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If phosphatases go up, memory goes down

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Abstract. Previous work has provided conclusive support for a role of various protein kinases in processes underlying learning and memory formation. While these processes are not yet established in full detail, it is interesting to entertain the idea of protein phosphatases being involved in such mechanisms as well. Recent advances in this respect have provided preliminary support of this view. From the pharmacological as well as the transgenic analysis, it appears that especially the calcineurin/inhibitor-1 cascade plays an important role in the transition of intermediate-term into long-term memory formation.

Key words. Hippocampus; synaptic plasticity; memory formation; protein kinase A; protein phosphatase 1 and 2A; calcineurin.

Antagonizing kinases

In the previous review, we concentrated on phosphorylation processes as a possible mechanism involved in at least some forms of memory formation. Since biochemistry has provided evidence for dephosphorylation within the same cells and even the same compartments of the cells, it appears quite logical to also expect phosphatases to play a part in the cellular mechanisms underlying learning. Although the exact nature of the kinase-phosphatase relationship remains unclear for many processes, it would be reasonable to assume that antagonizing actions are a prerequisite for the fine-tuning of information transduction and storage in the neuronal system.

Protein phosphatases are phosphotransferases that catalyze transfer of phosphoryl groups from phosphorylated side chains of amino acids to water molecules. Hampered by the lack of selective agonists and antagonists, investigations targeting phosphatase function have emerged only recently, and it is especially experiments on protein phosphorylation and dephosphorylation in synaptic plasticity research that have generated considerable interest in this subject. Both long-term potentiation (LTP) and depression (LTD) of synaptic plasticity are associated with increases in postsynaptic calcium, but what determines LTP as opposed to LTD remained elusive for quite some time. In a very detailed, though hypothetical account, Lisman [1] proposed that different calcium levels—generated by low- versus high-frequency stimulation—are responsible for the long-lasting changes in either direction. Because expression of LTP increases α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor activity by phosphorylation through calcium/calmodulin-dependent protein kinase II (CaMKII) [2], protein kinase A (PKA) [3] and protein kinase C (PKC) [4], it can be expected that LTD is paralleled by a decrease in AMPA receptor-mediated responses, and this decrease might be achieved via dephosphorylation. In other words, low frequency-induced LTD may be accompanied by the activation of specific phosphatases which have the ability to antagonize the action of the above-mentioned kinases. Searching for such candidate phosphatases, the group of Robert Malenka has provided compelling evidence for an essential role of phosphatases in LTD induction [5] with the calcineurin/inhibitor-1-phosphate cascade playing a major part [6]. Bath application of FK506 and cyclosporin A, both highly selective inhibitors of calcineurin, prevented LTD, and so did okadaic acid or caliculin A, both inhibitors of protein phosphatase 1 and 2A, respectively. Direct biochemical measurements of phosphatase activity after in vivo induction of LTD has supported this view and shown that both phosphatase 1 and 2A are activated for 1 h or much longer, respectively [7].

Calcineurin is a calcium-sensitive serine/threonine phosphatase that is enriched at synapses [8], especially in the hippocampal formation. Once activated, calcineurin can act either by directly dephosphorylating target proteins, or indirectly by activating the inhibitor-1-phosphate cascade (see fig. 1). In the latter case, calcineurin dephosphorylates inhibitor-1-phosphate so that inhibitor-1 can promote activation of protein phosphatase 1 (PP1). PP1 in turn can dephosphorylate a completely different set of target proteins, including *N*-methyl-D-aspartate (NMDA) and AMPA receptors [9, 10], the PKC substrates MARCKS, neurogranin and GAP-43 [11–13], as well as the transcription factor cyclic adenosine monophosphate responsive element binding (CREB) [[14]; for details, see Lamprecht, this issue]. Furthermore, the dephosphorylation site in inhibitor-1 is the site of phosphorylation by PKA.

Delineating this cascade has been a major step in understanding the role of phosphatases in synaptic plasticity. However, pharmacological application of phosphatase inhibitors very often requires long exposure for penetration of the compound into the cell. Also, results concerning LTP have been controversial, with some reporting no effect [6], some finding an enhancement [15, 16], and others reporting a deficit [17, 18]. Nevertheless, few reports have employed pharmacological tools in studies on learning and memory. They will be considered next.

Blocking phosphatases blocks memory formation

Convincing pharmacological evidence for a potential role of phosphatases in learning and memory formation is rather sparse. Up until now, there are few reports on one-trial aversive conditioning in 1-day-old chicks that have applied both okadaic acid as a blocker of protein phosphatase 1 and 2A [19], and cyclosporin A as an antagonist of calcineurin. This learning paradigm developed by Cherkin [20] makes use of the natural tendency of young chicks to peck beads. If a bead is coated with a bitter-tasting substance, for instance methylanthranylate, the animal shows a typical disgust reaction and refrains from further pecking at that bead. One trial is

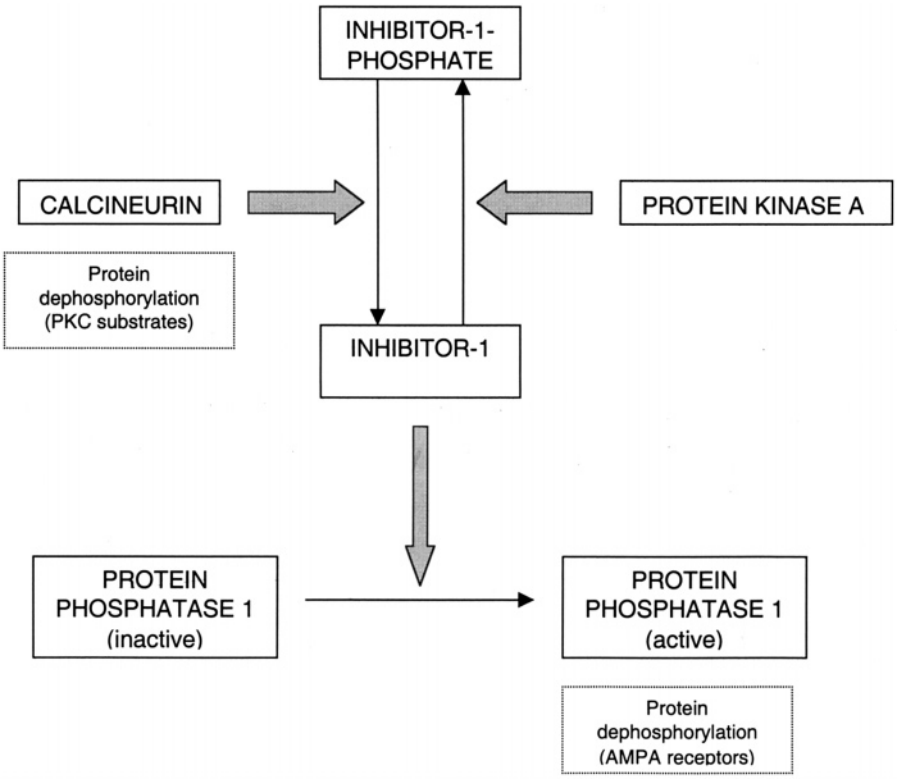

Figure 1. Biochemical cascades of calcineurin protein kinase A interplay believed to underlie its function in synaptic plasticity and memory formation. Phosphorylation of inhibitor-1 keeps it in an active state so that protein phosphatase 1 (PP1) is inhibited. Dephosphorylation of inhibitor-1, however, causes disinhibition, and this promotes activation of PP1. Both calcineurin and PP1 dephosphorylate different sets of target proteins and interact with other kinase pathways (see Micheau and Riedel, this issue).

usually sufficient to induce long-lasting memory (24 h and more), drug administration can take place either prior to or after the trial, and memory is usually tested at various time points after training. Based on pharmacological interventions, Gibbs and Ng [21] have presented a model with short-term, intermediate-term and long-term memory phases. It appears that transition of intermediate-term memory into longer-term memory requires activation of protein kinases (see Micheau and Riedel, this issue) thus prompting the idea of a potential antagonistic role of phosphatases as well. This issue has been addressed by Zhao et al. [22] by means of okadaic acid. They reported a dose- and time-dependent memory deficit in animals treated with ≥ 0.05 nM okadaic acid compared with DMSO-injected controls. The memory deficit appeared when animals were tested for retention 40–50 min post-training and persisted. These data implicate both protein phosphatase 1 and 2A in intermediate-term memory in chicks. Cyclosporin A, the calcineurin antagonist, had a U-shaped dose response curve, with 5 nM injected intracranially being most effective [23]. Memory deficits were apparent at 80 min or more post-training, suggesting a role of calcineurin in the stabilization of memory.

Genetic approach to phosphatase function

Very recently, a different approach has utilized gene-targeting techniques in order to determine phosphatase functions in synaptic plasticity and memory formation in both *Drosophila* [24] and mice [25, 26]. A strain of fruit flies carrying the *Su-var(3)6^{01}* mutation, which is known to affect the structural gene of the protein phosphatase 1 iso-enzyme, has been bred. Animals were tested in various learning paradigms, such as olfactory and visual associative tasks, as well as habituation of the landing response [24]. Associative learning of odours, for example in *Drosophila*, involves a training phase in a tube where two distinct odours are presented successively, one of which is followed by a shock. After a defined period post-training, animals were placed in a choice box with both odours present simultaneously. They were allowed to distribute freely before being cooled down and counted. The number in each odour compartment can then be used to calculate a performance index [27] as a measure of memory. When such training was applied, the *Su-var(3)6^{01}* mutation homozygotes and heterozygotes showed a clear performance deficit with increased memory decay compared with wild-type Canton-S controls. A quasi-uniform distribution (amnesia) was found 1.5 h and 15 min after training in an odour or visual associative task, respectively. Since mutants did not differ with respect to sensitivity to the sensory stimulus like odour or shock, it can be assumed that activity of protein phosphatase 1 is particularly important for short-term memory.

In an opposite approach, a truncated form of calcineurin was overexpressed in the forebrain of mice under the control of the CaMKII promotor [25, 26]. The resulting individuals (CN98) show no cross-motor abnormalities, and even basal synaptic transmission in hippocampal slices was normal. However, paired pulse facilitation was somewhat reduced in transgenics, suggesting presynaptic alterations. Slices were then exposed to various protocols of high-frequency stimulation in order to induce LTP. Transgenic slices, however, did not express tetanus-induced long-lasting LTP [25]. This form of long-term synaptic plasticity had previously been shown to depend on both PKA activation and protein synthesis [28]. The potentiation in transgenic slices decayed back to baseline within 3 h and was termed an 'intermediate' form of LTP [25]. This intermediate LTP depended on the activation of PKA, but not on protein synthesis [25]. It thus appears that overexpression of calcineurin alters the interaction between phosphatase and PKA in favour of the phosphatase. Providing more PKA via pharmacological means would, if this hypothesis were correct, overcome the deficit in the transgenics and reestablish normal LTP. This experiment has been done [25], and pharmacological application of the PKA activator sp-cAMP or the dopamin receptor agonist 6-Br-APB indeed generated normal LTP. Although it was claimed that this intermediate LTP has a novel and unique time course [25], it should be noted that such an intermediate form of LTP was proposed previously to be dependent on the activation of PKC (for review see [29]).

Collectively, these data strongly suggest that overexpression of calcineurin inhibits the expression of long-lasting LTP by antagonizing the action of PKA. The machinery to express lasting LTP, however, does exist, can be activated under certain circumstances and may become active in specific behavioural situations. At first glance, however, the prediction for learning experiments would be the following: Because lasting LTP is prevented, one would expect CN98 mice to have a deficit in long-term, but not short-term, memory. Owing to the ability to express intermediate LTP, the memory should be considerably longer than, for instance, in animals exposed to NMDA receptor blockade, in which LTP is abolished completely [30]. Finally, pharmacological activation of PKA may render CN98 mice normal in that the balance between kinase and phosphatase activity has been reestablished.

Clearly, much work needs to be done with these transgenic mice. However, first data on learning support at least some of the predictions put forward here. Since calcineurin was enriched in hippocampus, Mansuy et al. [26] specifically investigated learning forms that are

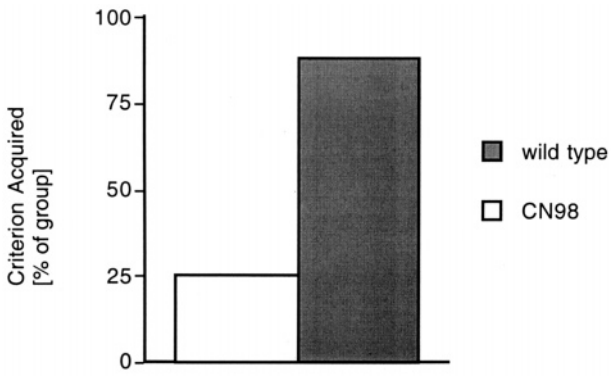

Figure 2. Transgenic mice overexpressing calcineurin are impaired in spatial learning. Only about 25% acquired criterion compared with wild-type littermates. Data taken from [26].

sensitive to hippocampal damage. Mice were tested in a spatial and a cued version of the Barnes maze [31]. This is a circular platform with 40 holes at the perimeter; one hole leads to an escape tunnel. Animals were placed in the center of the platform, and a bright light together with an aversive buzzer motivated the mice to find the escape tunnel. Identification of the correct hole is based on distal cues, and the position of the escape hole was kept constant for the spatial task. One trial was given per day for 40 days, and mice were trained to a criterion of three or fewer errors in five out of six consecutive trials. Only 25% of the transgenics met the learning criterion compared with 88% of the control littermates (see fig. 2). Whether such a deficit is due to an inability to learn about the spatial relationship of items and the escape hole, or due to some attentional or even visual or gross motor deficit remains elusive. Mansuy et al. thus went on to perform a cued task by rotating the platform between trials, but the position of the escape hole was marked by a visual cue behind the hole. Both transgenics and wild-type littermates acquired this task more readily with no difference between groups. These data therefore suggest a spatial rather than a general learning/memory deficit in CN98 mice overexpressing calcineurin. However, the deficit could be fully rescued when four trials (1.5 min intertrial interval) were applied per day instead of one. For Mansuy and colleagues these data indicate a short-term memory deficit in CN98 mice that is taxed by a single trial learning; but four trials overcome this deficit. An alternative, though not that exciting, interpretation would be that the quality of the reinforcer is different for both groups, with CN98 animals requiring stronger stimulation for learning. However, this somehow contradicts the LTP results, where a deficit after strong, but not weak, tetanic stimulation was found in hippocampal CA1 [25].

Using an object recognition task [32, 33], spontaneous activity can be used as a measure of memory, and hippocampal animals are known to be impaired in such learning tasks. Mice were allowed to explore a novel environment containing two objects for 15 min. At different time points post-training (30 min, 2 h, 24 h), they were replaced in the same environment now containing a third novel object, and the time spent exploring the novel compared with the familiar objects was recorded. It is to be expected that animals would spend more time exploring the novel objects. This was indeed the case for wild-type controls at all intervals. Calcineurin overexpression, however, caused a retention deficit after 24 h, but not 30 min. There was also a slight reduction in exploration after 2 h, which was not significant. These data clearly parallel the observations on LTP where the most severe deficit was obtained for long-lasting LTP.

Conclusions

With respect to a possible role of phosphatases in learning and memory formation, it appears somewhat overambitious to draw any firm conclusion from the limited set of data available to date. Clearly, pharmacological investigations on LTP using phosphatase blockers have yielded contradictory results, which are thus hardly comparable with results obtained in learning experiments. Nevertheless, there is increasing evidence for a potential role of phosphatases in memory formation. No deficit in acquisition learning has been reported so far, and this is certainly an interesting issue for future work. The deficit in acquisition of the Barnes maze should not be interpreted as a learning deficit, because only one trial per day was given with an intertrial interval of 24 h, which may be considered as long-term memory. Since inhibition of protein kinases often leads to memory deficits, an oversimplified prediction for phosphatase inactivation would have been a facilitation of memory. However, the reported memory deficits may not come as a surprise, because an imbalance in phosphorylation/dephosphorylation processes, for instance by overactivation of certain phosphatases, will necessarily disrupt the fine-tuning of neurons and result in deficits. What remains to be determined is the exact nature and time when phosphatase activation is mandatory for the emergence of long-term memories. It is thus imperative to investigate other behavioural paradigms and to apply both negative and positive reinforcers.

Determination of the exact functions of individual phosphatases is still far away. As for kinases, interactions with intracellular substrates are likely to be complex, and involvement in extensive cross-talk can be

expected. This cross-talk as well as the downstream modulation of transcription factors such as CREB; see Lamprecht, this issue) support this view. Furthermore, phosphatases may play an important part in the neuropathology of Alzheimer's disease (see Roloff and Platt, this issue), specifically with respect to the role of tau hyperphosphorylation and its contribution to the pathological state. As tau is phosphorylated via CaMKII and mitogen-activated protein kinase (MAP), normal functioning of the protein requires dephosphorylation by means of PP2A and calcineurin [34]. Thus, there is even clinical demand for a better understanding of the role of phosphatases in mechanisms underlying memory formation.

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1 Lisman J. (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. Proc. Natl. Acad. Sci. USA **86:** 9574–9578
2 Tan S. E., Wendthold R. J. and Söderling T. R. (1994) Phosphorylation of AMPA-type glutamate receptors by calcium/calmodulin-dependent protein kinase II and protein kinase C in cultured hippocampal neurons. J. Neurosci. **14:** 1123–1129
3 Roche K. W., Tingley W. G. and Huganir R. L. (1994) Glutamate receptor phosphorylation and synaptic plasticity. Curr. Opin. Neurobiol. **4:** 383–388
4 Cheng G., Rong X. W. and Feng T. P. (1994) Block of induction and maintenance of calcium-induced LTP by inhibition of protein kinase C in postsynaptic neurons in hippocampal CA1 region. Brain Res. **646:** 230–235
5 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
6 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
7 Thiels E., Norman E. D., Barrionuevo G. and Klann E. (1998) Transient and persistent increases in protein phosphatase activity during long-term depression in the adult hippocampus in vivo. Neurosci. **86:** 1023–1029
8 Kuno T., Mukai H., Ito A., Chang C. D., Kishima K., Saito N. et al. (1992) Distinct cellular expression of calcineurin Aα and Aβ in rat brain. J. Neurochem. **58:** 1643–1651
9 Tong G., Shepherd D. and Jahr C. E. (1995) Synaptic desensitization of NMDA receptors by calcineurin. Science **267:** 1510–1512
10 Traynelis S. F. and Wahl P. (1997) Control of rat GluR6 glutamate receptor open probability by protein kinase A and calcineurin. J. Physiol. **503:** 513–531
11 Liu Y. and Storm D. R. (1989) Dephosphorylation of neuromodulin by calcineurin. J. Biol. Chem. **264:** 12800–12804
12 Schrama L. H., Heemskerk F. M. J. and de Graan P. N. E. (1989) Dephosphorylation of protein kinase C phosphorylated B-50/GAP-43 by the calmodulin-dependent phosphatase calcineurin. Neurosci. Res. Comm. **5:** 141–147
13 Seki K., Cheng H.-C. and Huang K.-P. (1995) Dephosphorylation of protein kinase C substrates, neurogranin, neuromodulin and MARCKS, by calcineurin and protein phosphatases 1 and 2A. Arch. Biochem. Biophys. **316:** 673–679
14 Scwaninger M., Blume R., Krüger M., Lux G., Oetjen E. and Knepel W. (1995) Involvement of the Ca^{2+}-dependent phosphatase calcineurin in gene transcription that is stimulated by cAMP through cAMP response elements. J. Biol. Chem. **270:** 8860–8866
15 Wang J.-H. and Kelly P. T. (1996) The balance between postsynaptic Ca^{2+}-dependent protein kinase and phosphatase activities controlling synaptic strength. Learn. Mem. **3:** 170–181
16 Thomas M. J., Moody T. D., Makhinson M. and O'Dell T. J. (1996) Activity-dependent β-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. Neuron **17:** 475–482
17 Wang J.-H. and Stelzer A. (1994) Inhibition of phosphatase 2B prevents expression of hippocampal long-term potentiation. Neuroreport **5:** 2377–2380
18 Lu Y.-F., Tomizawa K., Morikawa A., Hayashi Y., Tokuda M., Itano T. et al. (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
19 Suganuma M., Fujiki H., Okabe S., Nishiwaki S., Brautigan D., Sagebritsen T. S. et al. (1992) Structurally different members of the okadaic acid class selectively inhibit protein serine/threonine but not tyrosine phosphatase activity. Toxicon **30:** 873–878
20 Cherkin A. (1969) Kinetics of memory consolidation: role of amnesic treatment parameters. Proc. Natl. Acad. Sci. USA **63:** 1094–2001
21 Gibbs M. E. and Ng K. T. (1977) Psychology of memory: towards a model of memory formation. Biobehav. Rev. **1:** 113–136
22 Zhao W., Bennett P., Sedman G. L. and Ng K. T. (1995) The impairment of long-term memory formation by the phosphatase inhibitor okadaic acid. Brain Res. Bull. **36:** 557–561
23 Bennett P. C., Zhao W., Lawen A. and Ng K. T. (1996) Cyclosporin A, an inhibitor of calcineurin, impairs memory formation in day-old chicks. Brain Res. **730:** 107–117
24 Asztalos Z., von Wegener J., Wustmann G., Dombradi V., Gausz J., Spatz H.-C. et al. (1993) Protein phosphatase 1-deficient mutant Drosophila is affected in habituation and associative learning. J. Neurosci. **13:** 924–930
25 Winder D. G., Mansuy I. M, Osman M., Moallem T. M. and Kandel E. R. (1998) Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. Cell **92:** 25–37
26 Mansuy I. M., Mayford M., Jacob B., Kandel. E. R. and Bach M. E. (1998) Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. Cell **92:** 39–49
27 Boynton S. and Tully T. (1992) latheo, a new gene involved in associative learning and memory in *Drosophila melanogaster*, identified from P element mutagenesis. Genetics **131:** 655–672
28 Huang Y.-Y. and Kandel E. R. (1994) Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learn. Mem. **1:** 74–82
29 Reymann K. G. and Staak. S. (1994) Molecular mechanisms underlying long-term potentiation: postsynaptic glutamate receptors and protein kinase C. In: Protein Kinase C in the CNS: Focus on Neuronal Plasticity, pp. 7–32, Canonico P. L., Scapagnini U., Pamparana F. and Routtenberg A. (eds), Masson Press, Milan
30 Morris R. G. M., Garrud P., Rawlins J. N. P. and O'Keefe J. (1982) Place navigation impaired in rats with hippocampal lesions. Nature **297:** 681–683
31 Barnes C. (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol. **93:** 74–104
32 Aggleton J. P. (1985) One-trial object recognition by rats. Quart. J. Exp. Psychol. **37:** 279–294
33 Ennaceur A. and Delacour J. (1988) A new one-trial test for neurobiological studies of memory in rats. 1. Behavioral data. Behav. Brain Res. **31:** 47–59
34 Goedert M. (1993) Tau-protein and the neurofibrillary pathology of Alzheimers disease. Trends Neurosci. **16:** 460–465