A form of long-lasting, learning-related synaptic plasticity in the hippocampus induced by heterosynaptic low-frequency pairing

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The late, transcription- and translation-dependent phase of long-term synaptic potentiation (L-LTP) at the Schaffer collateral synapse of the hippocampus is an experimental model of the synaptic plasticity underlying long-lasting memory formation. L-LTP is typically induced by homosynaptic tetanic stimulation; but associative forms of learning are likely to require the heterosynaptic pairing of stimuli. Here we describe L-LTP elicited by such heterosynaptic pairing at the Schaffer collateral synapse in mice. We find that repeated stimulation of one pathway at low frequency (0.2 Hz), which does not by itself induce synaptic potentiation, will produce long-lasting synaptic plasticity when paired with a brief conditioning burst applied to an independent afferent pathway. The induction of heterosynaptic L-LTP is associative and critically depends on the precise time interval of pairing: simultaneous, conjunctural pairing induces L-LTP; in contrast, delayed pairing induces short-lasting early-phase LTP. Heterosynaptically induced early-phase LTP could be depotentiated by repeatedly presenting unpaired test stimuli, whereas L-LTP could not. This heterosynaptically induced L-LTP requires PKA and protein synthesis. In addition, heterosynaptically induced L-LTP is impaired in transgenic mice that express KCREB (a dominant negative inhibitor of adenosine 3′,5′-cyclic monophosphate response element-binding protein-mediated transcription) in the hippocampus. These mice have previously been shown to be impaired in spatial memory but have normal L-LTP as induced by a conventional homosynaptic tetanic protocol. These data suggest that at least in some instances this L-LTP-inducing protocol may better model behaviorally relevant information storage and the in vivo mechanisms underlying long-lasting memories.

Long-lasting synaptic change is widely believed to underlie many forms of long-lasting memory (1), but it is increasingly clear that a synapse can support mechanistically distinct forms of long-lasting synaptic plasticity. For example, distinct forms of late-phase long-term synaptic potentiation (L-LTP) at the Schaffer collateral synapse of the hippocampus differ in their dependence on brain-derived neurotrophic factor (2, 3) and perhaps on their dependence on the transcription factor adenosine 3′,5′-cyclic monophosphate response element-binding protein (CREB) (4). In light of this observation, it is critically important to determine which forms of experimentally induced synaptic plasticity best recapitulate the endogenous mechanisms of information storage recruited in the hippocampus and in other structures by different forms of learning.

LTP can be separated into at least two temporal phases: early-phase LTP (E-LTP), which is independent of macromolecular synthesis and decays to baseline within 1–3 h of induction, and L-LTP, which requires transcription and translation and can persist for hours or days. Most studies of plasticity induce both E-LTP and L-LTP homosynaptically by patterned stimulation of a single input pathway (Fig. 1A2). E-LTP is typically induced by a single high-frequency tetanus (100 Hz, 1 S), whereas L-LTP is typically induced by three to four such tetanic trains (5, 6). We developed a protocol to induce transcription-dependent L-LTP by using heterosynaptic pairing (two input pathways, Fig. 1A2). The resulting plasticity has several characteristics that make it an attractive model for learning-related synaptic plasticity. In particular, we find that the timing between the paired stimuli critically regulates the duration of synaptic plasticity. This observation mirrors the importance of timing in CS-US pairing in behavioral studies and is consistent with the heterosynaptic rules for the long-term memory and memory-associated synaptic plasticity, which emerged from experiments in Aplysia (7, 8).

Materials and Methods

Transverse slices (400 μm) of acutely dissected mouse hippocampus were prepared from C57/B6 mice aged 4–8 weeks. In KCREB mice, slices from the dorsal hippocampus of littermate control and KCREB transgenic mice were used (4). Slices were maintained at 28°C in an interface chamber with continuous perfusion of artificial cerebrospinal fluid (124 mM NaCl/4 mM MgSO4/4 mM KCl/1.0 mM Na3HPO4/4 mM CaCl2/26 mM NaHCO3/10 mM d-glucose) at 1.5–2 ml/min, bubbled with 95% O2/5% CO2. In all experiments, the GABAergic inhibitor picrotoxin (20 μM) was present in the perfusion solution throughout the experiment. Experiments were started at least 2 h after the slice dissection. Extracellular recordings were made by using artificial cerebrospinal fluid glass electrodes (1–3 MΩ). Field excitatory postsynaptic potentials (EPSPs) were recorded in the Schaffer collateral–CA1 pathway of the hippocampus. Stimuli were delivered through bipolar stainless steel electrodes. Two stimulating electrodes were placed on either side of the recording electrode in stratum radiatum (Fig. 1A). Stim 1 was used as the test pathway, and Stim 2 was used as the conditioning pathway. The two afferent pathways were considered independent if no paired-pulse facilitation occurred when two stimuli were coupled in a 50-ms interval. For the measurement of the baseline synaptic response, 0.033-Hz (0.05-ms pulse duration) stimulation was used. In two pathway experiments, Stim 1 and Stim 2 were delivered with the same frequency (0.033 Hz) 10 s apart except during pairing. In other experiment, baseline EPSPs were sampled only in the test pathway (Stim 1) and Stim 2 was turned on only during the pairing.

Results

Pairing Single Volleys with Brief Conditioning Bursts in an Independent Pathway Induces Heterosynaptic L-LTP. We induced long-lasting synaptic potentiation in mouse hippocampal slices by
Pairing low-frequency stimulation of a test pathway (0.2 Hz) with bursts of stimuli, also delivered at low frequency, in a second, conditioning pathway (Fig. 1A). Such paired stimulation of two distinct afferent pathways has previously been used to induce E-LTP, as has pairing of low-frequency stimulation of a single afferent pathway with depolarization of the postsynaptic cell (9, 10). The magnitude and valence of such pairing-induced plasticity sensitively depend on the precise relative timing of the paired stimuli; by changing the interval between paired stimuli, different levels of E-LTP or synaptic depression (LTD) can be produced with identical electrical stimuli. This phenomenon has been termed spike timing-dependent plasticity (STDP) (11–17).
However, an STDP-like phenomenon has not previously been demonstrated for protein synthesis-dependent long-lasting plasticity (L-LTP).

We stimulated two independent afferent pathways, a test pathway (S1) and a conditioning pathway (S2), in stratum radiatum of acute hippocampal slices and recorded the postsynaptic responses in a population of postsynaptic CA1 neurons (Fig. 1A1). Test pulses delivered at 0.2 Hz to S1 (after a 30-min baseline) produced no change in the synaptic strength of either recorded pathway (Fig. 1B); these test pulses produced a brief, simple EPSP (Fig. 1B Top Right). When such test pulses were asynchronously paired 60 times with brief conditioning bursts in the second afferent pathway (the conditioning pathway, S2; conditioning bursts consisted of three pulses at 100 Hz; 100-ms interval between the S1 pulse and the first pulse in S2), the conditioning pathway was potentiated but the test pathway was not affected; that is, the two pathways remained independent of one another (Fig. 1C). Conditioning bursts produced a longer
and more complex EPSP (Fig. 1B Middle Right); asynchronous presentation of S1 test pulses and S2 conditioning bursts produced two distinct, nonoverlapping EPSPs (Fig. 1B Bottom Right).

Delivering test pulses to the test pathway (S1) and bursts to the conditioning pathway (S2) separated by only 0.1 ms (conjunctional pairing) produced a complex EPSP (Fig. 1D Inset). Repeating this pairing 60 times produced long-lasting potentiation of both the test pathway (Fig. 1D Left) and the conditioning pathway (Fig. 1D Right). The robust potentiation of the test pathway is striking because this pathway never saw stimulation—pairing-induced L-LTP depends on protein synthesis and on CREB-mediated transcription. (A) Conjunctual and delayed pairing were performed in the presence of the protein synthesis inhibitor anisomycin (20 μM, applied 20 min before until 90 min after pairing). This truncated L-LTP produced by conjunctional pairing (Upper, anisomycin (●), 118 ± 18% baseline 3 h after pairing; control (○), 168 ± 10%; n = 7 for each condition; P < 0.01). However, anisomycin had no effect on E-LTP produced by delayed pairing (Lower). (B) Pairing-induced L-LTP, but not high-frequency-induced L-LTP in the conditioning pathway, depends on CREB. Conjunctual pairing was induced in slices from dorsal hippocampus of KCREB-expressing transgenic mice (4) and littermate controls. Early potentiation in the test pathway was not altered in transgenic mice, but L-LTP was reduced (Upper, KCREB (●), 118 ± 6% baseline 3 h after pairing; littermate control (○), 150 ± 11%; n = 8 for each genotype; P < 0.05). L-LTP was normal in the conditioning pathway (Lower). (Inset) Representative EPSPs before and 3 h after pairing in control (Left) and KCREB (Right) mice. (Calibration, 2 mV, 10 ms.)

L-LTP Produced Heterosynaptically Depends on Activation of the N-methyl-D-aspartate (NMDA) Receptor, PKA, and Protein Synthesis. We found that L-LTP produced by pairing depends on the NMDA receptor. The NMDA blocker 2-amino-5-phosphonovaleric acid (APV) had no effect on the EPSP produced by stimulation of S1 but attenuated the more complex EPSP produced by a conditioning burst in S2 (Fig. 2A Left Inset). APV also attenuated the EPSP produced in response to conjunctional pairing (Fig. 2A Right Inset). This shows that the postsynaptic response to conditioning bursts in pathway S2 has a significant NMDA-dependent component and thus is likely to include significant calcium influx. Pairing in the presence of APV produced no E-LTP or L-LTP (Fig. 2A).

STDP depends on both the NMDA receptor and voltage-gated calcium channels (VGCCs) (12–17). To test whether this was true of the L-LTP produced by conjunctional pairing, we performed the pairing protocol in the presence of the VGCC inhibitor nifedipine (15 μM). This treatment did not affect LTP at 1 h, but it significantly reduced L-LTP at 3 h (Fig. 2B). Thus, pairing-induced L-LTP, like STDP, requires both the NMDA receptor and VGCCs. Interestingly, only the late phase of pairing-induced LTP requires VGCCs. This finding contrasts with STDP, in which E-LTP depends on VGCCs (12–16) but parallels the requirement for VGCCs of some forms of L-LTP produced by homosynaptic tetanization (18).

We found that changing the interstimulus interval dramatically affected the plasticity produced by this protocol. Once again, repeated pairing (that is, 60 pairings of S1 test stimuli and S2 conditioning bursts with a 0.1-ms interval between the test stimulus and the first impulse of the conditioning burst) produced robust L-LTP (Fig. 2C Upper, replicating Fig. 1D Left). However, when the interval between S1 and S2 was increased to 20 ms (delayed pairing), potentiation of comparable initial magnitude was produced but decayed to values indistinguishable from baseline within 90 min; that is, delayed pairing produced only E-LTP (Fig. 2C Lower).

Many forms of L-LTP and some forms of E-LTP (6, 19, 20) require the cAMP-dependent kinase PKA, which also is critical in a variety of invertebrate model systems to relay the synaptic stimuli required for long-lasting plasticity to nuclear events. In addition, disruption of PKA in genetically modified animals disrupts hippocampus-dependent learning and long-lasting alterations in the hippocampal representation of space (6). It is
therefore likely that the forms of hippocampal plasticity underlying long-term information storage require PKA. To see whether this is true of pairing-induced L-LTP, we performed our pairing and delayed-pairing protocols in the presence and absence of the PKA inhibitor KT5720 (1 µM). We found that both the L-LTP produced by conjunctual pairing and the E-LTP produced by delayed pairing were attenuated by PKA blockade; pairing-induced L-LTP of longer than 1 h was entirely eliminated (Fig. 2D). Both these forms of potentiation thus require PKA. It has been suggested that E-LTP as conventionally produced by tetanization avoids such a dependence on PKA because it saturates the elevation of cAMP and that gentler methods of induction are therefore more susceptible to PKA inhibition (19). By this hypothesis, the dependence of E-LTP as produced by delayed pairing on PKA is therefore consistent with its being induced by less robust, and possibly more physiological, stimulation than the robust homosynaptic stimulation more conventionally used to produce tetanus-induced E-LTP.

The defining feature of L-LTP is its requirement for gene induction and macromolecular synthesis. This requirement parallels the known dependence of various forms of long-lasting memory on macromolecular synthesis (21–23). To test the dependence of pairing-induced L-LTP on new protein production, we performed pairing in the presence and absence of the protein synthesis inhibitor anisomycin (20 µM). We found that anisomycin eliminated the late phase of pairing-induced L-LTP, although it did not affect the early phase (Fig. 3A Upper). In contrast, E-LTP produced by delayed pairing was not affected by anisomycin (Fig. 3A Lower). This finding confirms the mechanistic distinction first made in Fig. 2A, whereas delayed pairing produces only E-LTP, a small change in interstimulus interval causes conjunctual pairing to produce L-LTP, as defined both by duration and by pharmacological criteria.

Heterosynaptically Induced L-LTP Requires CREB-Mediated Gene Induction. Interference with the transcription factor CREB in area CA1 of the hippocampus disrupts long-term hippocampus-dependent memory but not standard forms of hippocampal long-lasting potentiation (4). To examine whether disruption of CREB-mediated gene induction would interfere with pairing-induced L-LTP, we examined LTP in slices from the dorsal hippocampus of transgenic mice expressing KCREB, a potent dominant inhibitor of CREB-family transcription factors, in area CA1. We have shown that these mice are deficient in hippocampus-dependent memory tasks but not in homosynaptic tetanus-induced forms of L-LTP (4). We found that inhibition of CREB-mediated transcription by KCREB significantly attenuated the L-LTP produced by conjunctual pairing (Fig. 3B Upper). In contrast, it did not affect the L-LTP produced in pathway S2, to which repeated conditioning bursts were applied (Fig. 3B Lower). This recapitulates the lack of effect of this transgene on L-LTP produced by more robust synaptic stimulation and emphasizes that pairing-induced L-LTP is mechanistically distinct. Because KCREB-expressing animals have a proven deficit in long-term hippocampus-dependent memory, our observation supports the idea that pairing-induced L-LTP may be a better model for the endogenous mechanisms of certain forms of long-term memory storage than homosynaptic, high-frequency protocols. Correlation between electrophysiology and other forms of learning will clearly be required to further substantiate this hypothesis.

Heterosynaptically Induced LTP Is Partially Depotentiated by Subsequent Unpaired Test Stimuli. Many forms of synaptic potentiation can be reversed, or depotentiated, by subsequent low-frequency stimulation. LTP produced by a 100-Hz tetanus is depotentiated by subsequent stimulation at 1–5 Hz, if it is applied within 10 min of the induction of potentiation (24, 25). However, this and similar examples use dramatically different stimulation for induction of LTP and its depotentiation. Because pairing-induced LTP is produced by low-frequency stimulation in the test pathway, in conjunction with conditioning bursts in the conditioning pathway, we wondered whether constant stimulation of the test pathway, first with conditioning bursts and then alone, could lead first to the induction of potentiation and then to its reversal. We found that when 60 pairings, by using the delayed protocol (20-ms interstimulus), were immediately followed by 60 unpaired stimuli to the test pathway at 0.2 Hz, the induced potentiation was rapidly depotentiated (Fig. 4A). In contrast, 60
unpaired stimuli delivered 5 min after delayed pairing were far less effective at depotentiation (Fig. 4B), confirming that for pairing-induced potentiation, as for tetanus-induced potentiation, a critical window exists within which depotentiation is possible. These data show that the same low-frequency synaptic stimulation can both potentiate and depotentiate this synapse, depending on the co-occurrence of other synaptic activity in the postsynaptic neuron. In contrast to the depotentiation of E-LTP produced by delayed pairing, the potentiation induced by conjunctural pairing could not be depotentiated by subsequent unpaired test stimuli (Fig. 4C).

Discussion

By using a modified STDP-like protocol to produce L-LTP, we have described a previously uninvestigated form of experimental plasticity that has several important characteristics. First, we have shown that heterosynaptic pairing can produce translation and transcription-dependent L-LTP. Second, variations in the interstimulus interval in our protocol control the duration of synaptic change. Finally, LTP induced by pairing can be, at least partially, depotentiated by the repeated application of unpaired test stimuli immediately after pairing, rather than by the application of a dramatically different stimulation for depotentiation as has been described previously.

A particularly interesting and important characteristic of pairing-induced plasticity is its heterosynaptic nature. Specifically, the potentiation produced at one population of synapses (S1) depends critically on the activity at other, independent synapses (S2). Such cooperativity between different synapses on the same postsynaptic neuron may be important for associative memory; indeed, it is central to the idea of a Hebbian synapse (26). The ability to model this feature for the transcription and translation-dependent long-lasting synaptic plasticity in a simple in vitro preparation is an important experimental advance.

Might this experimental paradigm better recapitulate the endogenous mechanisms of memory storage? Several considerations suggest that it does. First, the stimulation used to induce L-LTP by the pairing protocol is far less intense, and therefore possibly more physiological, than the tetanization conventionally used to induce L-LTP. This is true even in comparison with theta-burst protocols, because the stimulation at the potentiated test pathway (S1) consists simply of single pulses delivered at 0.2 Hz. Second, the precise timing of stimulation to the two afferent pathways in this pairing protocol critically regulates the duration of LTP; as has been explored in the discussion of STDP, such sensitivity dependence on spike timing may be an important characteristic of neural networks underlying associate learning and memory (11).

Third, the L-LTP produced by pairing requires several processes that have independently been shown to be required for hippocampus-dependent learning, including activation of the NMDA receptor, PKA signaling, and protein synthesis. In particular, pairing-induced L-LTP is impaired in transgenic mice with inhibited CREB and a deficit in long-lasting hippocampus-dependent memory; tetanus-induced L-LTP is normal in these mice (as is LTP induced directly by conditioning bursts in the conditioning pathway, S2, in our experiments; Fig. 3B Bottom). This correlation suggests that, if the memory deficit in these animals is indeed due to a disruption of hippocampal synaptic plasticity, pairing-induced L-LTP may be a better model for the important disrupted processes than are more conventional forms of L-LTP. Pairing-induced L-LTP merits close attention as a new in vitro model for the synaptic processes that underlie hippocampus-dependent learning and memory.

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