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Protein serine/threonine phosphatases in neuronal plasticity and disorders of learning and memory

Isabelle M. Mansuy[1] and Shirish Shenolikar[2]

[1] Brain Research Institute, Medical Faculty of the University Zürich, and Biology Department of the Swiss Federal Institute of Technology Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
[2] Molecular Pharmacology, Pfizer Global Research and Development, 2800 Plymouth Road, Ann Arbor, MI 48105, USA

Phosphorylation and dephosphorylation of cellular proteins by protein kinases and phosphatases represent important mechanisms for controlling major biological events. In the nervous system, protein phosphatases are contained in highly dynamic complexes localized within specialized subcellular compartments and they ensure timely dephosphorylation of multiple neuronal phosphoproteins. This modulates the responsiveness of individual synapses to neural activity and controls synaptic plasticity. These enzymes in turn play a key role in many forms of learning and memory, and their dysfunction contributes to cognitive deficits associated with aging and dementias or neurodegenerative diseases. Here, we review key modes of regulation of neuronal protein serine/threonine phosphatases and their contribution to disorders of learning and memory.

Introduction

Acquisition and storage of information in the mammalian brain rely on specific and persistent changes in the strength of individual synapses. These changes induce bidirectional and long-lasting alterations in synaptic plasticity – defined as long-term potentiation (LTP), long-term depression (LTD) and depotentiation – that make synapses highly adaptable to neuronal input [1,2]. These forms of plasticity share many components, among which the most important are protein kinases (PKs) and protein phosphatases (PPs) that function in highly integrated networks to control synaptic transmission. This review focuses on key regulatory features of the major serine/threonine PPs, which modulate >90% of dephosphorylation events in the mammalian brain. We discuss their role in the control of neurotransmission and plasticity, and new results that implicate them in memory disorders.

Targeting of serine/threonine PP functions in the mammalian brain

Over 99% of protein-bound phosphate in eukaryotic cells is found on serine (Ser) and threonine (Thr) residues. Phosphate is added to these residues covalently by specific PKs and removed by specific PPs. Although 418 human genes encode Ser/Thr PKs, only 15 genes are known to encode Ser/Thr PPs. PPs have evolved elaborate mechanisms to regulate activity of the nearly 100 000 phosphoproteins present in stimulated cells in a coordinated and timely manner. Among the PPs expressed by mammalian neurons [PP1, PP2A, PP2B (calcineurin), PP2C (PPM), PP4, PP5, PP6 and PP7], PP1, PP2A and calcineurin are the most abundant and dephosphorylate >90% of neuronal phosphoproteins. The functions of these enzymes have been studied using two classes of pharmacological inhibitors: (i) okadaic acid (OA) and calyculin, which inhibit primarily PP1 and PP2A (at different IC_{50} values) but can also inhibit PP4, PP5 and PP6 [3]; and (ii) the immunosuppressants cyclosporin-A and FK506, which inhibit calcineurin [4]. Most of these inhibitors act slowly in cells and are therefore generally used at higher concentrations than are required to inhibit PPs *in vitro* [5]. This makes it difficult to determine which PPs are involved in a response. For example, functions currently attributed to PP1 and PP2A based on inhibition by OA or calyculin A might also involve PP4, PP5 and/or PP6. More specific tools, such as small interfering RNA (siRNA) and new chemicals [6] to modulate PPs, should enable the functions of PPs to be better defined.

Ability of a PP to catalyze specific dephosphorylation is largely defined by association of its catalytic subunits with regulatory or targeting proteins. Three categories of endogenous PPs regulators are known. The largest comprises PP-binding proteins that recruit or tether PPs in unique subcellular compartments. Some regulators also function as scaffolds that bind PP substrates and facilitate their dephosphorylation. The importance of PP–substrate colocalization is best exemplified by the phosphoprotein Ca^{2+}/calmodulin-dependent protein kinase CaMKII, which is associated with the postsynaptic density (PSD), an actin-rich structure underlying the postsynaptic membrane. CaMKII autophosphorylated at Thr286 was first reported to be exclusively dephosphorylated by PP1 in isolated synaptosomes [7]. However it is now clear that CaMKII is controlled by different PPs in distinct subcellular compartments, with the PSD-bound CaMKII being dephosphorylated by PP1, and soluble or synaptosomal CaMKII being dephosphorylated by PP2A [8]. This substrate selectivity is mediated by the discrete subcellular

Corresponding authors: Mansuy, I.M. (mansuy@hifo.unizh.ch); Shenolikar, S. (shirish.shenolikar@pfizer.com).
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containment of PPs through their binding to regulatory and anchoring proteins. Interestingly, >60 PP1-binding proteins have been identified in mammalian cells. Many, including neurabin I, neurabin II (spinophilin), PP1 regulatory subunit 15A (PPP1R15A, previously known as GADD34), neurofilament-L, and A-kinase anchor proteins (AKAPs) such as yotiao (PRKA9), are present in neurons [9,10].

The second category of PP-binding proteins comprises substrates that bind directly to PPs to promote their own dephosphorylation. For example, PP1 binds to and dephosphorylates the retinoblastoma protein Rb1 [11], and calcineurin binds to the nuclear factor of activated T -cells (NFAT) transcription factors [12]. Likewise, most proteins bound to PP2C, PP4 and PP5 are substrates of these PPs [13–15]. The third and smallest category of PP-binding proteins includes endogenous inhibitors such as I-1 (also known as PPP1R1A), dopamine- and cAMP-regulated phosphoprotein, 32 kDa (DARPP-32, also known as PPP1R15B) and modulatory calcineurin-interacting proteins (MCIPs or calcipressins) [16]; proteins in this category inhibit multiple PP types and so might modulate multiple PP functions.

PP regulators have been identified by the presence of a conserved PP-binding sequence that potentially introduces competition among regulators for the target PP catalytic subunit. Many PP1 regulators contain a tetrapeptide [K/R][I/V]xF sequence that functions as a core PP1-binding site. Although other sites on PP1 regulators cooperate with this core motif to target specific PP1 isoforms, the presence of a common binding site prevents the anchoring of multiple regulators to a single PP1 catalytic subunit. However, heterotrimeric PP1 complexes containing more than one regulator have been identified [17–19]. Analysis of these complexes suggests that interactions of PP1 regulators, independent of their binding to a PP1 catalytic subunit, can expand the array of mammalian PP1 complexes (estimated to >100). PP2A also exists as heterotrimers formed by a catalytic (C) subunit (or subunits) associated with scaffolding (A) and regulatory (B) subunits [20]. B subunits direct the localization and/or function of PP2A complexes in cells. Stable complexes of PP2A with substrates also exist, and at least two mammalian proteins selectively inhibit PP2A activity *in vitro*. While the diversity of PP2A regulators appears smaller than for PP1, neurons can contain 50–100 different PP2A complexes. Some regulators possess broader specificity for PPs. For example, PP2A, PP4 and PP6 all bind to the α4 protein and might form elongation factor-2 (EF-2) phosphatases that regulate protein translation [21]. Thus, PPs can have overlapping functions and selective inhibitors become crucial in assessing their individual contributions to neuronal physiology. Emerging evidence also points to regulatory and anchoring partners for calcineurin [22], PP4 [23] and PP6 [21] but further studies are needed to define their role in the nervous system.

Whether involving substrates or regulators, the association of PPs with cellular partners focuses their functions and the scope of physiological events that they control. Growing evidence points to a dynamic exchange of PPs between interacting proteins. Mechanisms for PPs shuttling among regulators are only just being understood and might involve covalent modifications of regulatory (and catalytic) subunits to destabilize PP complexes transiently in response to specific physiological cues [10]. Some PPs regulators might be synthesized or degraded during specific stages of neuronal differentiation and brain development [24]. Early evidence suggests that the proteasome, which degrades cellular proteins in a ubiquitin-dependent manner, regulates neuronal functions of PP1 [25] and PP2A [26,27]. Together, a complex array of regulatory mechanisms orchestrates the assembly and disassembly of cellular PP complexes, their distribution within cells and their function in neurons during synaptic transmission, learning and memory.

Regulation of neurotransmitter receptors by PPs

Ser/Thr phosphorylation and dephosphorylation regulate presynaptic and postsynaptic events in excitatory and inhibitory neurons. At these sites, most relevant PP substrates include ligand-gated ion channels and G-protein-coupled receptors, whose functional properties, trafficking and synaptic organization are controlled by reversible phosphorylation [28]. The following sections focus on examples of neuronal PP1 targets that are linked to the control of synaptic plasticity.

Excitatory neurotransmission

Regulation of NMDA receptor responses Synaptic NMDA receptors are converted between phosphorylated and dephosphorylated states depending on synaptic stimulation. Following excitatory neurotransmission and Ca^{2+} influx, NMDA receptors are phosphorylated by protein kinase A (PKA) then rapidly dephosphorylated by calcineurin and/or PP1 and PP2A. Dephosphorylation of NR2A by calcineurin reduces the open time of individual channels [29] and desensitizes the receptor [30–32], reducing NMDA-receptor-mediated currents. In hippocampal neurons, the negative effect of calcineurin on NMDA receptors is modulated by activation of β2 adrenoceptors, which colocalize with calcineurin and PKA via the anchoring protein AKAP79 (also known as AKAP150) [33]. However, calcineurin also has positive effects on glutamate receptors and participates in NMDA-receptor-induced potentiation of metabotropic (mGluR5) receptor activity. It acts by dephosphorylating protein kinase C (PKC)-dependent sites on the mGluR5 C terminus and attenuating mGluR5 desensitization [34]. Calcineurin-mediated dephosphorylation also contributes to developmental mechanisms that modulate NMDA receptor subunit composition. In the developing cerebellum, it counteracts the TrkB and extracellular signal-regulated kinase (ERK1/2) cascade; this cascade upregulates the NR2C subunit, which then exchanges with NR2B to promote synaptic transmission from mature mossy fibers onto granule cells [35]. In the developing visual system, calcineurin functions in a homeostatic mechanism that limits NMDA receptor currents following early activation by light; it acts by dephosphorylating NR2A (at Ser900) and desensitizing glycine-independent NMDA receptors, which leads to activity-dependent shortening of receptor decay time

[36]. Finally, similar to calcineurin, PP1 diminishes NMDA-receptor-mediated synaptic currents in an activity-dependent manner in the hippocampus [37,38]. However in corticostriatal pathways, PP1 upregulates NMDA receptor activity through pathways involving casein kinase 1 (CK1) and mGluR1 signaling [39].

Regulation of non-NMDA glutamate receptors AMPA-type ionotropic glutamate receptors are regulated by activity-dependent phosphorylation and dephosphorylation of the GluR1 and GluR2 subunits. GluR1 phosphorylation by CaMKII and PKC (at Ser831 or Ser818) or by PKA (at Ser845) is associated with LTP, and potentiates channel conductance and open probability [40,41]. Phosphorylation of Ser845 and Ser818 also increases GluR1 surface expression and facilitates extrasynaptic trafficking, synaptic incorporation and receptor recycling following endocytosis [40,42,43]. By contrast, dephosphorylation of Ser831 and Ser845 by calcineurin or PP1, both of which are strategically positioned near AMPA receptors by targeting proteins, downregulates AMPA receptor activity and trafficking. Thus, calcineurin that is associated with AKAP79/150 (PRKA5) and synapse-associated protein SAP97 (also known as discs large homologue 1, or DLGH1) selectively dephosphorylates GluR1 at Ser845 [44]. It also dephosphorylates components of the endocytic machinery such as dynamin and amphiphysin [45,46] to regulate AMPA receptor internalization. Dissociation of a postsynaptic PP1–neurabin I complex following PKA-dependent phosphorylation of neurabin I (at Ser461) locally 'inactivates' PP1 [16,47] and inhibits dephosphorylation of postsynaptic proteins to reduce trafficking or insertion of AMPA receptor subunits into the synapse [48]. Interestingly, dephosphorylation of GluR1 at Ser845 favors AMPA receptor internalization [42,46,47], hinting that other GluR1-associated proteins are relevant substrates of the synaptic PP1–neurabin I complex. One potential candidate is stargazin, a PDZ-domain-containing protein required for AMPA receptor synaptic targeting, which is activated by phosphorylation by CaMKII and PKC and dephosphorylated by PP1 [49]. By contrast, a complex of PP1 with neurabin II (spinophilin) is the more likely GluR1 (Ser845) phosphatase, because loss of neurabin II function in mice impairs AMPA receptor dephosphorylation and hippocampal LTD [50–53]. Some synaptic PP1 pools might be inhibited by the potent inhibitors I-1 and DARPP-32 [54], which require PKA-mediated phosphorylation to be active. Both inhibitors are dephosphorylated and inactivated by calcineurin [55], generating a PP cascade whereby calcineurin activates PP1. By coupling increased PKA activity to suppression of PP1, I-1 and DARPP-32 constitute mechanisms for cross-talk between cAMP and Ca^{2+} signaling in neurons [56].

Activity-dependent neurotransmission by 5-hydroxytryptamine (5-HT) or dopamine also controls phosphorylation and dephosphorylation of AMPA receptors. For instance, in pyramidal neurons of prefrontal cortex, AMPA receptor activity is downregulated by activation of 5-HT_{1A} receptors through inhibition of PKA and subsequent PP1-catalyzed dephosphorylation of CaMKII [57]. Conversely, AMPA receptor activity is upregulated following activation of D_1 dopamine receptors, possibly by DARPP-32 activation and attenuation of PP1 activity [58].

Inhibitory neurotransmission

The β1–β3 subunits of $GABA_A$ receptors are phosphorylated by PKA and dephosphorylated by calcineurin or PP1 anchored to AKAP79/150 [59]; γ2 subunits are phosphorylated by PKC and dephosphorylated by calcineurin or PP1 anchored to yotiao [60]. PKA phosphorylation downregulates β1-containing $GABA_A$ receptors and activates β3-containing GABA receptors. Phosphorylation of β1 and β3 (at Ser409) or β2 (at Ser410) by PKC also downregulates $GABA_A$ activity. By contrast, $GABA_A$ receptor dephosphorylation requires the interaction of calcineurin with the γ2S subunit [61], or is catalyzed by PP1 and controlled by dopamine-activated mechanisms [62]. In pyramidal neurons of the prefrontal cortex, activation of D_4 dopamine receptors reduces $GABA_A$ receptor currents and synaptic transmission; this involves dephosphorylation of the β3 subunit, through concomitant inhibition of PKA and activation of PP1, which both associate with D_4 and $GABA_A$ receptors through yotiao [60,62]. Modulation of $GABA_A$ function by PP1 might also involve a complex formed between PP1α, phospholipase C (PLC)-related inactive protein type 1 (PRIP-1) and $GABA_A$-receptor-associated protein (GABARAP) [63]. PRIP-1, an inositol (1,4,5)-trisphosphate [Ins(1,4,5)*P*3]-binding protein, binds to and inhibits PP1. PKA-dependent phosphorylation of PRIP-1 within a PP1-binding domain (at Thr94 and Thr104) relieves inhibition and promotes dephosphorylation of the β3 $GABA_A$ subunit [64,65].

Positive and negative control of activity-dependent plasticity

Distinct synaptic states

NMDA-receptor-dependent LTP and LTD in the hippocampus might represent opposing modifications of a shared regulatory mechanism that depends on phosphorylation and dephosphorylation of common phosphoproteins to control synaptic strength, memory formation and memory storage [66]. Several PKs, including CaMKII, PKC, PKA, ERKs and mTOR, have been implicated in LTP expression and maintenance, whereas PP1 and calcineurin regulate LTD. Although common synaptic substrates of PKs and PPs have been identified, reversible phosphorylation predicts a sliding scale or continuum of synaptic strength that is unlikely to store adequate information about the diverse patterns of activity experienced by individual synapses. Physiological and pharmacological analysis of neurotransmission at individual synapses has suggested at least five functionally distinct synaptic states [67] (Figure 1) that can provide for higher information content of prior experience or activity, and so broaden the dynamic range of information stored in each neural circuit.

PPs and molecular diversity of synapses

The functional diversity of synapses might be achieved by expression and assembly of different heteromeric ion

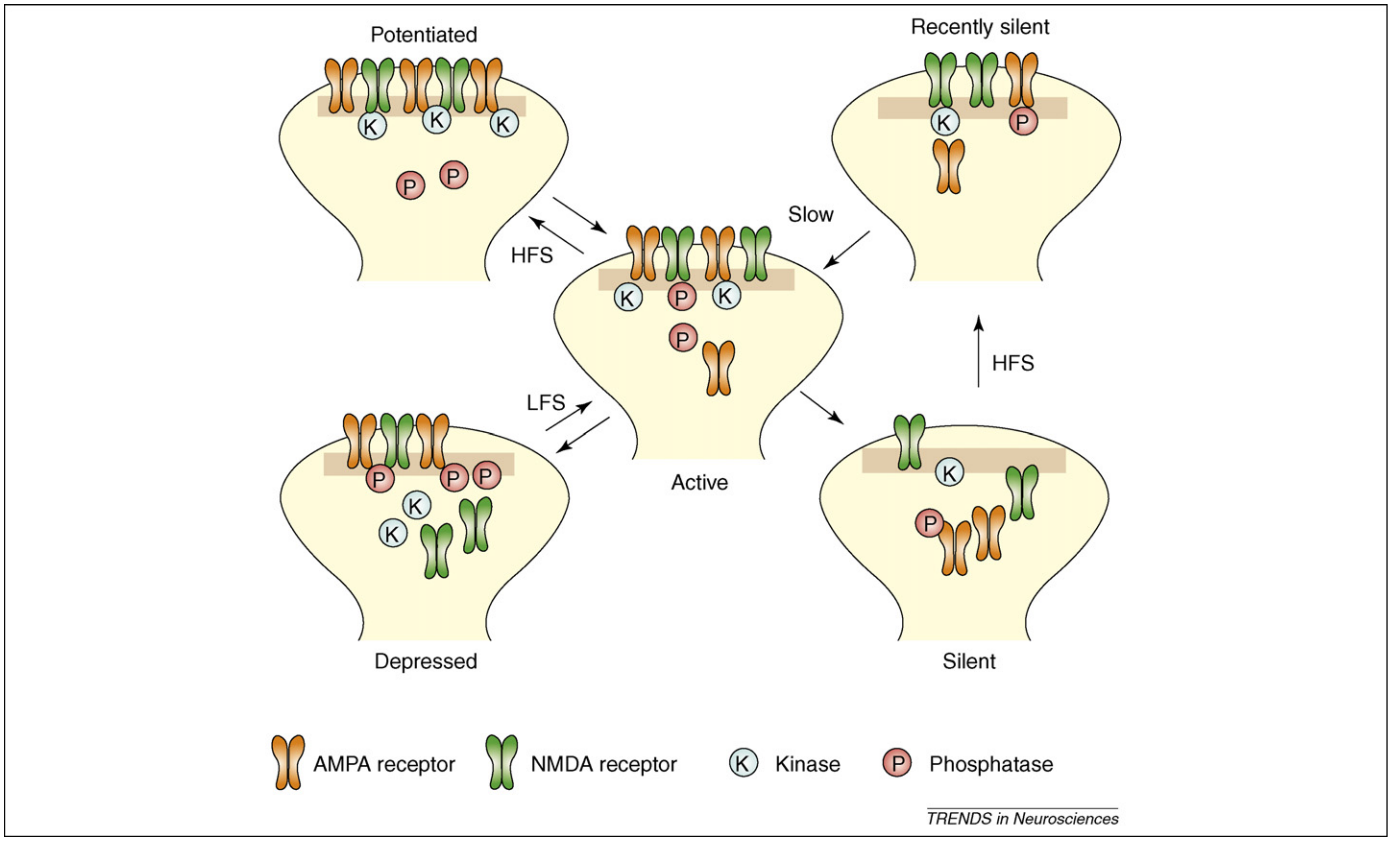

Figure 1. Molecular transitions of a hippocampal synapse. Recent studies suggest that PP1γ1 bound to neurabin I is required for insertion of AMPA receptors into hippocampal synapses. 'Silent' synapses display NMDA-receptor-mediated excitatory postsynaptic currents (EPSCs) and lack postsynaptic AMPA receptors. We speculate that silent synapses might also lack signaling components such as protein phosphatases (PPs) that facilitate AMPA receptor insertion. Lack of PPs and AMPA receptors is also consistent with the inability of silent synapses to undergo LTD. However, following a high-frequency stimulus (HFS), silent synapses are potentiated to a distinct 'recently silent' state in which they can show both NMDA-sensitive and AMPA-sensitive responses, which recruit both protein kinases (PKs) and PPs. The recently-silent synapses still lack the ability to be depressed but, through an unknown 'slow' mechanism, they spontaneously convert into 'active' synapses. During this slow process, receptors and signaling proteins might be reorganized or reassembled in a manner that converts the synapse into one that can undergo potentiation, depression or silencing. The active state is the most dynamic or plastic state of a hippocampal synapse. HFS further potentiates active synapses to recruit more AMPA receptors and PKs, through the localized translation and translocation of CaMKII (PKA, PKC and ERK are also activated) to enhance NMDA and AMPA currents in the 'potentiated' synapse. Emerging evidence suggests that LTP is associated with diminished PP activity by activation of phosphatase inhibitors such as I-1, by displacement from the postsynaptic density (PSD; shaded area underlying the postsynaptic membrane) or by ubiquitin-mediated degradation of the postsynaptic PP complexes. By contrast, LTD is associated with diminished AMPA-sensitive currents and increased synaptic recruitment of PPs in response to low-frequency stimulation (LFS). Following synaptic activity, both LTP and LTD revert the synapse back to the active state. PPs, specifically PP1 and PP2B, regulate depotentiation but the molecular events remain poorly understood. It should be noted that even among the potentiated and depressed synapses, there is considerable variability in synaptic transmission, indicating that additional molecular differences might further increase the range of synaptic responses.

channels (i.e. AMPA receptors and NMDA receptors) and/or by activation of distinct signaling cascades. AMPA receptors in the nervous system comprise four distinct GluR subunits (GluR1–GluR4), and the distinct NR1 and NR2 isoforms of NMDA receptors have unique functional properties. Different regions of mammalian brain also express distinct metabotropic glutamate receptor subtypes. Combinatorial assembly of receptors might provide diverse mechanisms to regulate synaptic plasticity in different brain regions. Furthermore, different signaling pathways utilizing distinct PKs and PPs that are activated by these receptors might also catalyze unique arrays of phosphorylation and dephosphorylation that define different synaptic states. For example, PP1, PP2A and calcineurin have all been identified as regulators of neuronal ion channels. PP2C binds to and dephosphorylates mGluR3 [68] and modulates neuronal Ca^{2+} channels [69]. PP2A, PP4, PP5 and PP6 control neuronal transcription and translation, and might define later stages of LTP and LTD.

Signaling capacity at individual synapses might be modified by dynamic expression and recruitment of signaling proteins to the PSD. Thus, CaMKII levels are increased in the PSD by LTP-inducing stimuli and activity through increased translation of dendritic mRNA and synaptic targeting of newly activated (autophosphorylated at Thr286) CaMKII [70,71]. These processes are negatively regulated by dephosphorylation of PSD-bound CaMKII by PP1 (which decreases affinity of CaMKII for the PSD and activation of CaMKII by Ca^{2+}/calmodulin [8]) and dephosphorylation of the RNA-binding protein cytoplasmic polyadenylation-element-binding protein 1 (CPEB1) [72]. In contrast to CaMKII, PP1 levels in dendritic spines are increased during LTD [73] and disruption of synaptic PP1 complexes inhibits LTD. In this regard, neurabins, which localize at spines through an N-terminal actin-binding domain and preferentially bind PP1γ1 (a major PSD-associated PP1 isoform [74]), might contribute to PP1 trafficking in spines. Phosphorylation of the actin-binding domain of neurabin II by CaMKII or PKA reduces its

affinity for F-actin, indicating that kinases implicated in LTP might dissociate PP1–neurabin II complexes from the actin-rich PSD. Thus, activity-dependent trafficking of CaMKII, PP1 and other signaling proteins might define the signaling capacity at individual synapses. Immunoelectron microscopy that identified mammalian synapses containing either PP1α or both PP1α and PP1γ1 indicated that only spines containing both PP1 isoforms contained dopamine D_1 receptors, which are known modulators of synaptic plasticity [75]. This argued for an ordered recruitment of PP1 isoforms and receptors during the functional maturation of spines.

Protein turnover provides another mechanism for molecular remodeling at spines [25,76] and for PP1 regulation. Synaptic activity induces changes in the level of neurotransmitter receptor subunits (e.g. NR1 and NR2B), scaffolding proteins (e.g. SAP102 and AKAP79/150) and signaling proteins (e.g. PP1) in spines. This is regulated in part by ubiquitination (in the case of AKAP79/150, Shank and the G-kinase-anchoring protein GKAP) and activity-dependent turnover of regulators, and is accompanied by altered synaptic signaling to downstream effectors such as the ERK–mitogen-activated protein kinase (MAPK) pathway and cAMP-response-element-binding protein (CREB) [25].

PPs in disorders of learning and memory

Consistent with their ability to constrain synaptic transmission and plasticity, PPs negatively regulate learning and memory processes. Thus, their activity needs to be controlled during and after these processes and PP alterations are implicated in memory disorders.

Modulation of PPs during learning and memory

Calcineurin activity and expression rapidly diminishes in the rodent medial prefrontal cortex after activation of spatial working memory [77] and in the hippocampus after fear conditioning, a form of associative memory for a tone–footshock or context–footshock pairing [78]. However unlike acquisition, memory extinction (a form of relearning, for instance induced for fear memory by presentation of a tone alone instead of tone–footshock) increases calcineurin expression and activity in lateral and basolateral amygdala (60–100%) and ventromedial prefrontal cortex (~60%), and is blocked by inhibition of calcineurin [79–82]. Nonetheless, downregulation of PPs during initial learning does seem to be required for proper processing and retention of information because induction of excess calcineurin impairs learning and memory retrieval in adult mice [83,84]. By comparison, increased PP1 activity due to elimination of DARPP-32, a PP1 inhibitor present in the striatum, impairs reversal learning in a discriminated operant task, possibly through altered dopaminergic neurotransmission [85]. However, knockout of the gene encoding I-1 has no effect on learning and memory, suggesting a distinct contribution of this factor to PP1 inhibition or the presence of compensating PP1-inhibitory mechanisms [86]. Conversely, partial reduction in calcineurin levels during training enhances learning in rodents and *Aplysia* [87–89]. Likewise, partial inhibition of PP1 during training improves spatial learning and recognition memory, and prevents age-related decline in these functions. Inhibition only after training also enhances memory [90], indicating that PP1-dependent constraint mechanisms are engaged both during and after learning and need to be repressed for establishment of long-term memory. However both calcineurin and PP1 pathways require minimal repression for acquisition of memory because more drastic or earlier elimination of these PPs by gene knockout or pharmacological inhibitors impairs learning [91,92]. Finally, perturbing PP1 anchoring by knockout of the gene encoding spinophilin also compromises conditioned taste aversion, a form of associative learning and memory [93], possibly as a result of altered formation and function of dendritic spines [51].

Changes in PPs associated with cognitive decline and dementia

During aging in humans and rodents, overall PP1 and calcineurin activity increases in the brain, which might contribute to learning and memory failures, cognitive decline and altered synaptic plasticity [94,95]. However, in synaptic terminals and cytosol of hippocampal pyramidal neurons, calcineurin activity might be attenuated owing to higher expression of an endogenous calcineurin inhibitor, Down syndrome critical region 1 (DSCR1 or calcipressin), and/or inactivation of calcineurin by oxidation and aggregation [96,97]. Alterations in PPs are associated with Alzheimer's disease (AD), the most common cause of memory deficits and dementia [98,99]. AD is characterized by neurofibrillary tangles, insoluble fibrils of hyperphosphorylated microtubule-associated protein tau, and amyloid-β (Aβ) plaques. Abnormal tau hyperphosphorylation might result from an imbalance between PKs and PPs, such as PP1, PP2A, calcineurin and PP5 [100,101]. All PPs are downregulated in AD brain [102] (although activation of calcineurin in AD has also been reported [103,104]), via mechanisms including decreased expression, upregulation of inhibitors (e.g. Cat53 for PP1 [105], I_1^{PP2A} and I_2^{PP2A} for PP2A [106], or DSCR1 for calcineurin [97,107]), or perturbation of regulatory proteins (e.g. prolyl isomerase Pin1 for PP2A [108]). Consistently, inhibition of PP1 and PP2A in rat brain induces both tau hyperphosphorylation and memory deficits [109]. Modulation of PPs might also dictate the toxicity of Aβ aggregates on synaptic functions and memory. PP1 favors Aβ formation and its inhibition stimulates the secretion of non-toxic soluble amyloid precursor protein (sAPP) from COS-1 cells and arrests Aβ production [110]. Likewise, calcineurin inhibition prevents Aβ-mediated cell death by blocking dephosphorylation of BCL2 antagonist of cell death (BAD) and by activating caspase-3 [111]. Calcineurin also contributes to Aβ-induced deficits in LTP induction and maintenance [112,113]. Calcineurin is activated by Aβ, through activation of the striatal-enriched tyrosine phosphatase (STEP, also known as PTPN5), which then dephosphorylates NR2B to reduce NMDA receptor surface expression and trafficking [114]. This effect is coupled with the blockade of CaMKII activation and GluR1 phosphorylation (at Ser831) [115], suggesting that Aβ induces a broad imbalance in activity of synaptic PPs and PKs.

Finally, learning associated with drug addiction is also negatively regulated by PPs, particularly calcineurin and PP1. In the conditioned place-preference test, inhibition of calcineurin by FK506 in the rat brain following training facilitates memory for the association between the subjective state produced by amphetamine and environmental cues that are present during that state [116]. By contrast, in the same task in mice, conditioned place preference was altered by increasing activity of calcineurin using a transgene, or increasing activity of PP1 by DARPP-32 knockout or double knockout of DARPP-32 and I-1 [117,118]. These results parallel impairment of amphetamine-dependent conditioned place preference by inhibition of MAPK activity in the nucleus accumbens [119], because MAPK activation by amphetamine depends on DARPP-32, an inhibitor of PP1 that is negatively regulated by calcineurin [56].

Concluding remarks

This review has focused on PPs and regulation of neuronal phosphoproteins that modulate synaptic plasticity, learning and memory. Readers have also been referred to several excellent reviews describing the broader underpinnings of PP structure and regulation. Great progress has been made over the past decade in highlighting the role of PPs in complex physiological events such as learning and memory. It has also become increasingly apparent that molecular determinants of memory formation are stored not only in individual neural circuits but also in specific connections or synapses. Because each neuron possesses hundreds to thousands of spines, biochemical mechanisms that record recent activity at individual synapses and the importance of activity-dependent changes in spine structure are only just being understood. The future challenge for neurobiologists is not only to understand links between PPs, synaptic plasticity and behavior but also to develop novel tools and technology, such as quantitative imaging of signaling events, that can track changes in PKs, PPs, phosphorylation and dephosphorylation that are associated with synaptic activity in individual spines. Only with the in-depth analysis of biochemistry and physiology of single synapses will we gain full appreciation of the signaling networks that modulate neural circuits, regulate learning and contribute to memory disorders.

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