinases	Activation	Moon et al., 1994; Wang and Salter, 1994; Suzuki and Okumura-Noji, 1995; Chen and Leonard, 1996; Rostas et al., 1996
Tyrosine phosphatase	Suppression	Wang et al., 1996
PP1	Suppression	Wang et al., 1994
PP2A	Suppression	Wang et al., 1994
PP2B	Suppression	Tong et al., 1995; Yakel, 1997
Metabotropic-glutamate receptors		
PKA	Suppression	Pedarzani and Storm, 1994
PKC	Suppression	Kawabata et al., 1996
GABA receptors		
$GABA_A$ receptor		
PKC	Suppression	Kellenberger et al., 1992; Leidenheimer et al., 1992
	Activation	Krishek et al., 1994
CaMKII	Activation	Machu et al., 1993; McDonald and Moss, 1994; Kano et al., 1996
PKG	Activation	McDonald and Moss, 1994; Leidenheimer, 1996
Tyrosine kinases	Activation	Moss et al., 1995
PP2B	Suppression	Martina et al., 1996
$GABA_C$ receptor		
PKC	Activation	Feigenspan and Borman, 1994
Glycine receptors		
PKA	Activation	Agopyan et al., 1993; Vaello et al., 1994
PKC	Suppression	Ruiz-Gomez et al., 1991
G protein-coupled receptors		
G protein-coupled receptor kinases	Desensitization	Benovic et al., 1987, 1991; Ambrose et al., 1993; Benovic and Gomez, 1993; Kunapuli and Benovic, 1993; Lefkowitz, 1993
PKC (mAChRs)		Haga et al., 1996
PIA (mAChRs)		Rosenbaum et al., 1987
Voltage-gated ion channels		
Calcium channels (VDCC)		
L-type, N-type, and likely P-type		
PKA	Activation	Hell et al, 1993, 1994; Levitan, 1994
PKC, CaMK-II, PKG		Hell et al, 1993, 1994
PP2B	Suppression	Chad and Eckert, 1986
Sodium channels		
PKA	Suppression	Costa et al., 1982; Smith and Goldin, 1992; Murphy et al., 1996
PKC	Suppression	Numann et al., 1991; West et al., 1991
PP2B	Activation	Chen et al., 1995
Potassium channels		
PKA	Suppression	Bosma et al., 1993
PKC	Activation	Furukawa et al., 1995
Tyrosine kinases	Suppression	Huang et al., 1993

table continues

Table 1
Continued

Receptors	Role	References
Other neuronal substrates		
Synaptic-vesicle proteins		
Synapsin 1		
CaMK II	NT release	Baines and Bennett, 1986; Fukunaga et al., 1995
CaMK I, MAP kinase, Cdk 5		Matsubara et al., 1996
Synaptotaginin		
CaMK II	NT release	Popoli, 1993
Rab 3A		
PKA		Fykse et al., 1995
CaMK II		Fykse et al., 1995
GAP-43		
PKC	Growth, Sprouting	Aigner et al., 1995
PP1, PP2A, PP2B	Antagonize PKC	Benowitz and Routtenberg, 1997
Nitric oxide synthase		
PKC, PKA, CaMK II	Suppression	Bredt et al., 1992
PP2B	Activation	Dawson et al., 1993
CREB		
PKA	Activation	Gonzalez and Montminy, 1989
CaMK I, CaMK II, CaMK IV	Activation	Sheng et al., 1991; Enslen et al., 1994; Sun et al., 1994
PP1, PP2A, PP2B	Suppression	Sun et al., 1994; Bito et al., 1996

PKA: cAMP-dependent protein kinase, PKC: Ca^{2+}/phospholipid-dependent protein kinase, CaM: calmodulin, CaMK: Ca^{2+}/calmodulin-dependent protein kinase, PP: protein phosphatase, PKG: cGMP-dependent protein kinase, NT: neurotransmitter, GABA: gamma-aminobutyric acid, NMDA: *N*-methyl-D-aspartate, MAP: microtubule-associated protein, Cdk: cyclin-dependent kinase.

Long-Term Depression

LTD is defined as a decrease in synaptic efficiency and could be another important mechanism by which neural networks store information.

In LTD, the role of phosphatases may be more significant than that of kinases. One form of LTD that has been observed in the hippocampus requires activation of postsynaptic NMDA receptors, a change in postsynaptic Ca^{2+} concentration, and activation of postsynaptic PP1 or PP2A. The induction of LTD is blocked by the extracellular application of either okadaic acid or calyculin A, two inhibitors of PP1 and PP2A (Mulkey et al., 1993). FK506 therefore blocked induction of LTD in rat visual cortex (Torii et al., 1995) and in hippocampus (Hodgkiss and Kelly, 1995a). Thus, LTD induction may require initial activation of CaN by Ca^{2+}/calmodulin. Presumably, CaN dephosphorylates and inactivates inhibitor-1, and this in turn increases PP1 activity and contributes to the generation of LTD (Malenka, 1994; Mulkey et al., 1994; Hodgkiss and Kelly, 1995b).

The contribution of phosphorylation in LTD formation has also been reported. Knockout experiments revealed in mice that a specific neuronal isoform of PKA type I regulatory subunit (RI beta) is required for both LTD and depotentiation (Brandon et al., 1995; Qi et al., 1996). Involvement of CaMK II in LTD has been demonstrated by the fact that bath application of KN-62, a CaMK II inhibitor, blocks low-frequency stimuli-induced depotentiation. This suggests that induction of depotentiation and

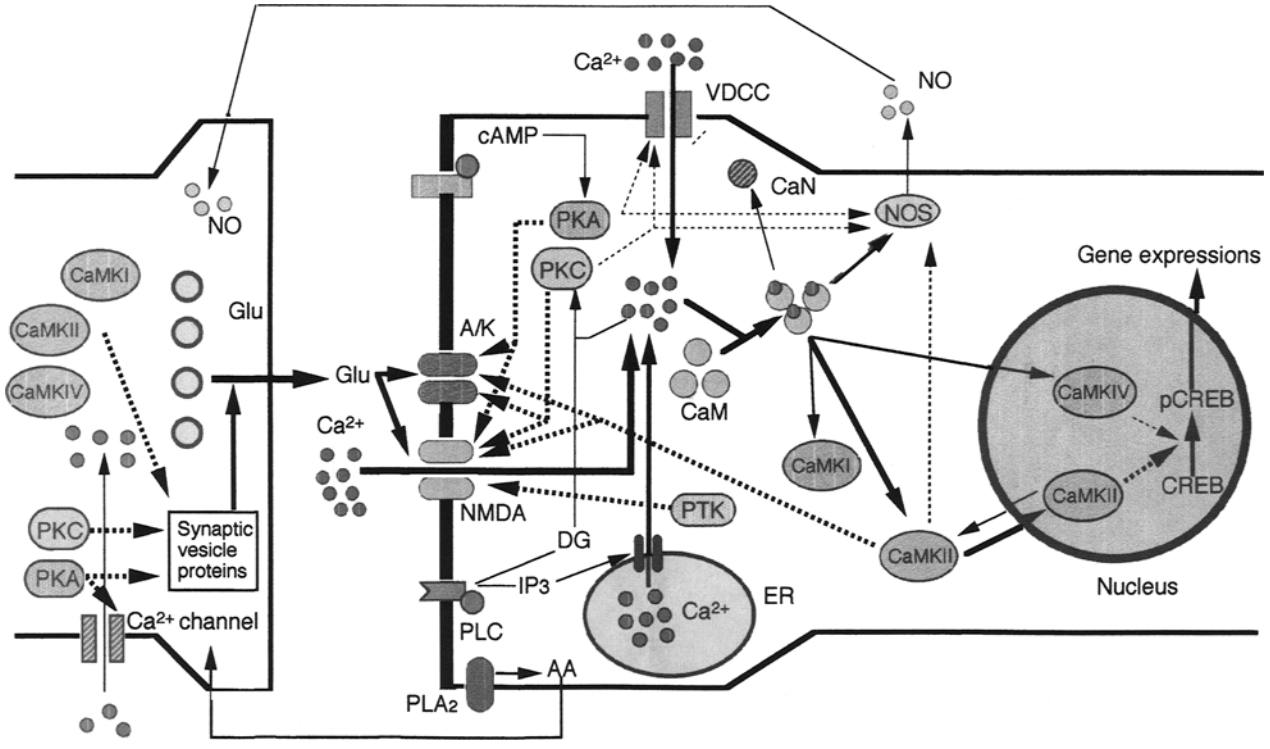

Fig. 1. Schematic drawing of the involvement of phosphorylation and dephosphorylation in LTP. Increase of intracellular Ca^{2+} to a higher level triggers the Ca^{2+}-dependent regulation cascade of kinases that causes LTP. CaN and other phosphatases antagonize the action of kinases. Arrows indicate signal transfer or stimulation of the target molecules. Dotted arrows indicate phosphorylation or dephosphorylation.

de novo LTD may require the same CaMK II-dependent mechanisms (Stanton and Gage, 1996). Linden and Connor demonstrated that inhibitors of PKC block LTD induction, whereas phorbol-12,13-diacetate, a PKC activator, alone is sufficient for LTD induction (Linden and Connor, 1991). Similarly, Stanton showed that transient PKC activation primes LTD (Stanton, 1995). PTKs have also been shown to be necessary for LTD at the parallel fiber-Purkinje cell synapse (Boxall et al., 1996) indicating that PTK-mediated LTD induction also exists.

Figure 2 summarizes the involvement of the kinases and phosphatases discussed in this review.

LTP and LTD in hippocampus have become the well-accepted models to study neuronal plasticity in the mammalian brain. However, there are pitfalls in relying exclusively on this approach to explain neuronal plasticity. First, the physiological significance of LTP and LTD in neuronal plasticity, especially in memory and learning, remains to be well established. Second, spatial and chronological coordination of both presynaptic and postsynaptic modifications are necessary for the induction and expression of LTP and LTD. Third, as there are both NMDA-dependent and NMDA-independent types of LTP present in hippocampus, it remains possible that other types of LTP and LTD exist in other parts of the brain. Additionally, multiple kinases and phosphatases are assumed to be simultaneously or chronologically involved in vivo. Specific inhibitors or stimulators of such enzymes, and molecular-genetic approaches (overexpression and knock-

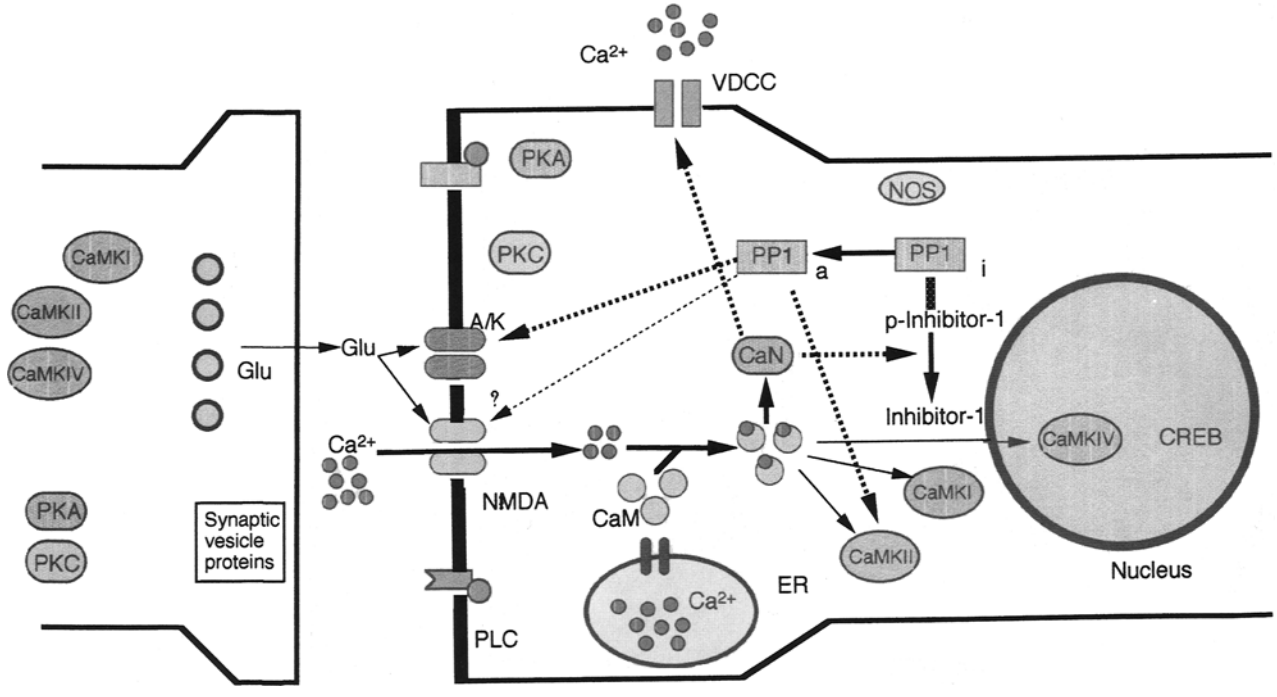

Fig. 2. Schematic drawing of the involvement of phosphorylation and dephosphorylation in LTD. Increase of intracellular Ca^{2+} to a lower level triggers CaN and thereby PP1. Arrows indicate signal transfer or stimulation of the target molecules. Dotted arrows indicate phosphorylation or dephosphorylation.

out experiments) are necessary to be developed for the analysis of the contribution of an individual enzyme in vivo. Finally, other factors such as neurotransmitters, growth factors, trophic factors, and cytokines may also regulate LTP and LTD.

Nonetheless, much progress in the analysis of LTP and LTD in the last two decades has brought a better understanding of neuronal plasticity and has provided powerful clues to explain many neuronal functions and disorders.

Conclusion

Regulatory roles of phosphorylation and dephosphorylation by kinases and phosphatases, respectively, upon neuronal target proteins are shown to be crucial in neuronal plasticity in the CNS. Phosphorylation is a relatively fast process in biological systems, and usually is second-to-minute order reaction catalyzed by kinases. Phosphorylated proteins are efficiently dephosphorylated by responsive phosphatases. This way of regulation is therefore reversible. Although phosphorylated state of target proteins is transient, the incorporated phosphate into the proteins can effectively produce functional changes on the phosphorylated molecules and subsequent signaling reactions are also affected. The relatively fast and reversible manner of regulation by phosphorylation and dephosphorylation is especially suitable to change the receptor affinity for ligands and the probability of opening of various channels and the quantum of neurotransmitter release. Especially in the CNS, quick and reversible responses are essential to alter or maintain the adaptive responses of neuronal-network functions.

Many kinases and phosphatases have been discovered, and many of them exist abundantly in the CNS, indicating that these

enzymes are highly utilized for the regulation of the CNS functions.

As discussed in the text, multiple kinases and phosphatases are involved in the regulation of the substrate function. Each kinase or phosphatase has diverse regulatory effects on various substrates, either positively or negatively. There are usually two or more phosphorylation sites in the substrate molecule. Some kinases share the phosphorylation sites, and the other phosphorylates the distinct site. Since both serine/threonine kinases and tyrosine kinases are involved in the regulation of many receptors and ion channels, phosphorylation on different sites of the molecules occurs, and this may exert differential regulation on the function. The function of the target proteins are therefore determined by the summation of phosphorylation status caused by different kinases on different phosphorylation sites. Targeted experiments, especially site-directed mutagenesis of the phosphorylation site lead to the answers of these questions. Further studies will be required to identify such cross-talk regulation mechanisms via multiple kinases and phosphatases in neuronal plasticity in the CNS.

Neuronal plasticity in the learning and memory functions has been demonstrated to be dynamically regulated via phosphorylation and dephosphorylation. The findings obtained in the learning and memory system may be applicable to study roles of kinases and phosphatases in the other plasticity mechanisms in postnatal development (O'Leary et al., 1994; Scheetz and Constantine-Paton, 1994; Friedlander et al., 1996), recovery from injury (Brodkey et al., 1993; Spear, 1996) and of aging (deToledo-Morrell et al., 1988; Cotman et al., 1990; Coleman et al., 1995) which may need different strategies for research.

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References

Agopyan N., Tokutomi N., and Akaike N. (1993) Protein kinase A-mediated phosphorylation reduces only the fast desensitizing glycine current in acutely dissociated ventromedial hypothalamic neurons. *Neuroscience* **56,** 605–615.

Aigner L., Arber S., Kapfhammer J. P., Laux T., Schneider C., Botteri F., Brenner H. R., and Caroni P. (1995) Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. *Cell* **83,** 269–278.

Ambrose C., James M., Barnes G., Lin C., Bates G., Altherr M., Duyao M., Groot N., Church D., and Wasmuth J. J. (1993) A novel G-protein coupled receptor kinase gene cloned from 4p16.3. *Human Nol. Gen.* **1, 697–703.**

Bahler M. and Greengard P. (1987) Synapsin I bundles F-actin in a phosphorylation-dependent manner. *Nature* **326,** 704–707.

Baines A. J. and Bennett V. (1986) Synapsin I is a microtubule-bundling protein. *Nature* **319,** 145–147.

Bear M. F. and Malenka R. C. (1994) Synaptic plasticity: LTP and LTD. *Curr. Opi. Neurobiol.* **4,** 389–399.

Ben-Ari Y., Aniksztejn L., and Bregestovski P. (1992) Protein kinase C modulation of NMDA currents: an important link of LTP induction. *Trends Neurosci.* 15, 333–339.

Benovic J. L. and Gomez J. (1993) Molecular cloning and expression of GRK6: a new member of the G-protein-coupled receptor kinase family. *J. Biol. Chem.* **268,** 19,521–19,527.

Benovic J. L., Onorato J. J., Arriza J. L., Stone W. C., Lohse M., Jenkins N. A., Gilbert D. J., Copeland N. G., Caron M. G., and Lefkowitz R. J. (1991) Cloning, expression, and chromosomal localization of beta-adrenergic receptor kinase 2. A new member of the receptor kinase family. *J. Biol. Chem.* **266,** 14,939–14,946.

Benowitz L. I. and Routtenberg A. (1997) GAP-43: an intrinsic determinant of neuronal development and plasticity. *Trends Neurosci.* **20,** 84–91.

Bito H., Deisseroth K., and Tsien R. W. (1996) CREB phosphorylation and dephosphorylation: a Ca^{2+}- and stimulus duration-dependent switch for hippocampal gene expression. *Cell* **87,** 1203–1214.

Bonovic J. L., Mayor F. Jr., Staniszewski C., Lefkowitz R. J., and Caron M. G. (1987) Purifica-

tion and characterization of the beta-adrenergic receptor kinase. *J. Biol. Chem.* **262,** 9026–9032.

Bosma M. M., Allen M. L., Martin T. M., and Tempel B. L. (1993) PKA-dependent regulation of mKv1.1, a mouse Shaker-like potassium channel gene, when stably expressed in CHO cells. *J. Neurosci.* **13,** 5242–5250.

Boxall A. R., Lancaster B., and Garthwaite J. (1996) Tyrosine kinase is required for long-term depression in the cerebellum. *Neuron* **16,** 805–813.

Brandon E. P., Zhuo M., Huang Y. Y., Qi M., Gerhold K. A., Burton K. A., Kandel E. R., McKnight G. S., and Idzerda R. L.(1995) Hippocampal long-term depression and depotentiation are defective in mice carrying a targeted disruption of the gene encoding the RI beta subunit of cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **92,** 8851–8855.

Bredt D. S., Ferris C. D., and Snyder S. H. (1992) Nitric oxide synthase regulatory sites. Phosphorylation by cyclic AMP-dependent protein kinase, protein kinase C, and calcium/calmodulin protein kinase; identification of flavin and calmodulin binding sites. *J. Biol. Chem.* **267,** 10,976–10,981.

Brodkey J. A., Gates M. A., Laywell E. D., and Steindler D. A. (1993) The complex nature of interactive neuroregeneration-related molecules. *Exp. Neurol.* **123,** 251–270.

Castillo P. E., Weisskopf M. G., and Nicoll R. A. (1994) The role of Ca^{2+} channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. *Neuron.* **12,** 261–269.

Chad J. E. and Eckert R. (1986) An enzymatic mechanism for calcium current inactivation in dialysed *Helix* neurones. *J. Physiol. Lond.* **378,** 31–51.

Chen C. and Leonard J. P. (1996) Protein tyrosine kinase-mediated potentiation of currents from cloned NMDA receptors. *J. Neurochem.* **67,** 194–200.

Chen L. and Huang L. Y. M. (1992) Protein kinase C reduces Mg^{2+} block of NMDA-receptor channels as a mechanism of modulation. *Nature* **356,** 521–523.

Chen T. C., Law B., Kondratyuk T., and Rossie S. (1995) Identification of soluble protein phosphatases that dephosphorylate voltage-sensitive sodium channels in rat brain. *J. Biol. Chem.* **270,** 7750–7756.

Coleman P. D., Rogers K. E., and Flood D. G. (1995) Neuronal plasticity in normal aging and Alzheimer's disease: a proposed intercellular signal cascade. Molecular and Cellular mechanisms of neuronal plasticity in normal aging and Alzheimer's disease, in *Progress in Brain Research,* vol 86 (Coleman P. D., Higgins G. A., and Phelps C. H., eds.), Elsevier, pp. 74–87.

Costa M. R. C., Casnellie J. E., and Catterall W. A. (1982) Selective phosphorylation of the alpha subunit of the sodium channel by cAMP-dependent protein kinase. *J. Biol. Chem.* **257,** 7918–7921.

Cotman C. W., Geddes J. W., Ulas J., and Klein M. (1990) Plasticity of exitatory amino acid receptors: implications for aging and Alzheimer's disease. Molecular and Cellular mechanisms of neuronal plasticity in normal aging and Alzheimer's disease, in *Progress in Brain Research,* vol 86 (Coleman P. D., Higgins G. A., and Phelps C. H., eds.), Elsevier, pp. 55–73.

Dawson T. M., Steiner J. P., Dawson V. L., Dinerman J. L., Uhl G. R., and Snyder S. H. (1993) Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. *Proc. Natl. Acad. Sci. USA* **90,** 9808–9812.

Deisseroth K., Bito H., and Tsien R. W. (1996) Signaling from synapse to nucleus: postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. *Neuron* **16,** 89–101.

DeRemer M. F., Saeli R. J., and Edelman A. M. (1992) Ca^{2+}-calmodulin-dependent protein kinases Ia and Ib from rat brain I. Identification, purification, and structural comparisons. *J. Biol. Chem.* **267,** 13,460–13,465.

deToledo-Morrell L., Geinisman Y., and Morrell F. (1988) Age-dependent alterations in hippocampal synaptic plasticity: relation to memory disorders. *Neurobiol. Aging* **9,** 581–590.

Devillers-Thiery A., Galzi J. Jl., Eisele J. L., Bertran S., Bertrand D., and Changeux J. P. (1993) Functional architecture of the nicotinic acetylcholine receptor: a prototype of ligand-gated ion channels. *J. Membr. Biol.* **136,** 97–112.

Enslen H., Sun P., Brickey D., Soderling S. H., Klamo E., and Soderling T. R. (1994) Characterization of Ca^{2+}/calmodulin-dependent protein kinase IV. Role in transcriptional regulation. *J. Biol. Chem.* **269,** 15,520–15,527.

Feigenspan A. and Bormann J. (1994) Modulation of GABAc receptors in rat retinal bipolar cells by protein kinase C. *J. Physiol. Lond.* **481,** 325–330.

Fournier F., Bourinet E., Nargeot J., and Charnet P. (1993) Cyclic AMP-dependent regulation of P-type calcium channels expressed in *Xenopus* oocytes. *Pflugers. Arch.* **423,** 173–180.

Fukunaga K., Muller D., and Miyamoto E. (1995) Increased phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II and its endogenous substrates in the induction of long-term potentiation. *J. Biol. Chem.* **270,** 6119–6124.

Fukunaga K., Stoppini L., Miyamoto E., and Muller D. (1993) Long-term potentiation is associated with an increased activity of Ca^{2+}/calmodulin-dependent protein kinase II. *J. Biol. Chem.* **268,** 7863–7867.

Funauchi M., Haruta H., and Tsumoto T. (1994) Effects of an inhibitor for calcium/calmodulin-dependent protein phosphatase, calcineurin, on induction of long-term potentiation in rat visual cortex. *Neurosci. Res.* **19,** 269–278.

Furukawa Y., Kim H. N., and Kubo T. (1995) Up- and down-modulation of a cloned *Aplysia* K^+ channel (AKv1.1a) by the activators of protein kinase C. *Zoolog. Sci.* **12,** 35–44.

Friedlander M. J., Harsanyi K., Dudek S., and Kara P. (1996) Developmental mechanisms for regulating signal amplification at exitatory synapses in the neocortex. Neural development and plasticity, in *Progress in Brain Research,* vol 108 (Mize R. R. and Erzurumlu R. S., eds.), Elsevier, pp. 245–262.

Fykse E. M., Li C., and Sudhof T. C. (1995) Phosphorylation of rabphilin-3A by Ca^{2+}/calmodulin- and cAMP-dependent protein kinases *in vitro. J. Neurosci.* **15,** 2385–2395.

Gasic G. P. and Hollmann M. A. (1992) Molecular neurobiology of glutamate receptors. *Ann. Rev. Physiol.* **54,** 507–536

Gianotti C., Nunzi M. G., Gispen W. H., and Corradetti R. (1992) Phosphorylation of the presynaptic protein B-50 (GAP-43) is increased during electrically induced long-term potentiation. *Neuron* **8,** 843–848.

Gonzalez G. A. and Montminy M. R. (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* **59,** 675–680.

Grant S. G., O'Dell T. J., Karl K. A., Stein P. L., Soriano P., and Kandel E. R. (1992) Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* **258,** 1903–1910.

Grant S. G. and Silva A. J. (1994) Targeting learning. *Trends Neurosci.* **17,** 71–75.

Greengard P., Valtorta F., Czernik A. J., and Benfenati F. (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science* **259,** 780–785.

Guy H. R. and Conti F. (1990) Pursuing the structure and function of voltage-gated channels. *Trends Neurosci* **13,** 201–206.

Haga K., Kameyama K., Haga T., Kikkawa U., Shiozaki K., and Uchiyama H. (1996) Phosphorylation of human m1 muscarinic acetylcholine receptors by G protein-coupled receptor kinase 2 and protein kinase C. *J. Biol. Chem.* **271,** 2776–2782.

Hell J. W., Yokoyama C. T., Wong S. T., Warner C., Snutch T. P., and Catterall W. A. (1993) Differential phosphorylation of two size forms of the neuronal class C L-type calcium channel alpha 1 subunit. *J. Biol. Chem.* **268,** 19,451–19,457.

Hell J. W., Appleyard S. M., Yokoyama C. T., Warner C., and Catterall W. A. (1994) Differential phosphorylation of two size forms of the N-type calcium channel alpha 1 subunit which have different COOH termini. *J. Biol. Chem.* **269,** 7390–7396.

Hodgkiss J. P. and Kelly J. S. (1995a) Only 'de novo' long-term depression (LTD) in the rat hippocampus in vitro is blocked by the same low concentration of FK506 that blocks LTD in the visual cortex. *Brain Res.* **705,** 241–246.

Hodgkiss J. P. and Kelly J. S. (1995b) Only 'de novo' long-term depression (LTD) in the rat hippocampus in vitro is blocked by the same low concentration of FK506 that blocks LTD in the visual cortex. *Brain Res.* **705,** 241–246.

Huang X. Y., Morielli A. D., and Peralta E. G. (1993) Tyrosine kinase-dependent suppression of a potassium channel by the G protein-coupled m1 muscarinic acetylcholine receptor. *Cell* **75,** 1145–1156.

Huang Y. Y., Li X. C., and Kandel E. R. (1994) cAMP contributes to mossy fiber LTP by initiating both a covalently mediated early phase and macromolecular synthesis-dependent late phase. *Cell* **79,** 69–79.

Hvalby ^OO., Hemmings Jr. H. C., Paulsen O, Czernik A. J., Nairn A. C., Godfraind J-M.,Jensen V., Raastad M., Storm J. F., Andersen P., and Greengard P. (1994) Specificity of protein kinase inhibito peptides and induction of long-term potentiation. *Proc. Natl. Acad. Sci. USA* **91,** 4761–4765.

Ikegami S., Kato A., Kudo Y., Kuno T., Ozawa F., and Inokuchi K. (1996) A facilitatory effect on the induction of long-term potentiation *in vivo* by chronic administration of antisense oligodeoxynucleotides against catalytic subunits of calcineurin. *Brain Res. Mol. Brain Res.* **41,** 183–191.

Jaffrey S. R. and Snyder S. H. (1995) Nitric oxide: a neural messenger. *Ann. Rev. Cell. Dev. Biol.* **11,** 417–440.

Kano M., Kano M., Fukunaga K., and Konnerth A. (1996) Ca^{2+}-induced rebound potentiation of gamma-aminobutyric acid-mediated currents requires activation of Ca^{2+}/calmodulin-dependent kinase II. *Proc. Natl. Acad. Sci. USA* **93,** 13351–13356.

Kawabata S., Tsutsumi R., Kohara A., Yamaguchi T., Nakanishi S., and Okada M. (1996) Control of calcium oscillations by phosphorylation of metabotropic glutamate receptors. *Nature* **383,** 89–92.

Kellenberger S., Malherbe P., and Sigel E. (1992) Function of the alpha 1 beta 2 gamma 2S gamma-aminobutyric acid type A receptor is modulated by protein kinase C via multiple phosphorylation sites. *J. Biol. Chem.* **267,** 25,660–25,663.

Kolaj M., Cerne R., Cheng G., Brickey D. A., and Randic M. (1994) Alpha subunit of calcium/calmodulin-dependent protein kinase enhances excitatory amino acid and synaptic responses of rat spinal dorsal horn neurons. *J. Neurophysiol.* **72,** 2525–2531.

Krishek B. J., Xie X., Blackstone C., Huganir R. L., Moss S. J., and Smart T. G. (1994) Regulation of GABAA receptor function by protein kinase C phosphorylation. *Neuron* **12,** 1081–1095.

Kuhn H. (1978) Light-dependent binding of rhodopsin kinase and other proteins to cattle photoreceptor membranes. *Biochemistry* **17,** 4389–4395.

Kunapuli P. and Benovic J. L. (1993) Cloning and expression of GRK5: a member of the G-protein-coupled receptor kinase family. *Proc. Natl. Acad. Sci. USA* **90,** 5588–5592.

Kusama T., Sakurai M., Kizawa Y., Uhl G. R., and Murakami H. (1995) GABA rho1 receptor: inhibition by protein kinase C activators. *Eur. J. Pharmacol.* **291,** 431–434.

Lai Y., Peterson B. Z., and Catterall W. A. (1993) Selective dephosphorylation of the subunits of skeletal muscle calcium channels by purified phosphoprotein phosphatases. *J. Neurochem.* **61,** 1333–1339.

Leidenheimer N. J. (1996) Effect of PKG activation on recombinant GABAA receptors. *Brain. Res. Mol. Brain. Res.* **42,** 131–134.

Leidenheimer N. J., McQuilkin S. J., Hahner L. D., Whiting P., and Harris R. A. (1992) Activation of protein kinase C selectively inhibits the gamma-aminobutyric acid A receptor: role of desensitization. *Mol. Pharmacol.* **41,** 1116–1123.

Lefkowitz R. J. (1993) G-protein-coupled receptor kinases. *Cell* **74,** 409–412.

Levitan I. B. (1994) Modulation of ion channels by protein phosphorylation and dephosphorylation. *Ann. Rev. Physiol.* **56,** 193–212.

Lin Y. F., Browning M. D., Dudek E. M., and Macdonald R. L. (1994) Protein kinase C enhances recombinant bovine alpha 1 beta 1 gamma 2L GABAA receptor whole-cell currents expressed in L929 fibroblasts. *Neuron* **13,** 1421–1431.

Linden D. J. and Connor J. A. (1991) Participation of postsynaptic PKC in cerebellar long-term depression in culture. *Science* **254,** 1656–1659.

Lisman J. E. and Goldring M. A. (1988) 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,** 5320–5324.

Lu Y.-F., Tomizawa K., Moriwaki A., Hayashi Y., Tokuda M., Itano T., Hatase O., and Matsui H. (1996) Calcineurin inhibitors, FK506 and cyclosporin A, suppress the NMDA receptor-mediated potentials and LTP, but not depotentiation in the rat hippocampus. *Brain Res.* **729,** 142–146.

Lynch M. A. and Voss K. L. (1991) Presynaptic changes in long-term potentiation: elevated synaptosomal calcium concentration and basal phosphoinositide turnover in dentate gyrus. *J. Neurochem.* **56,** 113–118.

Machu T. K., Firestone J. A., and Browning M. D. (1993) Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C phosphorylate a synthetic peptide corresponding to a sequence that is specific for the gamma 2L subunit of the GABAA receptor. *J. Neurochem.* **61,** 375–377.

Malenka R. C. and Nicoll R. A. (1993) NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci.* **16,** 521–527.

Malenka R. C. (1994) Synaptic plasticity in the hippocampus: LTP and LTD. *Cell* **78,** 535–538.

Martina M., Mozrzymas J. W., Boddeke H. W., and Cherubini E. (1996) The calcineurin inhibitor cyclosporin A-cyclophilin A complex reduces desensitization of GABAA-mediated responses in acutely dissociated rat hippocampal neurons. *Neurosci. Lett.* **215,** 95–98.

Matsubara M., Kusubata M., Ishiguro K., Uchida T., Titani K., and Taniguchi H. (1996) Site-specific phosphorylation of synapsin I by mitogen-activated protein kinase and Cdk5 and its effects on physiological functions. *J. Biol. Chem.* **271,** 21,108–21,113.

McDonald B. J. and Moss S. J. (1994) Differential phosphorylation of intracellular domains of gamma-aminobutyric acid type A receptor subunits by calcium/calmodulin type 2-dependent protein kinase and cGMP-dependent protein kinase. *J. Biol. Chem.* **269,** 18111–18117.

Moon I. S., Apperson M. L., and Kennedy M. B. (1994) The major tyrosine-phosphorylated protein in the postsynaptic density fraction is N-methyl-D-aspartate receptor subunit 2B. *Proc. Natl. Acad. Sci. USA* **91,** 3954–3958.

Moss S. J., Doherty C. A., and Huganir R. L. (1992) Identification of the cAMP-dependent protein kinase and protein kinase C phosphorylation sites within the major intracellular domains of the beta 1, gamma 2S, and gamma 2L subunits of the gamma-aminobutyric acid type A receptor. *J. Biol. Chem.* **267,** 14,470–14,476.

Moss S. J., Gorrie G. H., Amato A., and Smart T. G. (1995) Modulation of GABAA receptors by tyrosine phosphorylation. *Nature* **377,** 344–348.

Muller D., Buchs P. A., Stoppini L., and Boddeke H. (1991) Long-term potentiation, protein kinase C, and glutamate receptors. *Mol. Neurobiol.* **5,** 277–288.

Mulkey R. M., Endo S., Shenolikar S., and Malenka R. C. (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* **369,** 486–488.

Mulkey R. M., Herron C. E., and Malenka R. C. (1993) An essential role for protein phosphatases in hippocampal long-term depression. *Science* **261,** 1051–1055.

Murphy B. J., Rogers J., Perdichizzi A. P., Colvin A. A., and Catterall W. A. (1996) cAMP-dependent phosphorylation of two sites in the alpha subunit of the cardiac sodium channel. *J. Biol. Chem.* **271,** 28,837–28,843.

Nicoll R. A., and Malenka R. C. (1995) Contrasting properties of two forms of long-term potentiatio in the hippocampus. *Nature* **377,** 115–118.

Numann R., Catterall W. A., and Scheuer T. (1991) Functional modulation of brain sodium channels by protein kinase C phosphorylation. *Science* **254,** 115–118.

O'Dell T. J., Kandel E. R., and Grant S. G. (1991) Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. *Nature* **353,** 558–560.

O'Leary D. D., Ruff N. L., and Dyck R. H. (1994) Development, critical period plasticity, and adult reorganizations of mammalian somatosensory systems. *Curr. Opinion Neurobiol.* **4,** 535–544.

Omkumar R. V., Kiely M. J., Rosenstein A. J., Min K. T., and Kennedy M. B. (1996) 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,** 31670–31678.

Pasinelli P., Ramakers G. M., Urban I. J., Hens J. J., Oestreicher A. B., de Graan P. N., and Gispen W. H. (1995) Long-term potentiation and synaptic protein phosphorylation. *Behav. Brain. Res.* **66,** 53–59.

Pedarzani P. and Storm J. F. (1994) PKA mediates the effects of monoamine transmitters on the K+ current underlying the slow spike frequency adaptation in hippocampal neurons. *Neuron* **11,** 1023–1035.

Petrucci T. C. and Morrow J. S. (1987) Synapsin I: an actin-bundling protein under phosphorylation control. *J. Cell. Biol.* **105,** 1355–1363.

Popoli M. (1993) Synaptotagmin is endogenously phosphorylated by Ca^{2+}/calmodulin protein kinase II in synaptic vesicles. *FEBS Lett.* **317,** 85–88.

Qi M., Zhuo M., Skalhegg B. S., Brandon E. P., Kandel E. R., McKnight G. S., and Idzerda R. L. (1996) Impaired hippocampal plasticity in mice lacking the Cbeta1 catalytic subunit of cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **93,** 1571–1576.

Raymond L. A., Blackstone C. D., and Huganir R. L. (1993) Phosphorylation and modulation of recombinant GluR6 glutamate receptors by cAMP-dependent protein kinase. *Nature* **361,** 637–641.

Rosenbaum L. C., Malencik D. A., Anderson S. R., Tota M. R., and Schimerlik M. I. (1987) Phosphorylation of the porcine atrial muscarinic acetylcholine receptor by cyclic AMP dependent protein kinase. *Biochemistry* **26,** 8183–8188.

Rostas J. A., Brent V. A., Voss K., Errington M. L., Bliss T. V., and Gurd J. W. (1996) Enhanced tyrosine phosphorylation of the 2B subunit of the N-methyl-D-aspartate receptor in long-term potentiation. *Proc. Natl. Acad. Sci. USA* **93,** 10,452–10,456.

Ruiz-Gomez A., Vaello M. L., Valdivieso F., and Mayor F. Jr. (1991) Phosphorylation of the 48-kDa subunit of the glycine receptor by protein kinase C. *J. Biol. Chem.* **266,** 559–566.

Scheetz A. J. and Constantine-Paton M. (1994) Modulation of NMDA receptor function: implications for vertebrate neural development. *FASEB J. 8,* 745–752.

Severin S. E. Jr., Moskvitina E. L., Bykova E. V., Lutzenko S. V., and Shvets V. I. (1989) Synapsin I from human brain. Phosphorylation by Ca^{2+}, phospholipid-dependent protein kinase. *FEBS Lett.* **258,** 223–226.

Sheng M., Thompson M. A., and Greenberg M. E. (1991) CREB: a Ca^{2+}-regulated transcription factor phosphorylated by calmodulin-dependent kinases. *Science* **252,** 1427–1430.

Silva A. J., Stevens C. F., Tonegawa S., Wang Y. (1992a) Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science* **257,** 201–206.

Silva A. J., Paylor R., Wehner J. M., and Tonegawa S. (1992b) Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science* **257,** 206–211.

Smith R. D. and Goldin A. L. (1992) Protein kinase A phosphorylation enhances sodium channel currents in Xenopus oocytes. *Am. J. Physiol.* **263,** C660–C666.

Soderling T. R. (1993) Calcium/calmodulin-dependent protein kinase II: role in learning and memory. *Mol. Cell Biochem.* **127–128,** 93–101.

Son H., and Carpenter D. O. (1996) Protein kinase C activation is necessary but not sufficient for induction of long-term potentiation at the synapse of mossy fiber-CA3 in the rat hippocampus. *Neuroscience* **72,** 1–13.

Son H., Davis P. J., and Carpenter D. O. (1997) Time course and involvement of protein kinase C-mediated phosphorylation of F1/GAP-43 in area CA3 after mossy fiber stimulation. *Cell. Mol. Neurobiol.* **17,** 171–94.

Spear P. F. (1996) Neural plasticity after brain damage. Neural development and plasticity, in *Progress in Brain Research,* vol 108 (Mize R. R. and Erzurumlu R. S., eds.), Elsevier, pp. 391–408.

Stanton P. K. and Gage A. T. (1996) Distinct synaptic loci of Ca^{2+}/calmodulin-dependent protein kinase II necessary for long-term potentiation and depression. *J. Neurophysiol.* **76,** 2097–2101.

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

Stuhmer W. (1991) Structure-function studies of voltage-gated ion channels. *Annu. Rev. Biophys. Biophys. Chem.* **20,** 65–78.

Sun P., Enslen H., Myung P. S., and Maurer R. A. (1994) Differential activation of CREB by Ca^{2+}/calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. *Genes Dev.* **8,** 2527–2539.

Suzuki T. and Okumura-Noji K. (1995) NMDA receptor subunits epsilon 1 (NR2A) and epsilon 2 (NR2B) are substrates for Fyn in the postsynaptic density fraction isolated from the rat brain. *Biochem. Biophys. Res. Commun.* **216,** 582–588.

Timpe L. C., Schwarz T. L., Tempel B. L., Papazian D. M., Jan Y. N., and Jan L. Y. (1988) Expression of functional potassium channels from Shaker cDNA in *Xenopus* oocytes. *Nature* **331,** 143–145.

Tokuda M., Ahmed B. Y., Lu Y.-F., Matsui H., Miyamoto O., Yamaguchi F., Konishi R., and Hatase O. (1997) Involvement of calmodulin-dependent protein kinases I and IV in long term potentiation. *Brain Res.* **755,** 162–166.

Tong G., Shepherd D., and Jahr C. E. (1995) Synaptic desensitization of NMDA receptors by calcineurin. *Science* **267,** 1510–1512.

Torii N., Kamishita T., Otsu Y., and Tsumoto T. (1995) An inhibitor for calcineurin, FK506, blocks induction of long-term depression in rat visual cortex. *Neurosci. Lett.* **185,** 1–4.

Vaello M. L., Ruiz-Gomez A., Lerma J., and Mayor F. Jr. (1994) Modulation of inhibitory glycine receptors by phosphorylation by protein kinase C and cAMP-dependent protein kinase. *J. Biol. Chem.* **269,** 2002–2008.

Wadzinski B. E., Wheat W. H., Jaspers S., Peruski L. F. Jr., Lickteig R. L., Johnson G. L., and Klemm D. J. (1993) Nuclear protein phosphatase 2A dephosphorylates protein kinase A-phosphorylated CREB and regulates CREB transcriptional stimulation. *Mol. Cell Biol.* **13,** 2822–2834.

Wang J. H. and Stelzer A. (1994) Inhibition of phosphatase 2B prevents expression of hippocampal long-term potentiation. *NeuroReport* **5,** 2377–2380.

Wang L. Y., Orser B. A., Brautigan D. L., and MacDonald J. F. (1994) Regulation of NMDA receptors in cultured hippocampal neurons by protein phosphatases 1 and 2A. *Nature* **369,** 230–232.

Wang Y. T. and Salter M. W.(1994) Regulation of NMDA receptors by tyrosine kinases and phosphatases. *Nature* **369,** 233–235.

Wang Y. T., Yu X. M., and Salter M. W. (1996) Ca(2+)-independent reduction of N-methyl-D-aspartate channel activity by protein tyrosine phosphatase. *Proc. Natl. Acad. Sci. USA* **93,** 1721–1725.

Weisskopf M. G., Castillo P. E., Zalutsky R. A. and Nicoll R. A. (1994) Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. *Science* **265,** 1878–1882.

West J. W., Numann R., Murphy B. J., Scheuer T., and Catterall W. A. (1991) A phosphorylation site in the Na^+ channel required for modulation by protein kinase C. *Science* **254,** 866–868.

Yakel J. L. (1997) Calcineurin regulation of synaptic function: from ion channels to transmitter release and gene transcription. *Trends Pharmacol. Sci.* **18,** 124–134.

Yakel J. L., Vissavajjhala P., Derkach V. A., Brickey D. A., and Soderling T. R. (1995) 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,** 1376–1380.

Yin J. C., Del-Vecchio M., Zhou H., and Tully T. (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in Drosophila. *Cell* **81,** 107–115.