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The late maintenance of hippocampal LTP: Requirements, phases, 'synaptic tagging', 'late-associativity' and implications

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Abstract

Our review focuses on the mechanisms which enable the late maintenance of hippocampal long-term potentiation (LTP; >3 h), a phenomenon which is thought to underlie prolonged memory. About 20 years ago we showed for the first time that the maintenance of LTP – like memory storage – depends on intact protein synthesis and thus, consists of at least two temporal phases. Here we concentrate on mechanisms required for the induction of the transient early-LTP and of the protein synthesis-dependent late-LTP. Our group has shown that the induction of late-LTP requires the associative activation of heterosynaptic inputs, i.e. the synergistic activation of glutamatergic and modulatory, reinforcing inputs within specific, effective time windows. The induction of late-LTP is characterized by novel, late-associative properties such as 'synaptic tagging' and 'late-associative reinforcement'. Both phenomena require the associative setting of synaptic tags as well as the availability of plasticity-related proteins (PRPs) and they are restricted to functional dendritic compartments, in general. 'Synaptic tagging' guarantees input specificity and thus the specific processing of afferent signals for the establishment of late-LTP. 'Late-associative reinforcement' describes a process where early-LTP by the co-activation of modulatory inputs can be transformed into late-LTP in activated synapses where a tag is set. Recent evidence from behavioral experiments, which studied processes of emotional and cognitive reinforcement of LTP, point to the physiological relevance of the above mechanisms during cellular and system's memory formation.

Keywords: Protein synthesis-dependent late-LTP; Memory formation; Synaptic tagging; LTP reinforcement; LTP-phases

1. Introduction

The last 40 years have seen a growing interest in long-term potentiation (LTP), a cellular model of activity-dependent enhancement of synaptic transmission in the vertebrate brain. In first reports, Bliss and Coworkers (for review see Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973) described LTP in the dentate region of the hippocampus. The best studied form of LTP as well as the one which can be induced in the intact adult animal has been referred to as an 'associative, NMDA-receptor-dependent LTP'. The induction of LTP by brief high-frequency trains activates glutamatergic and modulatory, heterosynaptic inputs required for its prolonged maintenance. This particular form of LTP requires the activation of various kinases as well as the synthesis of plasticity-related proteins (PRPs) and thus consists of phases (Bliss and Collingridge, 1993; Frey et al., 1988; Krug et al., 1984; Matthies et al., 1990b; Otani et al., 1989; Reymann et al., 1988a,c).

While other forms of synaptic plasticity are unquestionably important, we shall hereafter discuss only the associative, NMDA receptor- and protein synthesis-dependent form and refer to it, for simplicity, as 'LTP'. Because of its long duration, input specificity and associative properties, LTP was widely used as a model for the investigation of basic memory mechanisms.

1.1. Proteins for memory formation

In 1974 Hansjuergen Matthies (Matthies, 1974) developed his hypothesis of neuronal mechanisms of memory formation

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in the mammalian brain: the assumed phases of short-term, intermediate, and long-term memory – their different time courses and decay times, different biological correlates, and different sensitivities to interventions – reflect corresponding cellular properties at the synaptic, synaptosomal, and nuclear level required for the regulation of memory formation (Matthies, 1974, 1989). Learning experiments revealed a biphasic occurrence of protein synthesis. It was suggested that the early phase of protein synthesis represents the synthesis of regulatory proteins. The regulatory proteins then control the formation of target proteins which, finally, remodel neuronal connectivity/efficiency during late stages of memory formation.

2. Protein synthesis-dependent consolidation of LTP

In 1984 Manfred Krug and colleagues (Krug et al., 1984) demonstrated for the first time that the maintenance of hippocampal LTP beyond 3 to 6 h in the DG in vivo can be blocked by anisomycin, a reversible translation inhibitor. The role of protein synthesis for the maintenance of LTP has then been replicated by various other laboratories (e.g., Fazeli et al., 1993; Frey et al., 1988; Nayak et al., 1998; Osten et al., 1996; Otani et al., 1989). Taken together, these first results led us to the hypothesis that LTP has stages or phases like memory consolidation and that the synthesis of proteins is required for the long-lasting maintenance of synaptic changes in efficacy (Frey et al., 1988; Krug et al., 1984; Matthies et al., 1990b; Reymann et al., 1988a,c).

2.1. Site of synthesis

Local protein synthesis inhibition in the entorhinal cortex demonstrated that the presynaptic somata are not the critical locus of protein synthesis for late-LTP. Only if anisomycin was applied into the DG, late-LTP was blocked (Otani and Abraham, 1989). A still open question is whether the molecular signals for initiating translation and the protein synthesis itself are localized at the nearby dendrites or distributed between dendrites and far away cell bodies. Strong stimulation that is used to induce long-lasting LTP certainly engages somatic signaling cascades. Evidence comes from our study where LTP was induced in dendritic stumps of CA1 pyramidal cells of hippocampal slices in vitro (Frey et al., 1989). In these experiments the cell-body layer, the major site of transcription and translation, was surgically removed from the apical dendrites. The isolated dendrites revealed a pronounced early-LTP in the field-EPSP, similar to that in intact slices. However, the potentiation decreased after about 3–4 h, thus showing the same lack of late-LTP as observed in complete CA1 neurons after inhibition of protein synthesis with anisomycin. These results indicate that the mechanisms responsible for late-LTP are located, at least partially, in postsynaptic compartments and somatic factors are needed for NMDA-receptor-dependent late-LTP in the CA1 region. If somatic signaling or even transcription is necessary for the synthesis, the question arises of how the input-specificity of late-LTP is achieved without elaborating protein-trafficking. This important question will be discussed later in the chapter on synaptic tagging. However, during the last few years, the picture with respect to the locus of protein synthesis became more complicated. Local application of the protein synthesis inhibitor emetine to either basal or apical dendrites revealed a specific contribution of protein synthesis in dendritic compartments irrespective of the contribution of somatic protein synthesis (Bradshaw et al., 2003). Anisomycin and cycloheximide block LTP already after 1 h even in isolated dendrites (Cracco et al., 2005; Vickers et al., 2005). A theta frequency stimulation applied to the Schaffer collateral pathway can also induce a form of late-LTP that is maintained even in isolated CA1 dendrites (Huang and Kandel, 2005). Translation of pre-existing mRNAs present in isolated dendrites (Steward and Schuman, 2001) might be sufficient to mediate potentiation or are necessary in addition to proteins or mRNA transported from the soma (for RNA see also below), at least to maintain partially early-LTP in distinct preparations. Besides postsynaptic protein synthesis, also a late NMDA-receptor-sensitive increase of presynaptic vesicle proteins was described (Lynch et al., 1994). It should be mentioned that besides local protein function in dendrites and presynaptic terminals the release of newly synthesized proteins into the extracellular space also might be important for late-LTP (Duffy et al., 1981; Fazeli et al., 1988; Pang and Lu, 2004).

All these findings might find their explanations by the need of local dendritic protein synthesis, or by drug site effects (see below), or paradigm/preparation/incubation-dependence (Sajikumar et al., 2005b).

2.2. De novo protein synthesis and posttranslational modification

The identification of the classes and functions of these proteins, which might themselves be receptors destined for insertion into the cell membrane or factors like kinases that enhance or inhibit the activity of existing molecular entities, is ongoing. If macromolecules are necessary for consolidation, the question arises whether similar if not identical molecules (e.g. PRP) are required in all synapses, a topic discussed in a later chapter. The foregoing results are consistent with the finding that the incorporation of radioactive-labeled amino acids into cytosomal proteins of hippocampal neurons is elevated for 1 h immediately after tetanization (e.g. Bullock et al., 1990; Duffy et al., 1981; Fazeli et al., 1988; Frey et al., 1991a). This transient enhancement of protein synthesis coincides with the time window after tetanization during which the inhibition of protein synthesis prevents the initiation of late-LTP. Learning experiments have shown a second peak in the elevation of leucine incorporation 8 h after training (Lössner et al., 1982). Later experiments supported these results for hippocampal LTP, revealing an increase in radioactive-labeled proteins shortly after tetanization and a subsequent processing and incorporation into synaptosomal membranes and postsynaptic densities 8 h after tetanization (Bullock et al., 1990; Frey et al., 1991a). Gel autoradiographs have

identified a complex and time dependent pattern of changes in protein synthesis (Fazeli et al., 1993). The discoveries of first candidates of such PRPs are discussed in the chapter on 'synaptic tagging'. Although the first stage of protein synthesis takes place immediately after tetanization, further post-translational processing of proteins during early-LTP is required for the induction of late-LTP. Recent confocal imaging analysis from our institute directly demonstrated the dendritic synthesis of a probe protein and its fast degradation as a prerequisite of late-LTP (Karpova et al., 2006). Furthermore, in addition to processing of functional proteins it can be assumed that late-LTP induction also leads to the synthesis of regulatory proteins involved in the long-lasting regulation of PRPs via gene expression.

Although, there seems to be evidence that late-LTP depends on de-novo protein synthesis, we have started a re-investigation of this topic in intact animals. We found that very often the non-specific side-effects of the used translation inhibitors on baseline potentials and LTP-induction were ignored. This side-effect may have caused a decline of LTP, i.e. preventing late-LTP rather by non-specific effects than by effects on protein synthesis. We have now applied structurally different protein synthesis inhibitors up to 4 h before late-LTP induction to circumvent non-specific side-effects during LTP-induction in vivo. Preliminary results revealed that in the freely behaving animal, a distinct level of PRPs seems always to be present in the neurons. However, this level of pre-existing PRPs will be fine-tuned during plastic events by modulatory neurotransmitters and thus, resulting in long-lasting changes of synaptic efficacy. For example, a dopamine-dependent fucosylation of hippocampal proteins is necessary for both late-LTP and long-term memory storage (Angenstein et al., 1992; Jork et al., 1979; Krug et al., 1991).

2.3. Role of transcription

The synthesis of mRNA is a necessary step in the establishment of mammalian long-term memory (for review see Alkon et al., 1991; Matthies, 1974; Matthies et al., 1990a). Initial studies have shown no influence of the mRNA-synthesis inhibitor actinomycin D (Otani et al., 1989). Later work from the laboratory of Kandel (Nguyen et al., 1994) demonstrated that a distinct LTP in hippocampal slices in vitro was prevented after 1 to 3 h when the inhibitors were applied during tetanization. A side effect of the drugs on mechanisms involved in LTP generation could not be excluded. Since the question of whether mRNA synthesis takes place in LTP is crucial for understanding the mechanisms underlying late-LTP, we performed experiments not subject to these shortcomings (Frey et al., 1993b, 1996). The effect of the mRNA-synthesis inhibitors actinomycin D as well as 5,6-dichloro-1-ß-D-ribofuranosyl benzimidazole (DRB) was studied in two different systems, the CA1 in vitro and the DG in vivo. To avoid unspecific effects of the drugs on LTP-induction, the irreversible drugs were applied sufficiently before tetanization. Although, we found that the population spike was affected by the drugs, the more relevant factor for synaptic efficacy – the fEPSP – showed, if any, only a delayed weak effect after about 6–8 h. Unfortunately, animals treated with irreversible mRNA-synthesis inhibitors can only be investigated for about 12 h before baseline potentials decline, and the animals often die after 24 h. Recently, the results in the CA1 in vivo were verified by the Manahan-Vaughan-laboratory (Manahan-Vaughan et al., 2000) and with respect to the DG these data are supported by the Abraham group (Otani et al., 1989). Thus, in the intact animal, as well as in slices, late-LTP can be maintained at least for the first 8 h under distinct circumstances, i.e. with inhibited mRNA-synthesis but an intact protein synthesis machinery (Sajikumar et al., 2005b). In other words, the protein synthesis-dependent first 8 h of LTP are maintained by pre-existing mRNA whereas later stages may require additional regulation of PRPs by gene expression. In line with these observations, a recent study by Vickers (Vickers et al., 2005) showed that late-LTP in slices was prevented by translational inhibitors after about 1–2 h whereas mRNA-synthesis inhibitors were only effective in blocking LTP starting after about 4–6 h. This suggests the requirement of gene expression to maintain LTP. Whether, it is only required to refill stores of ''housekeeping'' proteins including the PRPs remains open.

3. Early- and late-LTP phases, transmitters and intracellular signaling

3.1. LTP-induction by glutamate receptors

After distinguishing early- and late-LTP on the basis of their different sensitivity to protein synthesis inhibition we and others were interested to identify the transmitter receptors and second messengers involved in the induction of early- and late-LTP. It was already known that normally, a temporary co-activation of the NMDA- and AMPA receptors during tetanic stimulation and a concurrent influx of Ca^{2+} into the postsynaptic cell via NMDA-receptor operated channels is necessary for LTP induction (Herron et al., 1986). The postsynaptic depolarization following AMPA receptor activation is needed to overcome the Mg^{2+} block of NMDA receptor-related channels. We demonstrated that both the blockade of NMDA receptors or the inhibition of calmodulin (CaM)-dependent enzymes eliminates LTP not only from the beginning, but also the occurrence of late-LTP (Reymann et al., 1988b, 1989). Therefore, the activation of NMDA receptors and CaM-dependent enzymes is necessary for the induction of both early- and late-LTP. However, since activation of NMDA-receptors alone results only in decremental LTP in CA1 neurons (Collingridge, 1985; Kauer et al., 1988), other glutamatergic or non-glutamatergic receptors and coupled second messengers must be involved at least for the induction of processes enabling the late-LTP. If the early-LTP (<4 h) is not dependent on newly synthesized proteins it might be based on modifications of pre-existing proteins, e.g. by phosphorylation by protein kinases. Indeed, it was shown independently for the DG in vivo (Lovinger et al., 1987) and for the CA1 in vitro (Reymann et al., 1988b,c), that an inhibition of protein kinase

C (PKC) leads to loss of LTP after 1–2 h. Subsequent evidence with intracellular recording and delivery of inhibitors revealed that both postsynaptic PKC and CaMKII are required for the induction of LTP (Malinow et al., 1989).

3.2. Metabotropic glutamate receptors (mGluRs) and protein kinase C (PKC)

Since activation of NMDA-receptors usually results only in decremental and PKC-independent short-term potentiation other receptors and second messengers must be involved in the induction of LTP. After the mGluRs were identified as the glutamate receptor subtype also involved in PKC activation we and others could demonstrate that antagonists of mGluRs block distinct forms of LTP (Anwyl, 1999; Bashir et al., 1993; Behnisch and Reymann, 1993; Bortolotto and Collingridge, 1993, 1998, 2000; Reymann and Matthies, 1989; Riedel et al., 1995; Riedel and Reymann, 1993; Wilsch et al., 1998). Under certain conditions, e.g. within the DG in vivo, mGluR antagonists blocked LTP as expected with a similar delay as with PKC inhibitors. Complimentarily, a STP in the DG, induced by a weak tetanus, can be prolonged into LTP by application of mGluR agonists in between a time window of 30 min following the tetanus (Manahan-Vaughan and Reymann, 1996). This suggested the involvement of mGluRs in mechanisms of LTP complementary to NMDA receptor activation. Interestingly, both mGluR-antagonists and PKC-inhibitors suppress LTP more rapidly than the protein synthesis inhibitor anisomycin, usually by 1–3 h. Therefore, mGluRs and kinases as PKC seem to be important for at least the induction of a further, intermediate LTP stage (Fig. 1).

The activation of group I mGluRs stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis, resulting in the liberation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. Whereas IP3 triggers the release of calcium from endoplasmatic IP3-sensitive calcium stores, diacylglycerol was reported to activate PKC which on its part phosphorylates several substrates including NMDA receptor subunits resulting in a higher NMDA receptor-mediated Ca^{2+}-influx (Ben-Ari et al., 1992; Fitzjohn et al., 1996). One should also mention that the mGluR1/5 antagonist MCPG prevents the LTP-induced transient translocation of PKC gamma, but not that of PKC alpha/beta (Angenstein et al., 1999). Interestingly, many PKC isoforms, other kinases and their phosphorylation substrates have been shown to be important for LTP. Interestingly, glutamate receptors (GluR) themselves are phosphorylation substrates. Thus, GluR1 is phosphorylated by CaMKII, PKC and tyrosine kinases (for review: Soderling and Derkach, 2000), whereas PKA (see below) phosphorylates GluR6 and increases the glutamate response (Raymond et al., 1993).

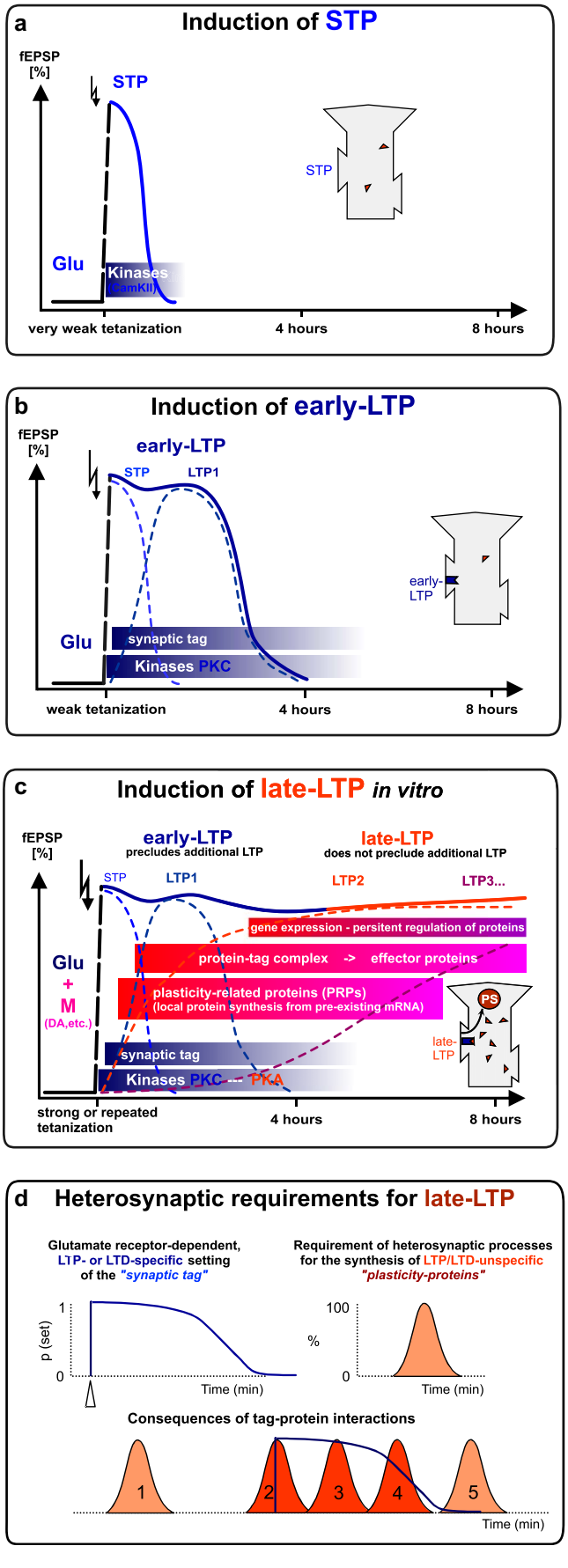
3.3. LTP expression by postsynaptic AMPA receptors

Similar to the test response before tetanization, the potentiated synaptic response seems also to be mediated mainly by the AMPA receptor, since it is blocked by respective antagonists (Davies et al., 1989). Thus LTP expression might be due to an increase in AMPA receptor function. Following tetanization, a slowly developing increase in AMPA-sensitivity was detected, which paralleled the conversion from STP to early-LTP. If a NMDA or group I mGluR antagonists or a PKC inhibitor was added to the perfusion medium during or immediately after tetanization, STP was not sustained and there was no increase in AMPA sensitivity (Davies et al., 1989; Reymann et al., 1988b; Sergueeva et al., 1993). This implies that the mGluR-PKC pathway is required to generate but not to maintain both the increase in AMPA receptor function and LTP1. With intracellular recordings the increase in AMPA sensitivity was already seen between the first five minutes after the tetanus (Fedorov et al., 1997). A very recent study has now shown that CA1 neurons very rapidly incorporate GluR2-lacking calcium-permeable AMPA receptors after tetanization (Plant et al., 2006) which could transiently sustain STP for 25 min. These receptors seem to be replaced by GluR2-containing AMPA receptors after approximately 25 min after LTP induction, which then allows at least LTP1 expression.

3.4. Non-glutamatergic ''reinforcing'' transmitters

Late-LTP in CA1 and DG can be prevented or simulated by inhibitors and activators, respectively, of opiods, aminergic, or metabotropic glutamatergic receptors coupled to the cAMP cascade (for review see Frey, 2001, 1997). We therefore suggested that late-LTP requires concomitant activation of different transmitter systems (Frey, 1997; Frey and Morris, 1998a; Matthies et al., 1990b). The typical LTP experiment, involving a brief period of high frequency stimulation (or, with intracellular recording, pairing of pre- and postsynaptic activation), overlooks the more likely situation in the behaving organism, where the activation of a population of glutamatergic synapses of an individual CA1 cell is likely to be accompanied by a dynamic activation of non-glutamatergic heterosynaptic inputs. The tetanization used in LTP studies, involving simultaneous field activation of hundreds of fibers, activates more than one kind of neurotransmitter input, and it is that cooperative action of inputs that induces late-LTP. We therefore favor the notion that a time-dependent convergence of two or more events is required for late-LTP.

Given the effects of dopamine on learning (Flood et al., 1980), we investigated whether dopamine is simultaneously released during LTP induction in the CA1 region of the hippocampus, and whether it is required for late-LTP (Frey et al., 1990, 1991b). The hippocampus is innervated by dopaminergic fibers that course through the mesolimbic pathway (Baulac et al., 1986), and there is evidence of the expression of the D5 receptor in CA1 pyramidal cells. The D5 receptor is related to the D1 dopamine receptor that is coupled to adenylyl cyclase. We demonstrated that, in addition to glutamate and possibly other neurotransmitters, dopamine levels increase during conventional LTP induction (Frey et al., 1990). To determine whether dopamine might be the additional activator necessary for late-LTP, we showed that it plays a crucial role in the initiation of the mechanisms responsible for late-LTP in the



apical dendrites of hippocampal CA1 neurons. When specific inhibitors of the dopaminergic D1 and D2 receptors were administered during tetanization, late-LTP was prevented (Frey et al., 1990, 1991b). The involvement of aminergic modulation of LTP has been proposed repeatedly (for review see Frey, 1997). However, it has been suggested that the influences of these transmitters are only modulatory. We have demonstrated that activation of dopaminergic inputs to hippocampal CA1 neurons plays a critical role, perhaps acting as a cellular switch to establish the late phase of LTP 2, since the time course of LTP decay after dopamine antagonists is similar to that seen after anisomycin. Dopamine-mediated glycoprotein processing by fucosylation is also necessary for late-LTP to occur (Angenstein et al., 1992). Other observations have demonstrated that the transient application of dopamine alone initiates a delayed increase in both the population spike (Gribkoff and Ashe, 1984) and the synaptic response, i.e., the field-EPSP (Sajikumar and Frey, 2004a). Later, it was shown that repeated application or transient application (Frey and Morris, 1998a; Huang and Kandel, 1995; Sajikumar and Frey, 2004a) of a D1/D5 receptor agonist in CA1 synapses in adult animals induces a delayed potentiation whose time course is similar to that found after administration of dopamine. These events may simulate the late phase of LTP. Similar results have been obtained by the application of norepinephrine in the DG (Stanton and Sarvey, 1985). In addition, our findings on the requirement of dopamine for late-LTP in apical dendrites of hippocampal CA1 neurons has recently been supported by

Fig. 1. Schematic illustration of separate phases of synaptic long-term potentiation based on the distinction of several putative mechanisms involved. The STP curve (a) corresponds to the potentiation remaining after PKC inhibition and represents an initial PKC-independent phase of potentiation. The LTP1 (b) curve was obtained by subtracting the polymyxin B-resistant potentiation and the LTP2 curve (see below) from the tetanized, non-treated group. LTP1 was blocked by PKC-inhibition. STP and LTP1 represent early-LTP (blue). The LTP2 curve is the difference between the non-treated and the anisomycin-treated group and represents a PKC- and protein synthesis-dependent late-LTP (red, (c)). Protein synthesis necessary for LTP2 starts earlier than the effect of anisomycin becomes visible, i.e. shortly after tetanization while STP and LTP1 are still in progress. Furthermore, tetanization and the induction of STP, LTP1, LTP2 involves the activation of characteristic enzymes. In the case of early- or late-LTP-induction a synaptic tag is set, whereas the induction of late-LTP requires the synthesis of PRPs and their specific processing for LTP2 and LTP3, the latter of which may involve the expression of genes. Note that the posttetanic potentiation lasting approximately 1 min is not considered in this scheme. Early-LTP requires the activation of glutamatergic receptors (Glu) whereas late-LTP in addition to glutamate also depends on the associative and synergistic activation of modulatory transmitter systems (M), such as dopamine in apical CA1 dendrites. Functionally, early-LTP precludes the further induction of LTP in the synaptic input whereas the establishment of late-LTP again allows the synaptic input to react with plasticity changes, i.e. it does not preclude the additional induction of early-LTP. In the right part of (a)–(c), hippocampal neurones are schematically presented. In (a) STP was induced in a synaptic input which did not affect the setting of a synaptic tag or the synthesis of PRPs. If early-LTP was induced (b), the synaptic tag (blue symbol) was activated whereas in the case of late-LTP (c), in addition to the tag, also the synthesis of PRPs was induced (PS, red symbols). The graphs schematically represent the change of the fEPSP related to pre-tetanic values. (d) represents the requirements and the dynamics of tags (blue) and PRPs (red) to induce late-LTP (see text for details; (d) adopted from Frey and Morris, 1998b).

studies from the Morris laboratory (O'Carroll and Morris, 2004).

Because the D1 receptor stimulates adenylyl cyclase and the D1-receptor blocker efficiently prevents late-LTP (Frey et al., 1991b), we explored whether the formation of cAMP is increased during LTP and whether late-LTP is dependent on cAMP-dependent protein kinase (PKA) or other cAMP-dependent processes. We found that the formation of cAMP was indeed transiently increased only after a threefold (i.e., repeated) tetanization used for LTP induction. This is the stimulation protocol required for reliable late-LTP (Reymann et al., 1985; Sajikumar et al., 2005b). This short-term elevation of cAMP was blocked by a D1/D5 antagonist, SCH 23390 and the NMDA-receptor blocker AP-5. Our observations reveal that application of PKA inhibitors are ineffective in blocking STP and LTP1 but do block LTP2–3 (Frey et al., 1993a; Matthies and Reymann, 1993). Transient application of a membrane-permeable cAMP analog or of other PKA activators initiated a delayed potentiation that seemed to simulate late-LTP (Frey et al., 1993a; Pockett et al., 1993; Selbach et al., 1997). These results suggest that the cAMP-dependent activation of PKA is required for late-LTP, probably LTP2 and LTP3.

3.5. The multi-stage model of LTP

Although the multiple phase model of LTP is now widely accepted, questions were raised whether the different time course and duration of LTP rather reflects only quantitative differences depending on tetanization strength. Indeed, experimental protocols used to elicit consolidation in LTP are usually based on increasing the intensity of inputs. For example, one 100 Hz tetanization is used for the induction of early-LTP and three spaced tetanizations are required to elicit the late-LTP (Huang and Kandel, 1994; Reymann et al., 1985). However, later studies revealed that also a single tetanus can lead to late-LTP if its stimulation intensity and the number of stimuli per tetanus are sufficiently high (Bortolotto and Collingridge, 2000; Sajikumar et al., 2005b). Obviously, the stimulation strength determines whether heterosynaptic inputs become activated by field stimulation which in turn has been shown to be required to induce late-LTP (see below). We prefer to distinguish between different phases of LTP instead of distinguishing just the induction and maintenance process as is the case with many other authors. The mechanisms which enable the late stages also have to be induced, probably as early as those underlying early-LTP. Interestingly, late-LTP can, in certain circumstances, be induced very shortly after the expression of the enhanced synaptic change that constitutes early-LTP. Thus, early-LTP might be a staging post on the way to a longer lasting change; alternatively, it could be a separate or parallel phase of LTP expression that is not required for late-LTP (see also chapters on 'synaptic tagging' and implications). Further evidence for a mechanistically separate early phase comes from findings demonstrating that after saturated LTP has been induced, an additional potentiation can be evoked by turning down the test intensity. Normally, it was thought that the induction of LTP precludes the further induction of LTP in the same input. However, we had shown in 1995 (Frey et al., 1995) that in a time window of approximately 3 h, i.e. during a period where early-LTP is expressed in these inputs, only STP can be induced, whereas later, beyond 4 h also a full-length LTP can be generated by further tetani. Thus, only during the maintenance of early-LTP the further induction of longer lasting LTP is precluded. However, if early-LTP was transformed into late-LTP, the same synapses further allowed LTP induction again, thus the induction of LTP was not precluded anymore. Even if the same principal expression mechanism as e.g. an increase in AMPA receptor function would be used for different LTP phases, different mechanisms seem to underlie the different phases. The main message is that LTP consolidation takes place not only as merely a function of more-of-the-same (i.e., transmitter), but also of coincidence of several transmitter inputs with several second messengers coupled to them. This corresponds with the natural situation, in which much of the information that we consolidate as long-term memory is distinguished from the non-consolidated one by virtue of context and association rather than intensity.

From all these results we proposed as early as in 1987 at a hippocampus-meeting in Hungary that what previously has been termed LTP is not a unitary phenomenon, but consists of at least three phases: i) STP, a decremental phase dependent on NMDA receptor activation and Ca^{2+}/calmodulin; ii) LTP1, an intermediate phase which depends on mGluR activation and phosphorylation of PKC- and CaMK-substrates; and iii) LTP2, a long-lasting component which requires the synthesis of proteins (Reymann et al., 1988c). As discussed before, there is some indication for an additional LTP3 phase, which depends on gene transcription (Fig. 1; see also Bliss and Collingridge, 1993; Frey et al., 1988; Krug et al., 1984; Matthies et al., 1990b; Otani et al., 1989; Reymann et al., 1988a,c). It seems likely that second messengers coupled to the above mentioned additional neurotransmitter receptors (mGluR, DA and others) such as postsynaptic PKC, PKA, CaMKII and MAPK act in parallel and/or in series to initiate the different LTP-phases. The different signal transduction systems are probably interacting at the level of transcription factors and activators initializing the synthesis of relevant proteins. Phosphorylation of other proteins might be necessary specifically to mark (tag) previously activated synapses before newly synthesized proteins arrive at synapse for reconstruction.

4. Synaptic tagging

4.1. Input-specificity via 'synaptic tagging' and associative properties

How pre-existing or newly synthesized PRPs interact with specific, activated synapses expressing LTP (or, also LTD, e.g. see Sajikumar and Frey, 2004a), but not with inactive synapses, is fundamental to the synapse-specificity thought critical for information processing and memory formation. We explain synaptic input-specificity by the concept of 'synaptic tagging', in which newly synthesized PRPs activated by

heterosynaptic interactions bind to recently potentiated, glutamatergic 'tagged' synapses thus maintaining LTP and input-specificity (Frey and Morris, 1997, 1998a,b). We had also shown that stimulation that normally leads to early-LTP could also induce late-LTP if a separate pathway had been strongly tetanized within a specific time window. Thus tetanization of a pathway can induce a LTP with variable persistence as a function of the prior history of activation of the neuron (Frey and Morris, 1998b). The tag is transiently active with an expected half-life of about 30 min in the intact animal. It has been also suggested that the PRPs are characterized by a specific, relatively short half-life of about 1–2 h (see for review Korz and Frey, 2004; Sajikumar et al., 2005b). Only in the event when both processes, the synapse-specific tag as well as PRPs are available, then the two can interact and transform early- into late-LTP at the stimulated synapses (see Fig. 1d; Frey and Morris, 1998b). The existence of tag- and PRP-dynamics therefore, determine an effective, functionally important time window during which a normally transient form of functional plasticity can be transformed into a long-lasting one. 'Synaptic tagging' can therefore also explain how a strong tetanus, producing protein synthesis-dependent LTP (late-LTP) in one pathway, can prolong the potentiation in an independent, weakly tetanized pathway that would normally have produced only early-LTP, however with a tag set (Frey and Morris, 1998b).

The late-associative time window is not only determined by the mechanistic half-life of tags and PRPs. Synaptic tags or tag-complexes can not only be passively deactivated by cellular degradational processes but also actively through distinct electrical stimulation such as by depotentiating stimuli within 5–10 min after the setting of the tag by specific stimulation (Sajikumar and Frey, 2004b; Stäubli and Scafidi, 1999).

With respect to heterosynaptic, neuromodulatory requirements to induce late-LTP, there is now growing evidence that in adult brain non-glutamatergic inputs, such as dopaminergic ones in the apical dendritic layer of CA1 pyramidal neurons, modulate the availability of PRPs in synergism with NMDA-receptor function, whereas the glutamatergic input alone mainly mediates the maintenance processes of LTP during early-LTP as well as the setting of the tag (for review see Frey, 2001). However, synergistic interactions with neuromodulatory inputs during establishment of early-LTP as well as setting the tag can also not be ruled out.

During the last few years our results on tagging were verified in various laboratories (Adams and Dudek, 2005; Barco et al., 2002, 2005; Dudek and Fields, 2002; Kauderer and Kandel, 2000; Young and Nguyen, 2005), and it was also shown that analog tagging processes can also occur in invertebrates' neurons (e.g. Martin et al., 1997). Furthermore, tagging was not only described for LTP but also LTD in mammals (Kauderer and Kandel, 2000; Navakkode et al., 2005; Sajikumar et al., 2005c; Sajikumar and Frey, 2004a) characterized by similar cellular and functional properties (Sajikumar and Frey, 2004a). Recently, the synaptic tagging model has been expanded to include functional interactions between LTP and LTD, referred to as 'cross-tagging' (Sajikumar et al., 2005c; Sajikumar and Frey, 2004a). 'Cross-tagging' describes the capability of late-LTP/late-LTD in one synaptic input (S1) to transform the opposite, protein synthesis-independent early-LTD/early-LTP in an independent synaptic input (S2) into its long-lasting form (Sajikumar and Frey, 2004a). Cross-tagging not only expands the repertoire of functional interactions between different synapses and afferent pathways, but also raises the following fundamental questions:

1) Cross-tagging requires that the transient synaptic tag must mark an activated synapse specifically for either LTP or LTD. Therefore the question arises, which molecules make tags LTP- or LTD-specific?
2) Cross-tagging studies should allow searches for the specificity of the tag-complex within determined brain structures. We have recently shown that PRPs can be process-specific for LTP in apical dendrites of CA1 pyramidal neurons (i.e., protein kinase Mζ (PKMζ); a persistently active PKC isoform that is synthesized during long-term potentiation and required for its maintenance (Ling et al., 2002)), as well as process-unspecific or regulatory (such as the PDE4B3; see Sajikumar et al., 2005c). Can further cross-tagging studies also identify LTD-specific molecules and can differences between different brain (sub)-structures be determined?
3) If PRPs are not restricted to a local synaptic site one can ask whether their protein synthesis is restricted to local dendritic compartments (Kelleher et al., 2004b; Martin et al., 2000; Steward and Schuman, 2001) or can the synthesis of PRPs affect distant synapses neuron-wide?

The latter question addresses the fundamental issue as to whether distinct functional neuronal compartments exist, or if induction of a late plastic event affects the entire pyramidal neuron. First evidence for functional compartmentalization has been recently presented by the Kandel laboratory (Alarcon et al., 2006).

4.2. Molecules mediating synaptic tags and the identity of PRPs

With respect to the first question, we have started to search for key-molecules and found that the induction of late-LTP in apical CA1-dendrites sets a process-specific synaptic tag that is mediated by calcium-calmodulin-dependent-kinase II (CaMKII), whereas induction of late-LTD sets tags whose process-specificity is mediated by mitogen-activated kinases (MAPKs, i.e., MEK-dependent extracellular-signal regulated kinases: ERKs) (Sajikumar et al., 2005a).

It is well known that both CaMKs and MAPKs are multi-functional enzymes important for LTP and LTD. Both enzymes are required for the induction of the long-lasting, protein synthesis-dependent forms, late-LTP and late-LTD, at least in area CA1 (but see Cooke et al., 2006). Besides these general functions, however, we have recently shown that MAPKs have a LTD-specific function, i.e., marking synaptic tags in a LTD-specific way to capture PRPs and thus to

transform early- into late-LTD. MAPKs, although involved in other processes required for both late-LTD and late-LTP, are not required for tagging processes during LTP. Interestingly, very similar results for MAPK-requirements were described for different forms of memory in Aplysia (Sharma et al., 2003). In contrast to MAPKs, CaMKII is specific for processes occurring at LTP-tags in synapses of apical dendrites in pyramidal CA1 neurons, i.e., it is required to capture or to process PRPs to ensure the transformation of early- into late-LTP (Sajikumar et al., 2005a). It still remains to be investigated, if LTD also depends on its process-specific PRPs and if different inputs into pyramidal neurons as well as other brain structures are characterized by the expression of structure-specific key-proteins mediating the tag and/or PRP-interactions. One hint comes from recent work where it was shown that CaMKII-activity is not required for late-LTP in the DG (Cooke et al., 2006).

MAPKs as well as CaMKs, may exert a general role for the synthesis of PRPs throughout the prolonged maintenance of the plastic events, most likely at restricted local dendritic sites, at least in CA1 (Kelleher et al., 2004a; Miller et al., 2002). This is supported by a persistent long-lasting activation of MAPKs during different phases of CA1-LTP (Ahmed and Frey, 2005), which is quite similar to the prolonged activation of CaMKII seen in some (Ahmed and Frey, 2005; Giovannini et al., 2001) but not other laboratories (Lengyel et al., 2004). The differences with respect to the prolonged phosphorylation of CaMKII could be explained by the different preparations, the induction of LTP-like phenomena (see Sajikumar et al., 2005b) or the limited specificity of the drugs used. The MAPK- as well as CaMK-family may exert various effects during plastic events being compartment-specific (Dudek and Fields, 2001), or more generally, in neuron-wide actions such as gene expression, as well as other processes which we do not yet fully understand. One interesting observation with respect to the multi-functional role of CaMKs during LTP has been recently described in transgenic mice with an enhanced expression of CaMKIV: these animals showed an enhanced NMDAR-dependent form of LTP caused by an increased incorporation of silent receptors into membranes (Marie et al., 2005). This result suggests that for a prolonged maintenance of LTP beyond 8 h, i.e., for a maintenance which requires both gene expression and protein synthesis (see LTP3 in Fig. 1c) (Frey et al., 1996; Manahan-Vaughan et al., 2000; Nguyen et al., 1994), CaMKIV-dependent processes may be directly involved in the generation of plasticity effectors maintaining LTP for prolonged periods at least in area CA1.

4.3. Restriction of associative properties to neuronal compartments

Hippocampal CA1 neurons receive their inputs in apical and basal dendrites from hippocampal neurons and from various extrahippocampal structures, including modulatory inputs from the amygdala, ventral tegmental area and many other brain regions (Amaral and Witter, 1998). Considering the intact organism, an associative interaction between information from system-wide inputs must be expected. Inputs containing spatial, contextual, relational information or information containing ‘‘system-relevant decisions’’ must be therefore associatively processed during the cellular formation of a memory trace within distinct neuronal nets. Such inputs can arrive as modulatory information integratively processed at the given neuronal compartment, e.g., by mechanisms of synaptic tagging and cross-tagging. However, apical and basal dendrites do not just differ with respect to their innervation but also in their morphology and physiological properties (Leung and Shen, 1995; Pike et al., 2000). Thus, many questions arise from these differences, such as whether system-relevant information processing and integration is restricted to single synapses, or to dendritic compartments, or to the whole neuron.

Recent results suggest that instead of the formation of a memory trace within a single synapse, functional synaptic populations within a dendritic compartment exist. These synaptic populations with their related intracellular messenger machinery could act as integrative units within neural networks (Engert and Bonhoeffer, 1997; Govindarajan et al., 2006; Kelleher et al., 2004b). Our hypothesis is that processes of synaptic tagging and cross-tagging should be locally restricted to more efficiently and reliably control systematic and organism-relevant information processing and computation (Frey, 2001; Polsky et al., 2004; Sajikumar et al., 2005c). Until now we had only investigated these processes in different synaptic inputs in the apical CA1-dendrites. Recently, Young and Nguyen (2005) have expanded this local restriction and described the first results of a negative interaction between stratum oriens stimulation and the availability to induce late-LTP in stratum radiatum of hippocampal pyramidal CA1 neurons. Dudek and Fields (2002) have shown that somatic action potentials can ‘‘prime’’ synaptic tagging at apical dendrites. In contrast to our hypothesis, the latter results suggest neuron-wide processes of synaptic tagging and cross-tagging. Recently, the Kandel-laboratory has shown that orthodromic induction of CA1-LTP is restricted to local, functional compartments in general. However, if stimulation is strong enough, a distinct spread of plastic events between compartments was described (Alarcon et al., 2006). In parallel, we had replicated these results and have expanded these studies by showing that the induction of LTP as well as LTD and their interactions, such as cross-tagging, is also restricted to functional compartments (Sajikumar et al., 2005a). Further studies are required to figure out the properties and functions of such local and intercompartmental interactions.

5. Physiological reinforcement of LTP

5.1. Structural and behavioral reinforcement

As we have shown in the previous chapters, the induction of hippocampal late-LTP requires the associative activation of heterosynaptic inputs, such as the activation of glutamatergic and dopaminergic receptors in area CA1 or glutamatergic and noradrenergic or muscarinergic receptors in the dentate gyrus (Frey, 2001). In searching for the physiological

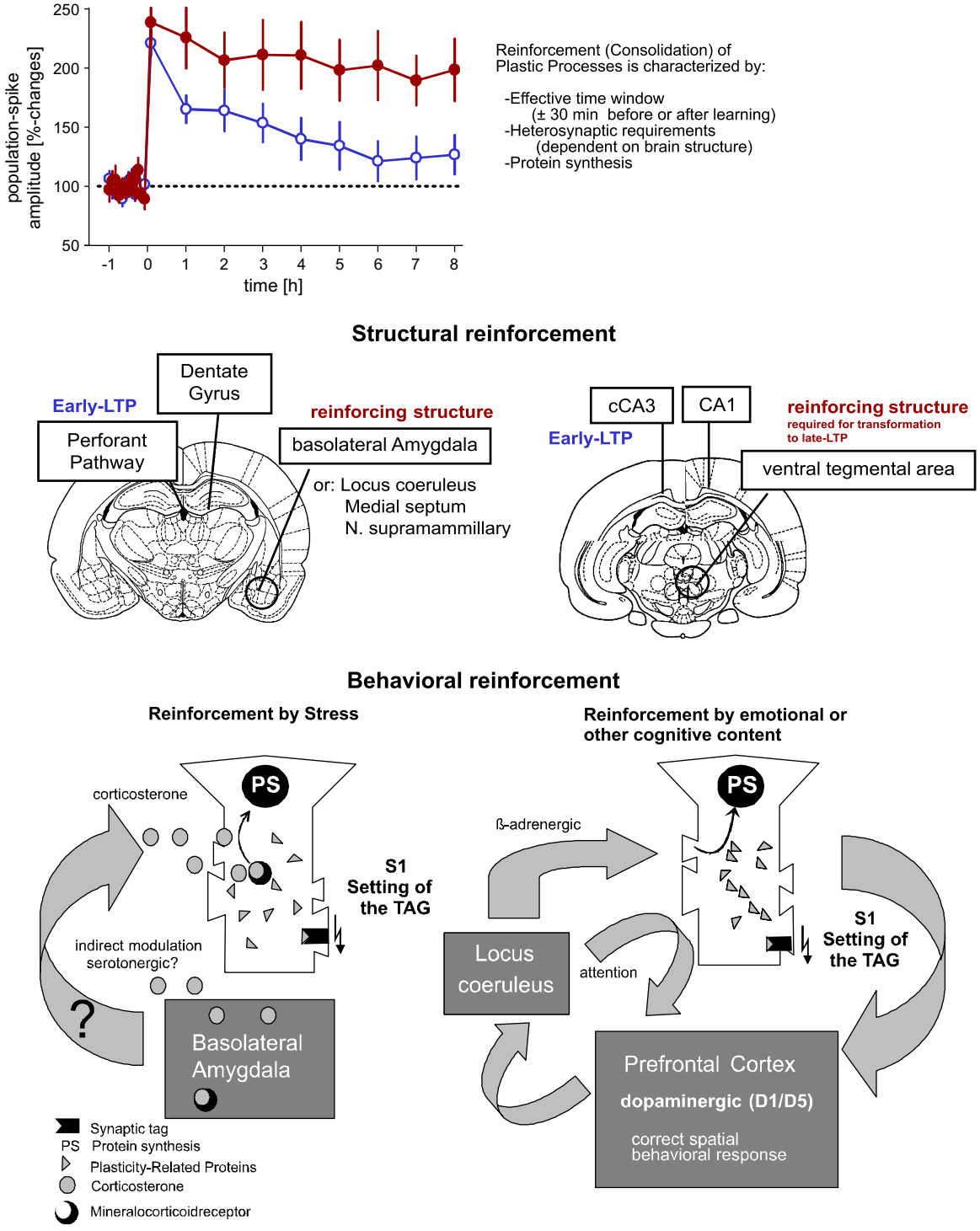


Fig. 2. Structural and behavioral reinforcement of LTP in vivo. Hippocampal early-LTP (blue curve in the upper graph, which sets a synaptic tag) can be reinforced into late-LTP (red curve) by the associative heterosynaptic stimulation of structure-specific, modulatory brain structures provided in the middle schemes for the DG-LTP or CA1-LTP, respectively. Similarly as to structural reinforcement, distinct behavioral paradigms (lower schemes) with different content can exert reinforcing effects on early-LTP (blue curve in the upper graph, which sets a synaptic tag at these inputs), thus transforming it into late-LTP (red curve) by heterosynaptic, associative interactions. The latter, most likely regulate the synthesis of PRPs which then interact with the synaptic tags, thus expressing late-LTP. High stress conditions (lower left scheme) revealed that a 2-min-swim in a water tank 15 min after a weak tetanus also prolongs early-LTP up to 24 h in naïve animals. The prolongation is dependent on protein synthesis but not on β-adrenergic activation. Using such a high stress paradigm, LTP is maintained by corticosterone induced activation of mineralocorticoid receptors (MR). Because the activated MR-complex acts as a transcription factor (circles) and modulates the activity of other factors, resulting translational products may include PRPs (triangles) being involved in the maintenance of LTP. Reinforcement (lower right scheme), such as by novelty detection typically is a rapid process that occurs after a single exposure to the arousing situation and can be blocked by a brief pre-exposure to the stimuli making them familiar. The term "emotional tagging" has been introduced to characterize this form of LTP-reinforcement related to the rapid consolidation of relevant new information (Richter-Levin and Akirav, 2003). LTP can also be protein-synthesis dependently reinforced by mastering a spatial task in

significance of the simultaneous activation of two different inputs, it should be noted that dopaminergic neurons are assumed to mediate, in part, the effect of biologically significant reinforcing stimuli during learning (Bischoff, 1986). Normal dopamine functioning also appears necessary for the establishment and maintenance of conditioned reinforcement, or incentive learning (Beninger, 1983). Thus, prolonged enhancement of synaptic efficacy during the processing of specific sensory information becomes understandable. Dopaminergic facilitation of learning is consistent with observations from Packard and White (1989), who demonstrated a role of dopaminergic receptors in memory facilitation on learning tasks sensitive to both hippocampal and caudate lesions. The question arises, whether this requirement of the joint activation of heterosynaptic inputs, i.e., of extrahippocampal structures in addition to intrahippocampal processes, can be detected during an animal's behavior and acquisition of information in combination with the induction of plasticity processes.

So far, only few data exist linking LTP as a physiological phenomenon involved in normal animal behavior (see above). In these studies it was expected that specific behavior may interfere with evoked population potential recordings, i.e. it was shown that novelty, for instance, can facilitate evoked potentials for a distinct time. This approach requires that behavior may drive a whole population of neurons within one direction. The question remains, if such a dramatic shift in the excitability of a well-known structure corresponds to a physiological processing of relevant information. Since 1995 we have tried a different approach taking into account the newly identified properties of late-LTP, i.e. its late associative properties. In a first approach study, our laboratory evaluated the effect of behavioral and motivational states, which is known to affect learning, on LTP induced by strong or weak tetanic stimulation in the DG of freely moving rats (Seidenbecher et al., 1995, 1997). The strong tetanization produced a ''saturated'' LTP, which lasted more than 24 h, and was not significantly different between the behavioral states. However, LTP induced by weak tetanization did differ across behavioral states. LTP which normally lasted only 5–7 h was clearly prolonged to more than 24 h if the water-deprived rats were allowed to drink during or up to 30 min after the tetanus. Similar results were obtained with application of foot shock instead of water. We concluded that both, appetitive and aversive stimuli act within a certain time window as reinforcers to consolidate LTP in a similar manner as in memory formation. Under low stress conditions such as during novelty detection or water delivery in water deprived rats, the transformation of early-LTP into late-LTP in the DG depends on the activation of β-adrenergic receptors (Seidenbecher et al., 1997) and on protein-synthesis (Straube et al., 2003). It seems plausible that during our strong field stimulation, the transmitter systems involved in this reinforcement (such as norepinephrine and dopamine) seem to be already activated by the electrical stimulation itself.

In later experiments we induced either in the CA1 or the DG a normally transient form of LTP and afterwards trained the animal with distinct behavioral paradigms within a time window, for which synaptic tagging was shown, expecting heterosynaptic influences on the normally transient potentiation. That means that behavioral content was investigated with respect to its ability to modify/prolong a normally transient form of plasticity. Our studies included phenomena which we investigated under the working hypothesis of: (1) 'structural reinforcement' or 'tagging'; and (2) 'behavioral reinforcement' consisting of 'emotional' and 'cognitive tagging'. The first topic investigates forms of plasticity in the DG and in the hippocampal CA1 region (by simultaneous recording of the population spike and the field-EPSP) in freely moving animals in response to direct stimulation of modulatory transmitter systems within distinct time intervals (Fig. 2, middle schemes). As mentioned above, the neuromodulatory systems as well as the timing for stimulation have been taken from our results in vitro, as already discussed in the previous chapters. Emotional tagging represents the possible reinforcement of electrically induced, normally transient forms of hippocampal LTP/LTD by emotional content, e.g. by swim stress, which is presented within similar time windows around LTP/LTD-induction as during structural tagging. Cognitive tagging represents a process, in which transient forms of plasticity are transformed into long-lasting forms by cognitive contents during learning, such as during reward-related learning in holeboard experiments or during specific spatial watermaze-training (Fig. 2, lower schemes).

5.2. Structural reinforcement: identifying modulatory brain structures

Specific associative electrical stimulation of modulatory systems within a distinct time interval can transform a protein-synthesis-independent early-LTP into a protein-synthesis-dependent late-LTP in the rat DG (Frey et al., 2001; Straube and Frey, 2003). The influence on hippocampal plasticity is thereby characterized by subtle structure-specific properties. So far, it has been shown that the basal lateral amygdala, the medial septum, the locus coeruleus as well as the nucleus supramammillaris (SuM; unpublished work) interfere specifically with DG-early-LTP. Depending on specific time windows within 30 min before or after DG-tetanization and the form of stimulation (e.g., high- or low-frequency stimulation) resulted in a reinforcement of early- into late-LTP (Fig. 2).

Since most of LTP-experiments in vitro were performed in the CA1-region of the hippocampus, the necessity to investigate processes in CA1 region in the intact animal cannot be replaced by DG-recordings. Thus, we were also interested in CA1 experiments in vivo which would allow us to work in

a holeboard. In contrast to emotional reinforcement this cognitive reinforcement appears only after repetitive training in the same environment and is related to the formation of a long-lasting spatial reference memory, suggesting that proteins being involved in memory consolidation also contribute to the maintenance of hippocampal LTP. Different cellular signalling is involved in emotional and cognitive reinforcement of LTP. Adopted from (Korz and Frey, 2004).

a similarly flexible manner as in vitro, i.e. to investigate associative interactions of modulatory inputs on different forms of LTP/LTD. During the last years we optimized stimulation and recording parameters for the CA1 which now have led to very reliable simultaneous recording of the field-EPSP and the population spike in freely moving animals for several weeks. To induce distinct forms of LTP we normally stimulate contralateral hippocampal substructures to avoid electrical-unspecific interactions with the ipsilateral site and to guarantee the stimulation of separate synaptic inputs (Hassan et al., 2006) and combine that with the activation of modulatory brain-structures. To study whether CA1-early-LTP in the intact animal can also be transformed into late-LTP we have first studied the effect of the electrical activation of the ventral tegmental area (VTA, a structure which innervates the CA1-region with dopamine) at different time points after tetanization of the CA1 (Fig. 2; unpublished work). Early-LTP was transformed into late-LTP in a protein-synthesis and D1-receptor-dependent manner when the VTA was stimulated within 30 min after CA1-tetanization. These results in vivo resemble our in vitro results.

5.3. Behavioral reinforcement: identification of content and interaction of modulatory brain structures

5.3.1. Reinforcement by high stress

Swim stress in the watermaze can result in the reinforcement of early- into late-LTP in the DG (Korz and Frey, 2003). This particular swim stress resulted in an enhanced phosphorylation of MAPK and CaMKII and the reinforcing effect on LTP by swim stress was mediated by mineralocorticoid receptors (MR) but not corticosterone or norepinephrine, muscarinergic or dopaminergic receptors. Our current working hypothesis is that strong emotional content such as high stress up-regulates the synthesis of PRPs in a more general way most likely through gene expression (for review see Korz and Frey, 2004, Fig. 2).

5.3.2. Reinforcement by other emotional or cognitive components

Reinforcement, such as by novelty detection typically is a rapid process that occurs after a single exposure to the arousing situation and can be blocked by a brief pre-exposure to the stimuli making them familiar. The term ‘‘emotional tagging’’ has been introduced to characterize this form of LTP-reinforcement related to the rapid consolidation of relevant new information (Richter-Levin and Akirav, 2003). Recently, we had shown that early-LTP can be transformed into late-LTP when early-LTP is associated with holeboard training in a specific manner, i.e., critical time windows within the learning curve determine the ability for reinforcement (Korz and Frey, 2004; Uzakov et al., 2005). During holeboard training, a significant improvement of learning and the consolidation of the reference memory after seven training trials can be detected. If the induction of early-LTP was related to that 7th trial (i.e., tetanization of the DG 15 min before the 7th trial) reference memory consolidation as well as LTP-reinforcement by holeboard-learning was detected, which was protein-synthesis-dependent. These results suggest that the same newly synthesized proteins involved in the formation of reference memory are also required for LTP maintenance. Memory consolidation in this model, as well as LTP-reinforcement were dependent on the heterosynaptic activity of noradrenergic as well as dopaminergic inputs. Recently, we were also able to describe a similar cognitive reinforcement of LTP by watermaze training after 5 training trials. After 10 trials, i.e. at a time point where the reference memory is already consolidated, no reinforcement was observed. However, if we removed the platform and the animals searched for it, a reinforcement of LTP appeared again (Korz and Frey, 2004).

6. Conclusions

6.1. Functional implications of compartmentalized plasticity

If we try to draw a more general picture when describing LTP-reinforcement processes in the intact animal we can ask the question: how is dendritic compartmentalization of synaptic plasticity related to mammalian behavior? We favor a model that is supported by our work in intact behaving animals (Frey et al., 2001; Korz and Frey, 2004): Under non-life-threatening situations, a restricted, compartmentalized regulation of PRPs is achieved by specific neuromodulatory inputs (e.g. by ß-adrenergic inputs to DG-neurons (Fig. 2) or by dopaminergic inputs at apical CA1 dendrites, Fig. 3). Depending on the task, more than one modulatory brain structure might be here involved, characterized by its own dynamics and associative cross-interactions with hippocampal neurons (Fig. 2). In contrast, the processing of very important information for the organism, like a life-threatening event with a high emotional or higher cognitive content, requires storing of information in a large number of neuronal networks by losing compartment-specificity. Basal dendrites with their close relation to the soma, and thus voltage-dependent-calcium-channel (VDCC)-dependent mechanisms (Fig. 3) or the activation of mineralocorticoid receptors which may act as transcription factors (Fig. 2), may regulate a more general, soma-wide synthesis of PRPs possibly via a direct activation of gene expression. These PRPs can then also be captured by synapses far away from the trigger of PRP-synthesis (Adams and Dudek, 2005; Dudek and Fields, 2002), and provides a cellular mechanism of a general cellular increase in the capacity to store relevant information at the cellular level. This is consistent with a partial spreading of tagging-processes from soma-near events to the apical dendrites (Alarcon et al., 2006). Furthermore, this could also explain the action of non-specifically acting stress hormones that up-regulate the synthesis of corticoid-receptor-dependent PRPs through a direct gene expression mechanism, which could provide the entire neuron with PRPs for a distinct period of time, which could then be captured at all activated synapses with a tag set. Such a mechanism was mentioned above by describing the induction of PRP synthesis via swim-stress activation of corticoid-receptors which resulted in the reinforcement of

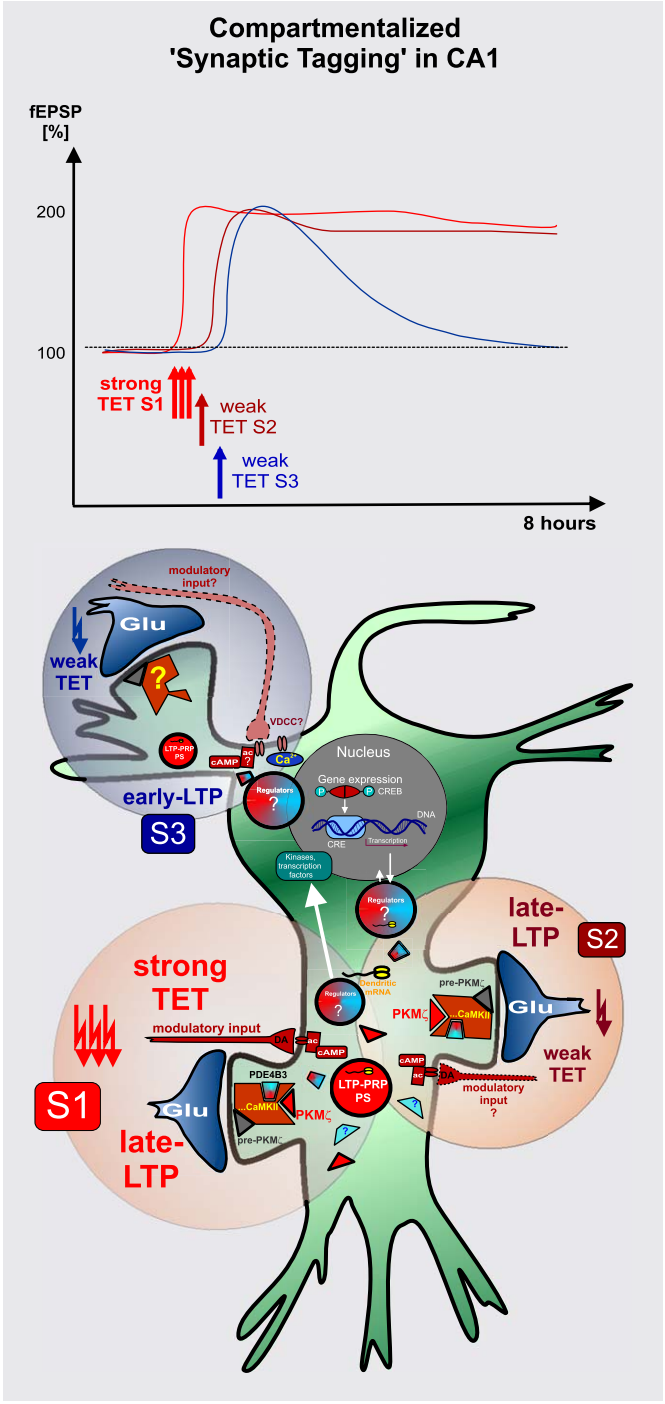


Fig. 3. Compartmentalized, cellular memory formation in a pyramidal CA1-neuron. The upper graph schematically illustrates compartmentalized ''synaptic tagging'' in CA1. Induction of late-LTP by a strong tetanus (strong TET) in a synaptic input S1 in the apical dendrites leads to late-LTP (red curve). If a weak tetanus (weak TET) was applied within a distinct time window to a separate synaptic input S2 in the apical dendrite the normally early-LTP was transformed into late-LTP by processes of synaptic tagging (dark red curve). However, weak TET to a synaptic input S3 in the basal dendrites – even if applied within the normally effective time window of tagging within a dendritic compartment – revealed that strong TET in apical S1 was ineffective in influencing early-LTP in S3 at basal dendrites (blue curve). Thus, tagging is restricted to distinct dendritic compartments. The lower scheme represents our cellular hypothesis of compartmentalized synaptic tagging in the hippocampal CA1 region. The induction of late-LTP (but also similarly late-LTD) in the apical synaptic input S1 activates postsynaptically via a heterosynaptic synergistic interaction of glutamatergic as well as dopaminergic receptors (modulatory input) a transient, protein synthesis-independent synaptic tag, which is made specific for LTP by CaMKII-dependent processes (dark red symbol at the postsynaptic site) and the synthesis of a pool of PRPs (triangles, trapeziums). PRPs consist of LTP-specific proteins such as PKMζ (red triangles) and regulatory proteins such as PDE4B4 (red-blue symbols). Both, the tag as well as the PRPs have a distinct half-life during that time window an interaction of the two can occur, thus, maintaining LTP in S1 for at least 8 h. If within this time window of available PRPs within this dendritic compartment a second, independent synaptic input S2 in the apical dendritic compartment is stimulated to set a synaptic tag (e.g. by inducing early-LTP via a weak TET), the latter can benefit from the compartment-restricted availability of PRPs, thus transforming the normally transient plastic form into an enduring one, in the shown example from early-LTP into late-LTP by processes of synaptic tagging and capturing of the PRPs provided through input S1. If within the same time window at a basal dendritic compartment S3 an early-LTP is induced even with a synaptic tag set (here early-LTP by a weak TET), these tags cannot benefit from the compartmentalized availability of PRPs in the apical dendrites, thus resulting in a transient expression of LTP, in early-LTP. Synaptic tagging, or better cross-compartmental tagging does not occur. It remains unclear, as to whether LTP-expression in synapses of the basal dendrites requires the same enzymes as in the apical dendrites (question marks in the corresponding parts). It remains also unclear, if basal plastic events require an activation of modulatory inputs. For a prolonged maintenance of a plastic event beyond 8 h the expression of genes has been shown to be obviously required which is also schematically represented in the figure.

early- into late-LTP (Korz and Frey, 2003 above). Compartmentalization and decompartmentalization is most likely specific for distinct brain structures depending on their morphological organization/cyto-architecture and function. The more general regulation of neuron-wide changes of plasticity-capacity in CA1 pyramidal neurons by basal dendritic synaptic events points to such a mechanism. Thus, in that case, it could also be that in addition to the above discussed modulatory mechanisms GABA-ergic neurons are specifically involved in the processing of information arriving at basal CA1-dendrites. For example, a fine-tuning of inhibition could lead to an enhanced depolarization of pyramidal cells through the activation of VDCCs located near the soma. Influx of calcium at this locus by GABA-ergic mechanisms could therefore also directly activate somatic protein synthesis and/or gene expression with a soma-wide impact on long-lasting plasticity events (Fig. 3). Indeed, our earlier work supports this assumption: Continuous blockade of GABA-ergic receptors in area CA1 resulted in a VDCC- and protein synthesis-dependent, long-lasting delayed-onset potentiation (Schollmeier et al., 1995).

6.2. Concept of 'cellular memory formation'

Finally, current results allow us to further develop the hypothesis of compartmentalized memory consolidation at the cellular level (Frey, 2001; Korz and Frey, 2004; Sajikumar et al., 2005c; Sajikumar and Frey, 2004a). We suggest that processing of encoding as well as consolidation of a memory trace takes place in functional compartments determined by late-associative interactions, i.e., synaptic tagging and synaptic cross-tagging (Frey and Morris, 1998a; Sajikumar and Frey,

2004a). The functional relevance of compartmentalized cellular memory formation is thereby determined by the physiological relevance of the afferent information. As described previously (Frey, 2001), in the intact animal afferent information might be automatically and transiently stored at glutamatergic synapses (e.g. by processes of early-LTP or early-LTD), irrespective of their late, final relevance for the organism in this context. However, if the system decided the information is relevant to be stored more permanently – within the time window of about 30 min for late-associative interactions – the system can regulate in an associative manner the synthesis of PRPs within distinct functional neuronal compartments by modulatory inputs such as dopamine or other neuromodulators, or neuron-wide by stress hormones to transform the normally transient events into long-lasting ones and by transmitting the information from one to another set of neurons (by interactions between synapses during tagging). The latter would result in the fine-tuning or construction of existing or new neuronal networks containing and further processing the encoded and consolidated information. The uncompartmentalized regulation of PRPs by stress could also fulfill a complimentary function and could affect the simultaneous consolidation of different compartment-specific memories by an arousal-dependent process.

Fig. 3 summarizes our current concept of cellular memory formation in a pyramidal CA1-neuron.

The induction of a late, protein-synthesis-dependent form of functional plasticity can exert dramatic long-lasting effects on a population of synapses within specific functional dendritic compartments. Hereby, the following specific rules can be described:

1) Specific afferent stimulation can induce either LTP or LTD at a specific set of synapses. The physiological meaning of the opposite way in storing information of LTP or LTD remains hereby still to be investigated. However, local processes guarantee the expression of only one form at a particular synapse over time by the possibility of setting a process-specific tag, i.e., CaMKII-dependent mechanisms for LTP, and ERK-dependent processes for LTD.
2) If the afferent stimulation was strong enough to induce a more enduring form of LTP/LTD, in adult animals the heterosynaptic activation/regulation of the synthesis of PRPs is required, consisting of process-specific PRPs (e.g., PKMζ for LTP, and perhaps other molecules for LTD) and process-unspecific regulatory proteins (e.g., PDE4B3). Process-specific PRPs with a specific half-life can be captured by synaptic tags within a time window, and may further regulate the processing of proteins as well as the induction of gene expression. The latter could require, in addition, the action of process-unspecific regulatory proteins which would lead to the synthesis of mRNAs most likely specific for the compartment from which the induction of gene expression was generated (Steward et al., 1998). Thus, this mRNA would be transported to the local, restricted dendritic compartment under normal conditions, or – in conditions with a high emotional or cognitive impact – this mRNA could provide the neuron with PRPs used neuron-wide for the transformation of early-into late-plasticity.
3) A time window of about 30 min in intact animals or of about 1–2 h in slices maintained at a temperature of 32 °C (see Sajikumar et al., 2005b) was detected for cross-synaptic late associative interactions to occur. Within this time interval – which is longer than that conventionally considered for heterosynaptic associative processes – heterosynaptic processes can induce/regulate the availability of a pool of the above mentioned local PRPs, which contains process-specific proteins (e.g., PKMζ) and process-non-specific proteins. The latter would be required for local processing at the synapses or the induction of a prolonged regulation of the availability of local sets of mRNAs by activation of gene expression.

The latter two properties may require a system-wide evaluation of the information to be consolidated, including extrahippocampal brain structures to determine the information as sufficiently relevant to store it more permanently. In that case, the activation of heterosynaptic inputs within the above time interval is sufficient to effectively transform the transient into a long lasting-event by regulating the availability of PRPs. We suggest that there are different kinds of neuromodulators fulfilling different but specific functions by regulating the synthesis of PRPs. Thus, as mentioned above, general modulators like stress hormones may interfere with neuron-wide, compartment-unspecific PRP-synthesis whereas dopaminergic inputs in the hippocampal CA1 carrying relational or contextual information may regulate local protein synthesis at apical dendrites. Other, as yet unknown modulators could be required for the synthesis of PRPs in the basal dendritic compartments.

Summarizing, the here described new properties of late-plastic events in hippocampal neurons support a new principle of cellular information storage, i.e., the idea of a ‘‘compartmentalized processing and storage of information’’ (Frey, 2001). This general idea was already outlined in more theoretical papers (Polsky et al., 2004) and was recently further developed by a theory of so-called ‘‘clustered engrams’’ by Tonegawa et al. (Govindarajan et al., 2006), in which it was proposed that these cellular mechanisms of information processing describe a general principle of long-term information processing and storage in the brain. We support these hypotheses and provided the first experimental evidence for the existence of functional clusters during cellular information storage by studying processes of hippocampal long-lasting functional plasticity. Recently, we were also able to describe similar dopaminergic associative interactions with hippocampal function during novelty detection as well as reward-related hippocampus-dependent long-term memory formation in humans using fMRI (Schott et al., 2004; Wittmann et al., 2005). These results strengthen the above assumptions and point to a general principle required for the formation of long-term memory.

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