Electrical Stimuli Patterned After the Theta-Rhythm Induce Multiple Forms of LTP

S. L. MORGAN AND T. J. TEYLER

Department of Neurobiology and Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272-0095

Received 13 October 2000; accepted in final form 6 June 2001

Morgan, S. L., and T. J. Teyler. Electrical stimuli patterned after the theta-rhythm induce multiple forms of LTP. J Neurophysiol 86: 1289–1296, 2001. The induction of long-term potentiation (LTP) by high-frequency stimulation is considered an acceptable model for the study of learning and memory. In area CA1 calcium influx through N-methyl-D-aspartate receptors (NMDARs; nmdaLTP) and/or L-type voltage-dependent calcium channels (vdccLTP) results in distinct forms of LTP. In the light of significant accumulation of knowledge about patterns of naturally occurring activity in the intact animal, we examined whether the application of stimuli patterned after natural activity induced nmdaLTP and/or vdccLTP. In rat hippocampal slices we examined LTP induced by three types of patterned stimulation short (S-TBS), long (L-TBS), and high-intensity long theta-patterned stimulation (HL-TBS). The patterns of stimulation were applied in control, nifedipine (blocks vdccLTP), d,l-2-amino-5-phosphonovaleric acid (APV; blocks nmdaLTP), or APV and nifedipine containing media. We found that S-TBS resulted in LTP that was completely attenuated in the presence of APV but was unaffected by nifedipine. Thus S-TBS results in the selective induction of nmdaLTP. L-TBS resulted in LTP that was completely blocked by APV and only partially blocked by nifedipine. Therefore L-TBS results in a compoundLTP consisting of both nmdaLTP and vdccLTP components. In the presence of APV, HL-TBS resulted in vdccLTP, and when APV and nifedipine were both present, LTP was completely blocked. Thus HL-TBS results in a vdccLTP in isolation when APV is present. We also examined saturation of S-TBS–induced LTP (nmdaLTP) by applying S-TBS at short intervals. When nifedipine was present, multiple S-TBS trains resulted in a substantially smaller final LTP as compared with controls. We conclude that multiple bursts of S-TBS eventually summate to result in compoundLTP. Stimuli patterned after innate rhythms in the hippocampus effectively induce nmdaLTP (S-TBS), compoundLTP (L-TBS), or vdccLTP (HL-TBS).

INTRODUCTION

Coincident activity in pre- and postsynaptic elements at many excitatory central synapses results in a long-lasting enhancement of synaptic responses known as long-term potentiation (LTP) (Bliss and Collingridge 1993). LTP is considered by many to be a likely candidate mechanism underlying at least some types of learning and memory. The correlative evidence linking LTP to learning and memory stems from several approaches including recording of behavioral LTP, pharmacological and/or physiological manipulation in behavioral experiments, and genetic manipulation (Martin et al. 2000). Compelling evidence linking LTP with learning and memory could result from the recording of LTP in the intact animal during behavior associated with learning. While there have been several reports of LTP recorded during learning in the intact animal, the results remain controversial (Ishihara et al. 1997; Jaffard et al. 1996; Moser et al. 1993). Another line of evidence links the pharmacological antagonism of receptors/channels necessary for LTP with impairments of learning and memory. Hippocampus-dependent tasks are impaired when antagonists that block LTP are infused into the hippocampus or into the lateral ventricles (Morris 1989). Saturation of hippocampal LTP by electrical stimulation prior to behavioral tasks also impairs learning and memory in rats (Moser et al. 1998). Genetic manipulation of N-methyl-D-aspartate glutamate receptors (NMDARs) to increase their conductance and/or total number results in improvements in learning and memory in specific tasks (Tang et al. 1999). Considered as a whole, the evidence presented above strongly indicates that LTP plays a role in learning and memory.

At least two forms of LTP coexist in hippocampal area CA1: voltage-dependent calcium channel–mediated LTP (vdccLTP), which is dependent on calcium influx via L-type voltage-dependent calcium channels (VDCCs) and N-methyl-D-aspartate glutamate receptor–mediated LTP (nmdaLTP), which is dependent on calcium influx via NMDA receptor channels (Grover and Teyler 1990). Physiological observations in the hippocampus and observations following pharmacological manipulation in the behaving animal have indicated differential and independent roles for each type of LTP in learning and memory (Borroni et al. 2000; Woodside et al. 1999). NMDARs and VDCCs are co-localized to the postsynaptic elements in area CA1 but have unique distributions (Magee and Johnston 1995; Magee et al. 1996; Moser et al. 1993). Another line of evidence links the pharmacological antagonism of receptors/channels necessary for LTP with impairments of learning and memory. Hippocampus-dependent tasks are impaired when antagonists that block LTP are infused into the hippocampus or into the lateral ventricles (Morris 1989). Saturation of hippocampal LTP by electrical stimulation prior to behavioral tasks also impairs learning and memory in rats (Moser et al. 1998). Genetic manipulation of N-methyl-D-aspartate glutamate receptors (NMDARs) to increase their conductance and/or total number results in improvements in learning and memory in specific tasks (Tang et al. 1999). Considered as a whole, the evidence presented above strongly indicates that LTP plays a role in learning and memory.

Under normal conditions, a low-frequency tetanus (~25 Hz) will result in nmdaLTP only, while a higher frequency tetanus (~100 Hz) will result in a compoundLTP consisting of both...
NmdaLTP and vdccLTP (Grover and Teyler 1990; Morgan and Teyler 1999). VdccoLTP can only be produced in isolation when using high-frequency tetani (~100 Hz) in the presence of an NMDAR antagonist. The physiological requirements for vdccoLTP induction are usually met by the contribution of the NMDARs to the depolarization of the postsynaptic membrane; thus vdccoLTP probably does not occur in isolation in the intact animal. When NMDARs are antagonized, only increasing postsynaptic depolarization with more intense or higher frequency tetani can induce vdccoLTP.

The two forms of LTP have distinct signal transduction cascades. NmdaLTP requires serine/threonine activation but is independent of tyrosine kinase activation (Cavus and Teyler 1996). Conversely, vdccoLTP is dependent on tyrosine kinase activation while remaining independent of serine/threonine activation (Cavus and Teyler 1996). These distinct signal transduction cascades suggest that dissimilar gene transcription and protein synthesis could be occurring in these two forms of plasticity, which could result in diverse cellular and network consequences (Bading et al. 1993; Ghosh and Greenberg 1995). NmdaLTP is readily reversible (de-potentiation) by applying long, low-frequency trains of stimuli that activate serine/threonine phosphatases (Cousens and Teyler 1996). VdccoLTP is more difficult to reverse by similar trains of low-frequency stimuli, suggesting a greater degree of permanence and a role in long-term memory storage (Martinez et al. 1997; Morgan et al. 2001). Thus physiological evidence suggests a divergent role for the two forms of LTP in learning and memory.

Recent behavioral experiments have also indicated distinct roles for these two forms of LTP in learning and memory (Borroni et al. 2000; Woodside et al. 1999). Antagonists of NMDARs and VDCCs at concentrations that block LTP in vivo affect the attenuation of memory in a hippocampal-dependent spatial task in different ways (Borroni et al. 2000). An NMDAR antagonist delayed reference memory acquisition, whereas a VDCC antagonist attenuated the retention of reference memory over a 7-day period (Borroni et al. 2000). When both a VDCC and NMDAR antagonist were applied simultaneously, working memory (21-h period) was impaired. These behavioral experiments suggest that nmdaLTP may differentially mediate short-term memory (STM) and vdccoLTP might mediate long-term memory (LTM) (Borroni et al. 2000; Woodside et al. 1999).

While high-frequency tetani have been the preferred method to induce LTP experimentally, these tetani do not resemble the innate activity in the intact animal during behavior correlated with learning. Recording in behaving animals has revealed a 3- to 7-Hz oscillation known as the theta rhythm (Klemm 1976). Some CA1 pyramidal cells discharge at high-frequency in short bursts phase locked with the ongoing theta-rhythm (theta bursts) during behavior correlated with learning (Hill 1978; Otto 1991; Ranck 1973). Theta burst stimuli (TBS) patterned after this intact activity is highly effective and perhaps optimal for the induction of LTP (Larson et al. 1986; Otto et al. 1991; Staubli and Lynch 1987). However, it remains unclear from previous studies which type or types of LTP were induced when using TBS in area CA1 of the hippocampus. If vdccoLTP and nmdaLTP are to be considered mechanisms important for learning and memory, they should be induced by activity that resembles that which occurs during learning in the intact animal. By using tetanic stimuli patterned after innate activity, we have examined this issue in hippocampal slices.

In behavioral experiments, nmdaLTP can be selectively blocked by antagonizing NMDARs. Whether depolarization sufficient to result in vdccoLTP occurs when NMDARs are blocked is unknown. If vdccoLTP cannot be induced in the absence of NMDAR currents, then application of NMDAR antagonists would result in a block of both forms of LTP. Alternatively, if vdccoLTP can be induced in the absence of NMDAR currents, this form of plasticity could occur in these conditions. Given the current hypothesis that vdccoLTP and nmdaLTP mediate different forms of memory, NMDAR antagonists could yield confounding results in behavioral experiments. This study details the induction of vdccoLTP in the absence of NMDAR currents by several types of patterned stimulation.

**Methods**

Detailed methods for slice preparation have been previously described and will be provided here in brief (Teyler 1980). Transverse hippocampal slices 400 μm thick were prepared from 50- to 65-day old male Long Evans rats. The slices were incubated for 1 h in an oxygenated holding chamber at room temperature. The slices were placed in an oxygenated (95% O₂-5% CO₂) heated (33–34°C) interface chamber and perfused with artificial cerebrospinal fluid (ACSF; ACSF contained in mM, 125.0 NaCl, 3.35 KCl, 1.25 NaH₂PO₄, 2.0 MgSO₄, 2.0 CaCl₂, 25.0 NaHCO₃, and 10.0 glucose). A concentric bipolar stimulating electrode and a glass micropipette-recording electrode (2–3 MΩ with 2 M NaCl) were placed in CA1 stratum radiatum to elicit and record population excitatory postsynaptic potentials (fEPSP). Field potentials were amplified (×100), band-pass filtered (1–2,000 Hz), digitized, and stored for later analysis. A test stimulus was applied every 30 s at a stimulus strength sufficient to produce a 1-mV fEPSP as measured in stratum radiatum.

LTP was always induced using TBS stimuli. TBS was applied after a minimum of a 30-min baseline period. TBS (Fig. 1) was either short-TBS (S-TBS), long-TBS (L-TBS), or high-intensity long-TBS (HL-TBS). S-TBS consisted of five trains of four pulses at 10 Hz separated by 200 ms repeated twice with an interburst interval (IBI) of 10 s. L-TBS utilized the same stimulation as S-TBS but was repeated six times separated by an IBI of 10 s. The amplitude of the S-TBS and the L-TBS trains was sufficient to elicit a 0.75- to 1-mV population spike as measured in the cell body layer. HL-TBS was the same as L-TBS except the stimulus intensity was increased to yield a population spike of 2.5–3 mV as measured in the cell body layer. In one experiment four bouts of S-TBS were applied at 30-min intervals.

Patterned stimuli were presented either in the presence or absence of 50 μM (±) D,L-2-amino-5-phosphonovaleric acid (APV) and/or 30 μM nifedipine. Drugs were bath applied for 30 min prior to and 10 min after tetanus with a perfusion rate of about 1 ml/min. In the experiment in which multiple bouts of S-TBS were applied, nifedipine was applied 30 min prior to the first tetanus and continued for the duration of the experiment. Experiments with light-sensitive drugs were performed in the dark. All drugs were purchased from Sigma/RBI. Data were analyzed by measuring the initial slope of the fEPSP of all sweeps. Responses 10 min before tetanus were averaged and established as a baseline response. Posttetanic responses were expressed as a percent change of the baseline fEPSP slope. Student’s t-tests were used to compare the changes in slope of the experimental and control groups various times posttetanus as indicated. Reports of short-term potentiation (STP) are an average of responses across the initial 5 min of the posttetanus period. Reports of LTP are an average of the last 10 min of the recording period. In some figures representative sweeps are taken from the time point(s) as indicated. The significance level was set at the P < 0.05 level.
RESULTS

S-TBS

Introduction of S-TBS in control media \((n = 7)\) resulted in a large, rapidly developing STP \((102.8 \pm 11.5\%\), mean \pm SE\) that swiftly decayed over the first 15 min posttetanus. The potentiation then remained stable for the duration of the 60-min recording period. The average increase in the initial slope of the fEPSP as measured in the last 10 min of the recording period was 66.4 \pm 11.5\% (Fig. 2). The time course of LTP induced by S-TBS in the present study closely paralleled the time course of high-frequency LTP in previous studies (Cavus and Teyler 1996). Application of S-TBS stimulation consisted of 5 bursts separated by 200 ms repeated 2 times with an interval of 10 s between each burst. The average increase in the initial slope of the fEPSP as measured in the last 10 min of the recording period was 66.9 \pm 22.7\%.

To determine whether both nmdaLTP and vdccLTP (compoundLTP) could be induced by theta burst stimulation, a longer theta burst stimulation was applied. L-TBS stimulation in control media \((n = 6)\) resulted in a large, rapidly developing STP \((170.2 \pm 37.9\%\). The potentiated response stabilized within the first 5 min of the posttetanus recording period and remained stable for the duration of the recording period (Fig. 3). The average increase in the initial slope of the fEPSP as measured in the last 10 min of the recording period was 170.2 \pm 37.9\%. The magnitude of this compoundLTP appears to be larger than in comparable studies using high-frequency tetanus trains (Cavus and Teyler 1996). The LTP induced by L-TBS in control conditions was completely attenuated when APV was applied prior to tetanus (Fig. 3; \(n = 6\)) as measured 5 min post tetanus \((23.4 \pm 13.4\%\)) or 60 min posttetanus \((6.6 \pm 6.9\%\). There was a significant difference in the amount of LTP induced by L-TBS in the presence of APV as compared with control conditions, \(P < 0.05\). This result taken alone suggests that the LTP induced by L-TBS was completely mediated by NMDARs. Importantly, however, L-TBS in the presence of nifedipine \((n = 6)\) resulted in a rapidly developing potentiation that stabilized within the first 5 min of the posttetanus recording period \((97.4 \pm 26.95\%\) and remained stable for the duration of the recording period. The average increase in the initial slope of the fEPSP as measured in the last 10 min of the recording period was 66.9 \pm 22.7\%.

There was a significant difference in the amount of potentiation elicited by L-TBS in the presence of nifedipine as compared with control, \(P < 0.05\). These latter results indicate that L-TBS induces a compoundLTP that consists of two components, nmdaLTP and vdccLTP. Under L-TBS conditions the compoundLTP is completely blocked by APV but only attenuated.

L-TBS

To determine whether both nmdaLTP and vdccLTP (compoundLTP) could be induced by theta burst stimulation, a theta burst stimulation was applied. L-TBS stimulation in control media \((n = 6)\) resulted in a large, rapidly developing STP \((170.2 \pm 37.9\%\). The potentiated response stabilized within the first 5 min of the posttetanus recording period and remained stable for the duration of the recording period (Fig. 3). The average increase in the initial slope of the fEPSP as measured in the last 10 min of the recording period was 170.2 \pm 37.9\%. The magnitude of this compoundLTP appears to be larger than in comparable studies using high-frequency tetanus trains (Cavus and Teyler 1996). The LTP induced by L-TBS in control conditions was completely attenuated when APV was applied prior to tetanus (Fig. 3; \(n = 6\)) as measured 5 min post tetanus \((23.4 \pm 13.4\%\)) or 60 min posttetanus \((6.6 \pm 6.9\%\). There was a significant difference in the amount of LTP induced by L-TBS in the presence of APV as compared with control conditions, \(P < 0.05\). This result taken alone suggests that the LTP induced by L-TBS was completely mediated by NMDARs. Importantly, however, L-TBS in the presence of nifedipine \((n = 6)\) resulted in a rapidly developing potentiation that stabilized within the first 5 min of the posttetanus recording period \((97.4 \pm 26.95\%\) and remained stable for the duration of the recording period. The average increase in the initial slope of the fEPSP as measured in the last 10 min of the recording period was 66.9 \pm 22.7\%.

There was a significant difference in the amount of potentiation elicited by L-TBS in the presence of nifedipine as compared with control, \(P < 0.05\). These latter results indicate that L-TBS induces a compoundLTP that consists of two components, nmdaLTP and vdccLTP. Under L-TBS conditions the compoundLTP is completely blocked by APV but only attenuated.
by nifedipine, indicating that nmdaLTP is required for the induction of vdccLTP and consequently compoundLTP. Further, the time course of the compoundLTP induced in this study with L-TBS was similar to the time course of the compoundLTP induced with high-frequency tetani (Cavus and Teyler 1996; Grover and Teyler 1990).

High-intensity long-theta patterned stimulation

HL-TBS in the presence of APV \((n = 5)\) resulted in STP \((51.2 \pm 10.6\%)\) and a slowly developing potentiated response that stabilized at about 10–12 min posttetanus (Fig. 4). The potentiated response remained stable for the duration of the recording period. The average increase in the initial slope of the fEPSP as measured in the last 10 min of the recording period was \(67.3 \pm 14.4\%). \) In the presence of APV and nifedipine \((n = 5)\) HL-TBS failed to result in a significant STP \((7.9 \pm 7.8\%)\) or LTP as measured across the last 10 min of the recording period \((-1.7 \pm 5.1\%); \) Fig. 4).

Interaction of nmdaLTP and vdccLTP

Although nmdaLTP and vdccLTP utilize separate cellular mechanisms for altering synaptic gain, blockade of NMDARs can block both forms of LTP as shown in Fig. 3. In Fig. 5 we see that S-TBS, which induces only nmdaLTP, is blocked by APV as expected. However L-TBS, which induced a compoundLTP (both nmdaLTP and vdccLTP), was also blocked by APV. Under these conditions, vdccLTP is not induced due to the lack of sufficient postsynaptic depolarization, normally contributed by the NMDARs, which is required to activate VDCCs under these tetanus conditions. The additional depolarization normally required for vdccLTP induction can be supplied by increasing the intensity of the theta burst stimulation (HL-TBS, Fig. 4).

In control media, S-TBS induced nmdaLTP that was completely blocked by APV and unaffected by nifedipine (Fig. 2). L-TBS induced a compoundLTP, but when nifedipine was present, vdccLTP was blocked, leaving only nmdaLTP in isolation (Fig. 3). As can be seen in Fig. 6, the magnitude of
Plasticity following multiple bouts of S-TBS in control and nifedipine groups

Results of the examination of the level of STP induced by the first and then subsequent S-TBS trains in control and nifedipine conditions follow. Reports of STP are the average of slopes from the 5-min period following an S-TBS as compared with the average of responses across the last 10 min of the preceding period. In control conditions \((n = 8)\) STP induced by the first \((97.1 \pm 24.2\%\), second \((63.0 \pm 19.7\%\), and third \((17.2 \pm 20.7\%\) S-TBS train was of a progressively smaller magnitude than the STP induced by the preceding S-TBS train \((P < 0.05; \text{Figs. 7 and 8})\). The fourth S-TBS resulted in a STP \((22.4 \pm 21.5\%\) that did not significantly differ from the STP produced by the third S-TBS \((P > 0.05)\).

With nifedipine present \((n = 9)\) STP induced by the first \((104.4 \pm 23\%)\) S-TBS train was significantly larger than the STP that resulted from the second \((49.1 \pm 21.8\%)\) S-TBS train \((P < 0.05)\). The STP induced by the third \((49.2 \pm 22.7\%)\) and fourth \((25.4 \pm 26.6\%)\) S-TBS were not significantly different from the STP resultant from the second S-TBS \((P > 0.05; \text{Fig. 8})\). With APV present STP induced by the second \((29.3 \pm 15.6\%)\), third \((13.3 \pm 14.2\%)\), and fourth \((9.2 \pm 16.7\%)\) S-TBS were not different from the STP induced by the first S-TBS \((29.5 \pm 13.0\%)\).

Reports of LTP are an average increase in initial slope of the EPSP taken across the final 10 min of that post–S-TBS period as compared with the initial baseline period. In the control condition LTP induced by the first \((84.9 \pm 11.9\%)\), second \((156.0 \pm 19.7\%)\), and third \((174.3 \pm 20.7\%)\) S-TBS trains were of a larger magnitude than the LTP induced by the previous S-TBS train \((P < 0.05; \text{Fig. 9})\). LTP induced by the fourth \((171.3 \pm 21.5\%)\) S-TBS train was not different from LTP resultant from the prior S-TBS train \((P > 0.05)\). With nifedipine present, LTP induced by the first \((64.5 \pm 15.8\%)\), second \((75.5 \pm 14.6\%)\), third \((92.5 \pm 18.5\%)\), and fourth \((94.3 \pm 15.0\%)\) S-TBS trains resulted in small but not significant increases in slope as compared with the previous S-TBS \((P < 0.05; \text{Figs. 7 and 9})\). Following the final S-TBS, LTP in the control group was larger than LTP generated when nifedipine present.
from the LTP resultant from the first S-TBS (12.6% ± 1.6%) S-TBS was not significantly different from the amount of LTP produced by the prior S-TBS in the same condition. The pound sign (#) above a bar indicates a statistically significant difference between the control and nifedipine groups for that post–S-TBS period. The 1st S-TBS results in an LTP of similar magnitude indicating that this LTP is dependent on NMDARs only (nmdaLTP). By the 2nd S-TBS in the control condition, VDCCs began to contribute to the resultant compoundLTP. When nifedipine was present, the 2nd S-TBS resulted in a much smaller LTP that was dependent on NMDARs only.

DISCUSSION

The results of the present study show that S-TBS results in nmdaLTP. Similarly, Ito et al. (1995) have found that patterned stimulation similar to S-TBS results in an nmdaLTP that does not involve L-type VDCCs. Conversely we have found that L-TBS results in a compoundLTP consisting of two components: nmdaLTP and vdccLTP. The present results indicate that vdccLTP does not require long trains of high-frequency activity, rather it can be induced by stimuli patterned after physiological activity. Further, we have shown that when NMDARs are antagonized pharmacologically both forms of LTP are effectively blocked when L-TBS is used to induce LTP (Fig. 3). Thus, in experiments in which NMDAR antagonists are used to antagonize nmdaLTP, investigators may find that vdccLTP does not require long trains of high-frequency activity, which may be similar to the level in the present experiment. Thus even when NMDARs are experimentally antagonized, vdccLTP may be induced in the behaving animal. However, it seems that when NMDAR antagonists are administered, at least a partial block of vdccLTP may occur since the depolarizing current normally supplied by the NMDARs is no longer present. This type of indirect partial blockage of vdccLTP may confound results from behavioral and electrophysiological experiments as these forms of LTP have disparate mechanisms and functions.

In the intact animal CA1 pyramidal cells fire in complex spike bursts that are phase locked to the theta-rhythm during behavior that is correlated with learning (Hill 1978; Ranck 1973). In the experiments described in this paper, we have used TBS of varying lengths to approximate this innate bursting pattern. The results of this study indicate that S-TBS results in an nmdaLTP as it was unaffected by nifedipine and completely blocked by APV (Fig. 2). This form of LTP has been implicated in STM (<21 h) in a hippocampal-dependent behavioral task (Borroni et al. 2000; Woodside et al. 1999). Further, L-TBS induced a compoundLTP composed of nmdaLTP and vdccLTP (Fig. 3). L-TBS was meant to mimic more prolonged complex spike bursting such as might occur when an animal was subjected to intensive or over-training (Barnes 1979). VdccLTP has recently been implicated in LTM (>7 days) in behaving animals performing a spatial task (Borroni et al. 2000; Woodside et al. 1999). Thus it seems that more intense or prolonged complex spike activity could result in a form of plasticity implicated in LTM.

The application of multiple S-TBS trains further supports the idea that a single S-TBS results in nmdaLTP. The first S-TBS resulted in a similar LTP in both the control and the nifedipine groups (Figs. 7 and 9). Nifedipine had no effect on LTP induced by the first S-TBS train indicating that VDCCs are not involved in LTP induced by this stimulation (Fig. 7). As the number of S-TBS increased, a difference between the amount of LTP in the control and nifedipine groups became evident (Figs. 7–9). In the nifedipine group, following the second S-TBS, the amount of STP to each subsequent S-TBS remained approximately the same (Fig. 8). In all groups the amount of STP decreased almost linearly until the fourth S-TBS train (Fig. 8). The amount of STP induced in either nifedipine or control conditions was similar following each S-TBS train. Our measure of synaptic potentiation across the first 5 min of the post–S-TBS period likely includes mechanisms associated with posttetanic potentiation (PTP) and STP. PTP is a largely presynaptic mechanism that underlies a short lasting (~0.05–4 min) potentiation (Schulz and Fitzgibbons 1997). The most often cited mechanism for PTP induction is elevation of presynaptic calcium and sodium. The end result of the elevation of these two ions intracellularly is an increase in neurotransmitter vesicle release probability (Fischer et al. 1997). PTP is unaffected by APV, further indicating strictly presynaptic mechanisms (Swandulla et al. 1991). STP is not well understood but is generally accepted to result from a transient elevation of intracellular calcium and activation of serine/threonine kinases (Malenka et al. 1988; Schulz and Fitzgibbons 1997). Since STP was not produced following delivery of the fourth S-TBS train in control or nifedipine conditions, the mechanisms of STP become saturated with repeated LTP induction. Our results indicate that the mechanisms of STP and LTP may overlap. In contrast, Schulz and...
Fitzgibbons (1997) have found that saturation of LTP in high calcium medium does not always occlude STP induction. The high calcium medium or the increased number of high-frequency stimuli used to saturate LTP in the latter experiment could explain the difference in results.

Multiple S-TBS in the control group resulted in a much larger degree of LTP as compared with the nifedipine group (Fig. 9). The nifedipine group reached near maximal potentiation after one or two theta bursts, while the control group did not reach maximum magnitude until after the third theta burst (Figs. 7 and 9). This is best explained by the recruitment of VDCCs in the control group and the lack of participation of VDCCs in the nifedipine group. It seems that by the second or third theta burst the threshold for VDCC activation was reached. It is likely that as nmdaLTP is induced and the postsynaptic response to a given presynaptic signal increases, the threshold for VDCC activation is approached.

The present results indicate that when nmdaLTP is repeatedly induced, eventually a compoundLTP (nmdaLTP and vdccLTP) results. Given the recent evidence that in hippocampal area CA1 nmdaLTP may mediate STM (<21 h) while vdccLTP may mediate LTM (>7 days), it appears that moderate presynaptic activity would normally result only in STM (nmdaLTP). If further moderate presynaptic activity occurred, the STM (nmdaLTP) could then be converted to a LTM (compoundLTP).

The hippocampus is thought by some to be primarily involved in the encoding of STM or working memory. These STM would then later be transferred to various cortical targets (Teyler and DiScenna 1986). Cortical LTP is mediated by NMDARs and VDCCs and can by induced by physiologically relevant patterned stimulation of CA1, which results in LTP within its rhinal cortical targets (CousSENS and Otto 1998).

High-frequency tetanic stimulation of hippocampal neurons can also result in LTP in the prelimbic area of the prefrontal cortex (Laroche et al. 1990). If both cortical LTP and hippocampal LTP share similar mechanisms, it would not be surprising if they both subserved similar functions. However, in at least some cortical areas, LTP seems to have dissimilar characteristics (Teyler 1989). Therefore it is possible that LTP in different brain areas is a variation on a theme and that each variation might play a unique role in learning and memory. The behavioral experiments that have been conducted on the role of nmdaLTP and vdccLTP have utilized systemic injections rather than localized ventricular or hippocampal infusion. As a result, the hippocampal versus neocortical site of action cannot be determined.

If vdccLTP does subserve LTM (>7 days), why then is this type of plasticity present in the hippocampus, a structure that is thought to serve primarily to encode and store only STM (Barnes 1979)? It has been suggested that the role of the hippocampus is to record and store an “index” of cortical targets activated during experiential events (Teyler and DiScenna 1986). This index could then be reactivated to recall prior events or for the recognition of previously experienced information. Presumably, the encoded index would need to remain intact either permanently or until such time as the experiential event could be transferred to the cortex and become independent of the hippocampus (Teyler 1989). The association of cortical areas likely occurs by the induction of cortical LTP. VdccLTP could be the primary mechanism that serves to “permanently” encode an index of activated cortical targets necessary for recall of experiential information. Support for the permanence of vdccLTP in the hippocampus comes from experiments in long-term depression (LTD) or depotentiation. LTD is a long-lasting decrease in synaptic efficacy that is induced normally with long trains of low-frequency stimuli; alternatively similar stimuli can depotentiate or reverse potentiated responses (Martinez et al. 1997; Morgan et al. 2001).

Significantly nmdaLTP and not vdccLTP can be readily depotentiated, perhaps suggesting that nmdaLTP could be quickly and easily reversed, while vdccLTP could have a greater relative permanence, as it cannot be readily depotentiated. Alternatively, since cortical representations themselves are quite plastic, vdccLTP in the hippocampus may be required to accurately reference the hippocampal index to a malleable cortical representation (Buonomano and Merzenich 1998). One way to begin to understand the precise role of these forms of LTP in the hippocampus and/or the cortex might be to perform a variety of behavioral tasks in which the various forms of LTP are selectively antagonized in various independent loci.

Our experiments confirm that theta patterned activity is sufficient to induce LTP in area CA1. Further, short bursts of this activity induced only nmdaLTP, while longer bursts resulted in a compoundLTP consisting of nmdaLTP and vdccLTP components. In the intact animal, these two forms of LTP act synergistically with nmdaLTP always being induced prior to vdccLTP. Experimentally, vdccLTP can only be induced in isolation by high-intensity long theta patterned activity in the presence of APV, a condition that never occurs in the intact animal. These results have serious implications for experiments in which nmdaLTP is selectively antagonized, as both forms of LTP may effectively be blocked. Such implications have far reaching consequences when considering any distinct role that nmdaLTP or vdccLTP may have in learning and memory.

This work was supported in part by National Institute of Mental Health Grant MH-57892.

REFERENCES


J Neurophysiol • VOL 86 • SEPTEMBER 2001 • www.jn.org


