Neuroscience 126 (2004) 83–94

SUBTHRESHOLD CONTRIBUTION OF *N*-METHYL-D-ASPARTATE RECEPTORS TO LONG-TERM POTENTIATION INDUCED BY LOW-FREQUENCY PAIRING IN RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS

N. V. KRASTENIAKOV, M. MARTINA AND R. BERGERON*

Department of Psychiatry, Department of Cellular and Molecular Medicine, University of Ottawa and Ottawa Health Research Institute, Ottawa, Ontario, Canada K1Y 4E9

Abstract—Long-term potentiation (LTP) is a use-dependent and persistent enhancement of synaptic strength. In the CA1 region of the hippocampus, LTP has Hebbian characteristics and requires precisely timed interaction between presynaptic firing and postsynaptic depolarization. Although depolarization is an absolute requirement for plasticity, it is still not clear whether the postsynaptic response during LTP induction should be subthreshold or suprathreshold for the generation of somatic action potential. Here, we use the whole-cell patch-clamp technique and different pairing protocols to examine systematically the postsynaptic induction requirements for LTP. We induce LTP by changes only in membrane potential while keeping the afferent stimulation constant and at minimal levels. This approach permits differentiation of two types of LTP: LTP induced with suprathreshold synaptic responses (LTP_{AP}) and LTP induced with subthreshold excitatory postsynaptic current (EPSCs; LTP_{EPSC}). We found that LTP_{AP} (>40%) required pairing of depolarization ($V_m \geq -40$ mV, for 40–60 s) with four to six (0.1 Hz) single synaptically initiated action potentials. LTP_{EPSC} was of smaller magnitude (<30%) and required pairing of depolarization to −50 mV (60 s) with six subthreshold EPSCs. The *N*-methyl-D-aspartate receptor (NMDAR) antagonists aminophosphonovaleric acid and 7-chlorokynurenic acid consistently blocked LTP_{EPSC} but were ineffective in preventing LTP_{AP}. Robust, NMDAR-independent LTP is obtained by stronger postsynaptic depolarization that converts the EPSCs to suprathreshold somatic action potentials. Purely NMDAR-dependent LTP is obtained by pairing mild somatic depolarization with subthreshold afferent pulses to the postsynaptic cell. Our results indicate that the degree of postsynaptic depolarization in the presence of single afferent pulses determines the type and magnitude of LTP.

*Corresponding author. Tel: +1-613-729-0722; fax: +1-613-729-1266.
E-mail address: rbergeron@ohri.ca (R. Bergeron).

Abbreviations: ACSF, artificial cerebrospinal fluid; AMPAR, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; APV, aminophosphonovaleric acid; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; LTD, long-term depression; LTP, long-term potentiation; LTP_{AP}, LTP induced with action potentials; LTP_{EPSC}, LTP induced with subthreshold excitatory postsynaptic currents; NMDAR, *N*-methyl-D-aspartate receptor; VDCC, voltage-dependent calcium channel; V_m, membrane potential; 7-Cl-KYN, 7-chlorokynurenic acid.

Key words: action potential, depolarization, EPSC, glutamate, patch clamp, 7-chlorokynurenic acid.

Long-term potentiation (LTP) of synaptic transmission in the mammalian hippocampus is a model for studying the cellular and molecular mechanisms of learning and memory (Bliss and Lømo, 1973; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). LTP is consistent with the theoretical assumption that the brain stores information in a form of use-dependent synaptic modification (Hebb, 1949; Brown et al., 1990). In the Schaffer collateral/commissural synapse onto CA1 pyramidal cells, LTP and its counterpart long-term depression (LTD) are Hebbian and require *N*-methyl-D-aspartate receptor (NMDAR) activation and elevation of postsynaptic calcium (Lynch et al., 1983; Malenka et al., 1988; Brown et al., 1990; Cummings et al., 1996).

LTP induction requires two events: synapse activation accompanied by transmitter release and depolarization of the postsynaptic membrane (Kelso et al., 1986; Malinow and Miller, 1986; Bliss and Collingridge, 1993). The requirement for sufficient membrane depolarization reflects the fact that for synaptic modifications to take place cooperative interaction between multiple afferents must be present (McNaughton et al., 1978; Lee, 1983; Debanne et al., 1996). This type of depolarization-mediated 'cooperativity' has been demonstrated in the hippocampus by increasing the number and rate of discharging presynaptic neurons or by directly depolarizing the postsynaptic cell (McNaughton et al., 1978; Lee, 1983; Kelso et al., 1986; Malinow and Miller, 1986; Gustafsson et al., 1987; Debanne et al., 1996). However, the degree of depolarization during induction alters the postsynaptic action potential threshold, which in turn may influence the specific mechanism of synaptic strengthening (Scharfman and Sarvey, 1985; Magee and Johnston, 1997; Markram et al., 1997; Thomas et al., 1998; Pike et al., 1999; Sabatini et al., 2002). On the other hand, it has been suggested that the degree of postsynaptic depolarization during induction may determine the direction of synaptic change: mild depolarization leads to LTD, while LTP is induced with stronger depolarization (Kerr and Abraham, 1995; Goda and Stevens, 1996; Feldman et al., 1998; Ngezahayo et al., 2000; Shouval et al., 2002). Thus, the induction requirements for plasticity in CA1 pyramidal cells remain unclear.

The 'pairing protocols' for LTP induction allow better control over the postsynaptic voltage during coincidental

0306-4522/04$30.00+0.00
doi:10.1016/j.neuroscience.2004.03.034

presynaptic and postsynaptic activity. A 'pairing protocol' usually couples a constant rate of afferent stimulation with a steady depolarization, provided by the somatic intracellular or patch electrode (Kelso et al., 1986; Gustafsson et al., 1987; Stricker et al., 1996). Mechanistically, the depolarization provided by the voltage-clamp reflects the direct measurement of the 'cooperativity' requirement. However, this measurement is usually confounded by the depolarization produced by afferent stimulation, which may differ in duration, frequency or intensity. Reducing the rate and number of presynaptic pulses during induction allows better separation of synaptically evoked depolarization from that provided by voltage-clamp. Low-frequency pairing also permits the differentiation and direct examination of two different types of input specific LTP: LTP induced by pairing depolarization with postsynaptic action potentials (LTP_{AP}) and LTP induced by pairing depolarization with subthreshold excitatory postsynaptic currents (LTP_{EPSC}).

The goal of the present study is to examine the induction requirements for low-frequency LTP. During the course of the experiments we established the induction requirements and basic properties of LTP_{AP} and LTP_{EPSC}. We also describe the effect of NMDAR antagonism on both of them.

EXPERIMENTAL PROCEDURES

Preparation of hippocampal slices

All experimental procedures were conducted in strict accordance with the international, Canadian (CCAC) and institutional (OHRI) guidelines for the ethical use of laboratory animals. Every effort was made to minimize the number of animals used and their suffering. Coronal brain slices containing the hippocampus were obtained from Sprague–Dawley rats (21–28-days old). Prior to decapitation, the animals were anesthetized with isoflurane inhalation. The brain was removed and placed in an oxygenated (95% O_2/5% CO_2) physiological solution, artificial cerebrospinal fluid (ACSF) at 4 °C, containing (in mM) 126 NaCl, 2.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$ and 10 glucose. The osmolarity of the ACSF was adjusted to 300 mOsm and the pH to 7.2. A block containing the region of interest was prepared, and sections (300 μm) were obtained with a vibrating microtome (Leica VT 1000S, Wetzlar, Germany). The slices were stored for 1 h in an oxygenated chamber at room temperature before they were used for the experiments.

Data recording

For recording, the slices were transferred to a submerged-type recording chamber and held securely in place by a nylon mesh. Oxygenated and warmed (32–34 °C) ACSF was continuously superfused at a rate of 2.5 ml min^{-1}. Current- and voltage-clamp recordings were obtained with a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA, USA) under visual control using differential interference contrast and infrared video microscopy (IR-DIC; Leica DMLFSA).

Whole-cell recordings were obtained with borosilicate pipettes filled with a solution containing (in mM) 130 K-gluconate, 10 HEPES, 10 KCl, 2 $MgCl_2$, 2 ATP-Mg and 0.2 GTP-tris(hydroxymethil) aminomethane. The pH was adjusted to 7.2 and osmolarity to 280–290 mOsm. The pipettes had resistances of 5–8 MΩ when filled with that solution. Electroresponsive properties of neurons were studied in a current-clamp by applying 500 ms current pulses from rest. The amplitude of the current pulses was varied in fixed increments of 10 pA. The input resistance was estimated in the linear portion of the current-voltage plots. The evoked postsynaptic response was studied in voltage-clamp from −65 mV holding potential. Voltages have not been corrected for the theoretical liquid junction potential (approximately 10 mV). Aminophosphonovaleric acid (APV) was purchased from RBI (Natick, MA, USA) and 7-chlorokynurenic acid (7-Cl-KYN) was purchased from Tocris (Bristol, UK).

Single electrode whole-cell patch-clamp is prone to error when recording continuously from long multibranched neurons. To minimize space and point clamp errors and maximize uniformity throughout the experiments, the following measures were taken: First, for the analysis, only cells that met the following criteria were included: resting membrane potential (V_m) of −65 to −68 mV; spike amplitude of 85–120 mV; input resistance of 90–140 MΩ; spike-afterhyperpolarization of 4–9 mV amplitude. Second, recordings with series resistance higher than 20 MΩ were discarded. Third, recordings were discarded if during the experiment, V_m changed spontaneously by more than 2 mV, spike amplitude by more than 8 mV and series resistance by more than 10%. Fourth, excitatory postsynaptic current (EPSC) activation ($\tau<2$ ms) mediated mainly by amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) was monitored throughout each experiment.

Electrical stimulation

Schaffer collaterals/commissural afferents were stimulated by concentric bipolar electrodes (Frederick Haer Co, Brunswick, ME, USA) placed in the stratum radiatum. Baseline post-synaptic responses were evoked by 100 μs constant current pulses (0.1 Hz) delivered through a current output isolation unit (A-365; World Precision Instruments, Sarasota, FL, USA). Stimulation intensity (0.02–0.15 mA) was adjusted according to EPSC amplitudes and spike threshold.

LTP induction

High- and low-frequency LTP induction protocols were used. High-frequency induction (high-frequency LTP) consisted of single (100 or 250 ms) 100 Hz trains paired with or without postsynaptic depolarization ($V_m=-50$ mV). Low-frequency induction (low-frequency LTP) consisted of different types of somatic depolarization ($V_m=-60$, −50, −40, −30, −20 mV) paired with single afferent pulses (0.1 Hz). Stimulation intensity for all induction paradigms was the same as during the baseline period.

The central goal of the study was to examine the threshold requirements for LTP induced by single afferent pulses. To achieve this, it was necessary first to identify the electrophysiological parameters of CA1 pyramidal cells that may influence the impact of synaptic potentials. This was done with the help of current clamp I–V plots and depolarizing steps in the voltage-clamp mode applied from $V_m=-65$ mV. The action potential threshold (approximately −49 mV) was determined by the voltage preceding each spike. This value was relatively stable despite the irregular firing behavior of pyramidal cells. The threshold was further tested and confirmed by prolonged voltage clamp pulses, conditions that mimicked closely our low-frequency induction. In our suboptimal voltage clamp conditions the peak of sodium action potentials appeared at approximately −30 mV. Despite the usual variability EPSCs were clamped well and peak amplitudes were inversely related to the holding current (V_m from −65 to 0 mV). The holding current for the subthreshold induction ranged from 0.03 to 0.2 nA whereas suprathreshold induction required ≥0.2 nA.

The scope of the study is limited to the Hebbian LTP which requires co-occurrence of synapse activation by glutamate and postsynaptic depolarization (Kelso et al., 1986; Brown et al., 1990). At the Schaffer collateral CA1 synapses, LTP is input

specific and confined to the set of stimulated synapses (Brown et al., 1990; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). In control experiments, the Hebbian requirements and the locus of LTP expression were examined directly. Subthreshold or suprathreshold somatic depolarization of variable duration applied in the absence of afferent stimulation did not result in any lasting changes in EPSC amplitude. Both control and potentiated EPSCs were reduced to the same amplitude by 20 μM 6-nitro-7-sulfamoylbenzo(f)-quinoxaline-2,3-dione, proving that the synaptic enhancement was due to changes in AMPAR activity at the stimulated pathway. In addition, several EPSC parameters indicative for input specificity were monitored throughout the experiments. LTP was accepted as monosynaptic (input specific) if EPSCs before and after induction exhibited short constant latency, identical initial slope and were of similar duration.

Data analysis

The aim of our analysis was to measure EPSC potentiation and determine whether it is dependent on depolarization and type of postsynaptic response during induction. A procedure was followed that helped establish an upper limit of EPSC potentiation (maximal LTP) and eliminated from our sample complexities related to the phenomenon of EPSC-spike potentiation. Initially, stimulus location and intensity was adjusted such that active sodium conductances appeared at response amplitudes between 200 and 300 pA. Next the intensity was reduced by approximately 50% to elicit EPSC of approximately 100 pA. In our conditions, this procedure ensured an upper limit of EPSC potentiation of approximately 70%. We typically observed potentiation of 50–60% even during the most intense induction protocols and LTP of over 40% was considered in the text as maximal. In three experiments, LTP exceeded 100% but in those cases, EPSC amplitude did not reach a stable value and those recordings were discarded.

The peak EPSC amplitude was measured with respect to its baseline a few ms before the stimulus artifact. Before induction, an initial baseline of current data lasting from 20 to 30 min was collected. After the induction period, responses were monitored for at least 30 min post-treatment. In the graphs that summarize the average experiments, individual values represent the time-matched mean value normalized first to each cell baseline and then across all subjects. Sample traces in the summary graphs are 5-min averages taken just before induction and between 10 and 15 min after induction. The normalized values from all rats were used to calculate ±S.E.M. LTP magnitude was calculated from the averaged data 10 min before induction versus 20–30 min post-induction. The same time windows were used for statistical comparisons with two-tailed, unpaired Student's *t*-test ($P<0.01$).

RESULTS

Depolarization enables LTP during brief high-frequency afferent stimuli

Tetanic stimulation applied to the Schaffer collaterals is a standard procedure for LTP induction in CA1 pyramidal cells (Bliss and Collingridge, 1993). We examined the effects of depolarization on high-frequency LTP with the help of single, 100 Hz, brief (25 and 10 pulses) trains that did not induce LTP when applied alone. The synaptic responses during the tetanic stimulation were continuously monitored (Fig. 1A–D, lower traces). When the cell was clamped at −65 mV during the trains, we did not observe potentiation of the EPSC (Fig. 1A, C). However, when the cell was clamped at −50 mV for the duration of the trains, potentiation invariably was observed (Fig. 1B, D). Pooled data illustrate that LTP was obtained only when the trains were paired with somatic depolarization to −50 mV (Fig. 1E). In both cases, potentiation was significant (57.43±8.4%, $n=5$, $P<0.01$ for a 25 pulse train and 52.31±7.6%, $n=4$, $P<0.01$, for a 10 pulse train) and of maximal magnitude (see Experimental Procedures). Stronger depolarization than that provided by the afferent stimulation must be therefore required for enabling LTP during brief high-frequency synaptic stimulation.

We observed that for LTP induction, the cell must fire prolonged trains of action potentials. We also noticed that in different cells, the bursts were of variable duration. The prolonged action potential discharge during the train led to the buildup of additional depolarization (Fig. 1B, D, lower trace). Thus, depolarization enabled the cell to fire persistently and at levels well above the applied holding potential. The after-depolarization observed here was similar to that reported previously for hippocampal (Jensen et al., 1996) and cortical (Sjöström et al., 2001) pyramidal neurons. The mechanism responsible for the build-up of membrane
after-depolarization involves interaction between the membrane time constant and voltage-gated persistent Na^+ currents (Azouz et al., 1996; Jensen et al., 1996). The spike after-depolarization buildup during high-frequency stimulation may have contributed to LTP as previously shown for cortical pyramidal neurons (Sjöström et al., 2001).

From the high-frequency LTP experiments summarized in Fig. 1, we conclude that: 1) somatic depolarization enables LTP under conditions where the afferent stimulation alone is insufficient; 2) lowering the threshold for postsynaptic spiking is one possible mechanism depolarization may have contributed to LTP; 3) LTP induction is associated with prolonged trains of action potentials, which lead to the buildup of prolonged after-depolarization; 4) postsynaptic voltage requirements for LTP cannot be determined precisely with the help of high-frequency stimulation due to the variability in postsynaptic spiking and more importantly due to the variability in amplitude and duration of spike after-depolarization.

Minimal pairing requirements for low-frequency LTP of maximal magnitude

We used low-frequency pairing protocols to examine the postsynaptic voltage requirements for LTP without the confounding effect of massive, high-frequency induced spike after-depolarization. We tested a number of pairing protocols that differed in duration (1, 5 and 10 min), degree of depolarization ($V_m=-60$, −50, −40, −30, −20 and 0 mV), and frequency (0.05, 0.1 and 0.2 Hz) of afferent stimulation (data not shown). During these experiments, it was observed that LTP was most easily obtained when the postsynaptic cells fired action potentials during induction (LTP_{AP}). It was also determined that 60 s of depolarization during which the postsynaptic cell fired consistently in response to the afferent pulses (0.1 Hz) resulted in LTP_{AP} of maximal magnitude. Pairing depolarization to −40 mV with six, five and four single synaptically initiated action potentials for 60, 50, and 40 s respectively, resulted in LTP_{AP} of maximal

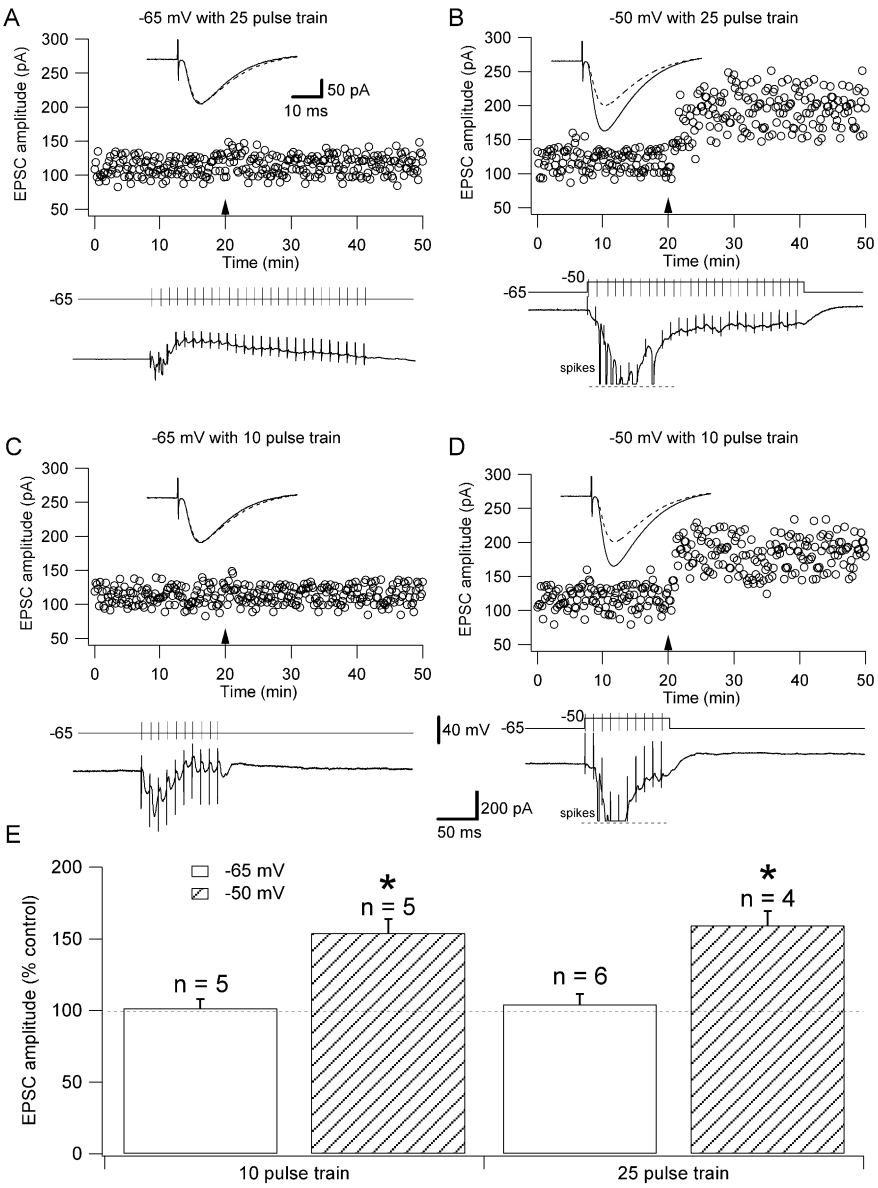

Fig. 1. Effects of postsynaptic depolarization on brief, high-frequency synaptic stimulation. (A) failure of LTP during high-frequency afferent stimulation. Individual experiment in which single train (25 pulses, 100 Hz) was given to the Schaffer collaterals. Top graph shows response amplitudes before and after the train (arrow). Induction protocol and synaptic responses during induction (lower traces) are shown. Inset, superimposed 5-min response averages taken immediately prior to (dotted line) and between 10 and 15 min after induction. (B) Depolarization to −50 mV co-applied with the train results in LTP. Prolonged firing and spike after-depolarization buildup (lower trace) during induction seems necessary for high-frequency LTP. (C) Similar results were obtained with 10-pulse train (100 Hz). Synaptic stimulation alone did not induce any potentiation. (D) Postsynaptic spikes and spike after-depolarization rescue LTP induced by pairing 10-pulse train with depolarization to −50 mV. (E) Pooled data illustrate the effects of depolarization to −50 mV on LTP during brief high-frequency afferent stimulation. Error bars indicate S.E.M.

magnitude (Fig. 2A–C). Potentiation was: 52.86±7.2%, $n=7$, $P<0.01$, for six action potentials; 52.23±7.8%, $n=6$, $P<0.01$, for five action potentials; 51.1±7.1%, $n=5$, $P<0.01$, for four action potentials (Fig. 2F, G). Pairing −40 mV with three and two action potentials (Fig. 2D, E) for 30 and 20 s, resulted from rapid reduction of LTP_{AP} size for three action potentials (21.35±9.1%, $n=5$, $P<0.01$), to complete loss of potentiation for two action potentials (1.27±5.6%, $n=4$; Fig. 2F, G). We conclude that the minimal requirements for obtaining LTP_{AP} of maximal magnitude is $V_m=-40$ mV paired with four to six synaptically initiated single action potentials (0.1 Hz).

Postsynaptic voltage and the induction of LTP_{AP}

The postsynaptic voltage requirements for LTP_{AP} were examined by pairing depolarization of constant duration (60 s) but of different amplitude ($V_m=-40$, −30 and −20 mV) with six suprathreshold synaptic responses (0.1 Hz;

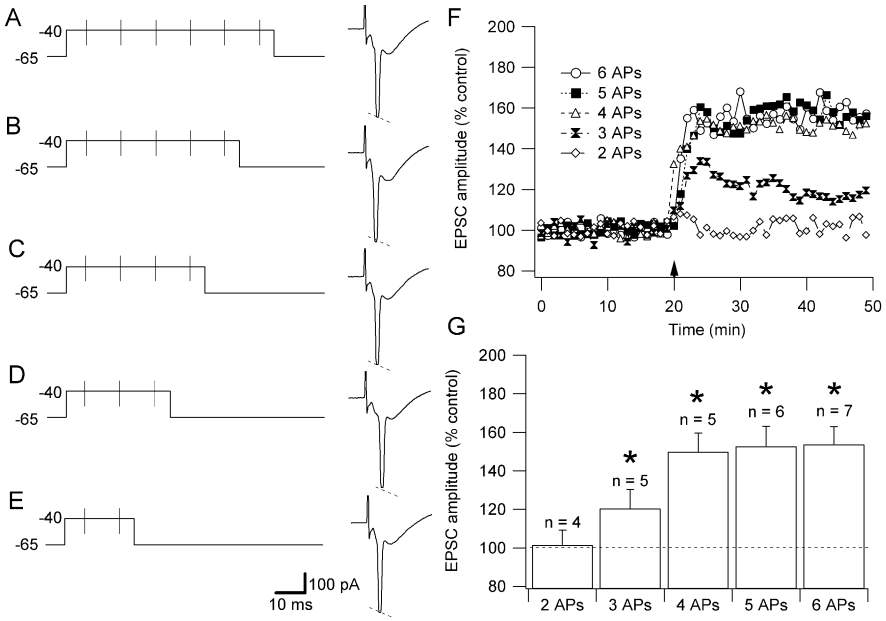

Fig. 2. Minimal pairing requirements for LTP of maximal magnitude. (A) Pairing depolarization to −40 mV with six single (0.1 Hz) suprathreshold synaptic responses leads to LTP of maximal magnitude. Low-frequency induction protocol and a whole cell single response during induction are shown. (B–E) Four different pairing protocols and representative synaptic responses. Depolarization to −40 mV paired with suprathreshold synaptic responses, sequentially reduced from five to two. (F) Summary graph showing potentiation time course under the different induction conditions. (G) Pooled data indicate potentiation for the different pairing protocols. Pairing depolarization to −40 mV with four, five or six synaptically initiated action potentials resulted in LTP of maximal magnitude. Reducing further the number of suprathreshold responses paired with depolarization to three and two led to gradual loss of potentiation.

Fig. 3A–D). In all three conditions, potentiation was of similar and maximal magnitude: 52.86±7.2%, n=7, P<0.01, for −40 mV; 57.17±8.1%, n=5, P<0.01, for −30 mV and 58.22±8.9%, n=5, P<0.01 for −20 mV, respectively (Fig. 3D). These results indicate that once the threshold for action potentials is reached, very little or no additional potentiation is added even when the cell is further depolarized. In the presence of membrane depolarization ($V_m \geq -40$), the action potential threshold is also the threshold for obtaining maximal LTP_{AP}.

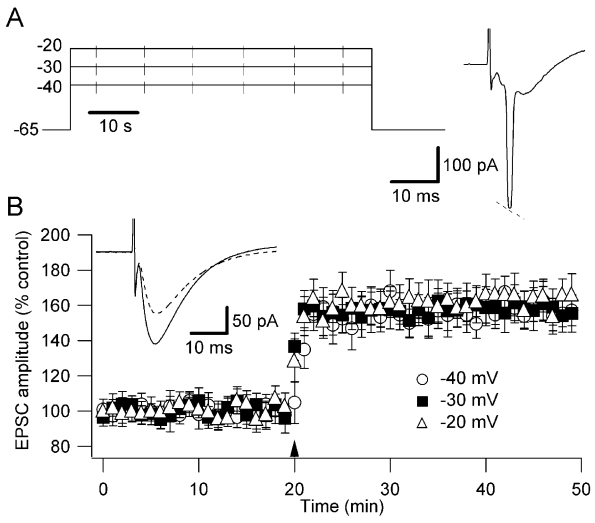

Fig. 3. Membrane voltage and the induction LTP_{AP}. Pairing three different types of somatic depolarization with suprathreshold synaptic responses are used to examine the effects of V_m on LTP_{AP}. (A) Pairing −20, −30 and −40 mV with six single suprathreshold responses. On the right, single synaptic response during induction from sample experiment. (B) Summary graph shows potentiation magnitude for the different induction protocols. There is no significant difference in LTP_{AP} under the three induction conditions. Inset, superimposed averaged traces showing the response before (dotted line) and after pairing from sample experiment.

Pairing postsynaptic depolarization with subthreshold EPSCs results in LTP of submaximal magnitude (LTP_{EPSC})

It has been demonstrated that blockade of the postsynaptic somatic action potential does not prevent the induction of LTP (Kelso et al., 1986; Golding et al., 2002). Although this finding indicates that the generation of somatic sodium spikes is not required for LTP, little is known about LTP obtained with depolarization that is subthreshold for postsynaptic spikes. Here we paired small depolarization with subthreshold EPSCs to examine the properties of LTP obtained in the absence of sodium spikes (LTP_{EPSC}; Fig. 4). Pairing somatic depolarization to −60 mV with six EPSCs (0.1 Hz) did not result in significant potentiation (2.79±4.7%, n=7; Fig. 4A, D). Depolarization to −50 mV was associated with two different types of postsynaptic response during the afferent shocks: either EPSCs or action potentials (Fig. 4B, C). However, the action potentials in most cases did not persist during the entire induction time and a significant number of failures were observed. To control for this difference, we analyzed the cells that fired during induction separately from the cells that did not

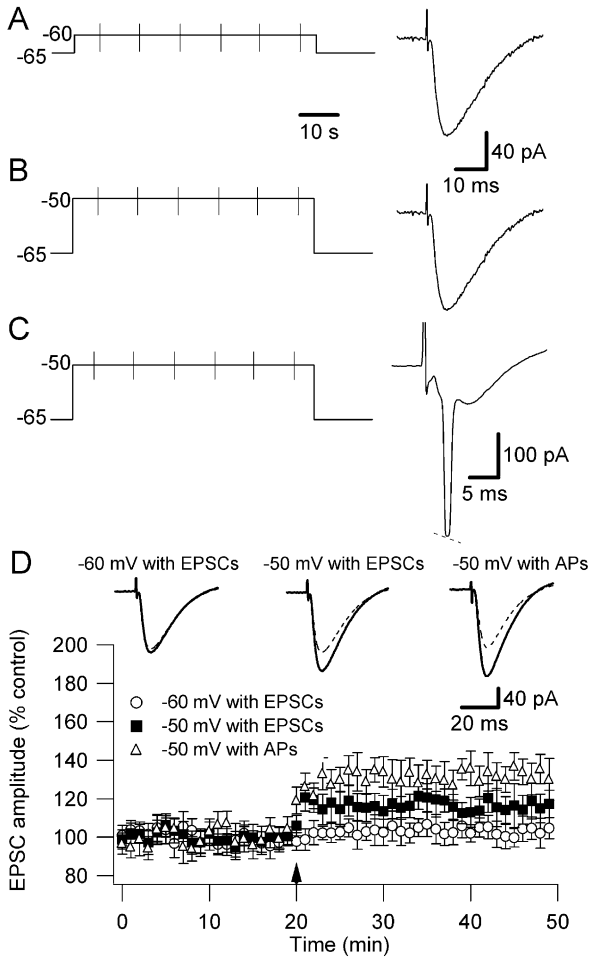

Fig. 4. Pairing postsynaptic depolarization with subthreshold EPSCs results in LTP_{EPSC}. (A) Pairing depolarization to −60 mV with six subthreshold EPSCs. Induction protocol and single synaptic response during induction from sample experiment are shown. (B and C) Pairing postsynaptic depolarization to −50 mV with single afferent pulses may result in subthreshold or suprathreshold responses. (B) Pairing −50 mV with six subthreshold EPSCs. (C) Pairing −50 mV with six suprathreshold EPSCs. (D) Summary graph shows potentiation time course under the three induction conditions. Insets, averaged traces from individual experiments represent the evoked EPSCs before (dotted lines) and after pairing.

fire. Pooled data indicate that for both groups, potentiation was present: 16.4±5.9%, n=5, P<0.01, for the purely LTP_{EPSC} group and 29.64±7.9%, n=6, P<0.01, for the spiking group (Fig. 4B–D).

We found that the size of LTP_{EPSC} obtained with −50 mV is approximately 16%. Since the upper limit of LTP in our conditions is approximately 70%, LTP_{EPSC} (V_m=−50 mV) magnitude translates into 20–25% of total potentiation. LTP_{EPSC} was found to be voltage-dependent since depolarization to −60 mV did not induce any potentiation while at V_m=−50 mV potentiation was maximal. With further increase of the depolarization, pure LTP_{EPSC} was more difficult to obtain due to the appearance of postsynaptic spiking. These results suggest that postsynaptic firing is not a prerequisite for LTP and synapse modification can take place under conditions where local depolarization and synaptic activation coexists with enhanced perisomatic inhibition (Yuste and Tank, 1996; Häusser et al., 2000; Golding et al., 2002).

The effect of NMDAR antagonists APV and 7-Cl-KYN on LTP_{AP} and LTP_{EPSC}

Next we examined the effects of APV (50 μM) on LTP_{AP} and LTP_{EPSC} (Fig. 5). During the experiments, it was observed that although APV did not change baseline EPSC amplitude, it increased the threshold for action potential during depolarization. Pyramidal cells, voltage clamped at −40 mV, usually spike in control ACSF in response to afferent stimulation (Figs. 2 and 3). In the presence of APV, approximately 50% of the cells did not spike (n=6, data not shown). Depolarizing the cell to −50 mV was often associated with irregular spiking in response to the stimulus. The occasional spiking was absent in the presence of APV (n=8, data not shown). The effects of APV on the action potential threshold are consistent with a reduction of inward cationic currents mediated by NMDAR (Mayer et al., 1984; Nowak et al., 1984). In the pooled data for the effects of APV on LTP_{EPSC}, cells that did not fire during depolarization to −40 mV were analyzed together with the cells depolarized to −50 mV (Fig. 5A, B).

In the presence of APV, no potentiation was observed when the postsynaptic cell did not spike during depolarization. Pairing depolarization (V_m=−60, −50 and −40 mV) with six subthreshold EPSCs in the presence of APV was found to have no lasting effect on EPSC amplitude (0.62±5.7%, n=4 for −60 mV and 1.27±5.8%, n=6 for −50/−40 mV, respectively; Fig. 5A, C). However, APV did not prevent LTP induced by pairing depolarization to −40 mV with six synaptically initiated spikes (LTP_{AP}; 51.17±7.6%, n=4, P<0.01; Fig. 5A, B). The magnitude of LTP_{AP} obtained in the presence of APV was not significantly different from that obtained in control ACSF (Fig. 5C). With stronger background depolarization (V_m≥−40 mV), APV cannot prevent postsynaptic firing and the induction of maximal LTP_{AP}. Thus, at subthreshold induction conditions, APV through its action on NMDARs increases the threshold for LTP_{AP} and reliably blocks LTP_{EPSC}.

To our knowledge this is the first study to report the resistance of LTP induced by single afferent pulses to NMDAR antagonism. Therefore, we further examined the role of NMDARs in the induction of both types of LTP with the help of 7-Cl-KYN, a selective antagonist for the strychnine-insensitive glycine site of NMDAR (Kemp et al., 1988; Kemp and Priestley, 1991). Glycine is required for NMDAR activation and antagonist blockade of the glycine site prevents NMDAR-mediated responses (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Lerma et al., 1990). 7-Cl-KYN (3 μM) applied during the pairing mimicked the effect of APV on both types of LTP. Blockade of the glycine site during induction eliminated the potentiation obtained by pairing depolarization with subthreshold EPSCs (1.1±5.9%, n=4 for −60 mV and 0.89±6.2%, n=5 for −50 mV; Fig. 5B, C). Increasing depolarization to −40 mV in most cases did not prevent postsynaptic firing and the induction of strong LTP (51.67±8.9%, n=5, P<0.01;

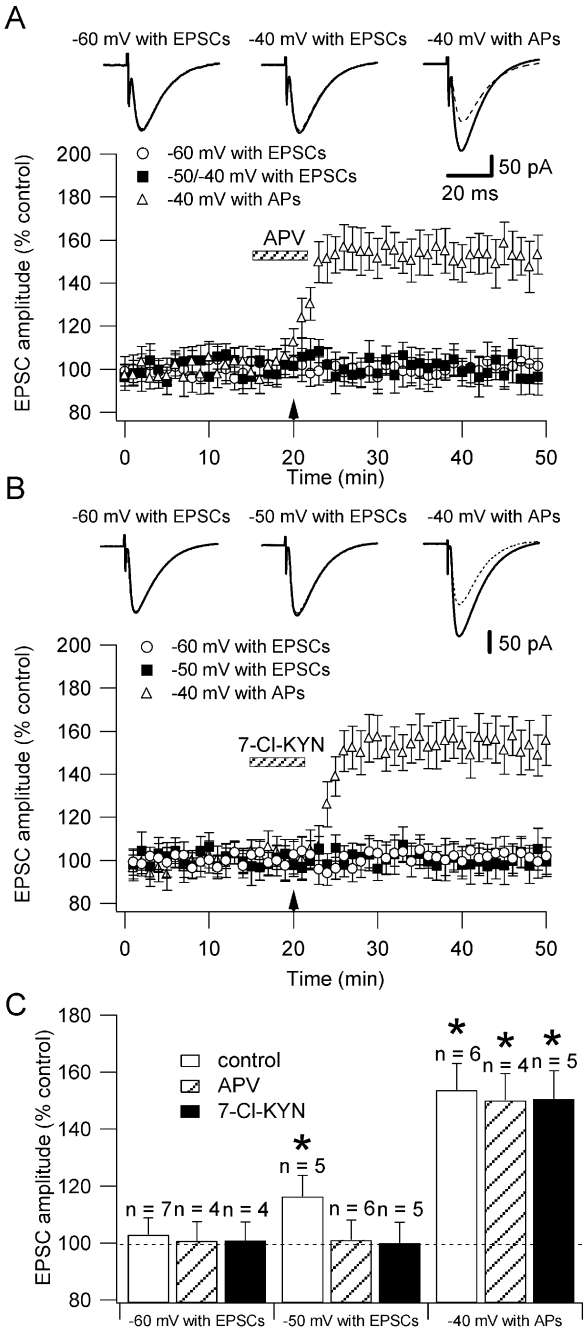

Fig. 5. Blockade of NMDA receptors prevents LTP_{EPSC} but has no effect on LTP_{AP}. (A) Graph showing the effects of APV on LTP_{EPSC} and LTP_{AP}. APV blocks LTP induced by pairing depolarization with subthreshold EPSCs but is ineffective in preventing LTP induced by pairing depolarization with suprathreshold synaptic responses. Insets, averaged sample traces show EPSCs before (dotted lines) and after LTP induction for the three pairing protocols. (B) 7-Cl-KYN (3 μM) has similar to APV effects on LTP_{EPSC} and LTP_{AP}. Pairing depolarization with subthreshold EPSCs in the presence of 7-Cl-KYN did not result in potentiation. 7-Cl-KYN did not prevent LTP induced by pairing depolarization with postsynaptic action potentials. (C) Summary data show the magnitude of potentiation in control slices and in the presence of APV and 7-Cl-KYN. Although pairing to −60 mV was not associated with potentiation, NMDAR antagonists were also tested with this protocol.

Fig. 5B, C). In the cases where postsynaptic spiking was absent, LTP did not develop ($n=3$, data not shown). Blockade of LTP_{EPSC} with 3 μM 7-Cl-KYN, which is in the concentration range (0.3–10 μM) previously shown to block the glycine site of NMDARs (Kemp et al., 1988; Izumi et al., 1990; Kemp and Priestley, 1991; Parsons et al., 1993) indicates that the glycine site activation is required for LTP_{EPSC}.

From the experiments with APV and 7-Cl-KYN we conclude that: 1) LTP obtained by pairing depolarization with EPSCs (LTP_{EPSC}) is purely NMDAR-dependent; 2) LTP_{EPSC} requires small depolarization (≤15 mV); 3) bath perfused NMDAR antagonists cannot prevent the development of input specific LTP when the depolarized cell fire action potentials in response to single afferent pulses; 4) NMDAR antagonism has no effect on the magnitude of LTP induced by pairing depolarization ($V_m \geq -40$ mV) with synaptically initiated single action potentials.

Thus, by pairing different degrees of somatic depolarization with single sub- or suprathreshold synaptic responses, two different types of LTP can be differentiated: the purely NMDAR-dependent LTP_{EPSC}, which is of submaximal magnitude and LTP_{AP}, being of maximal magnitude and resistant to NMDAR antagonism.

DISCUSSION

Pairing requirements for low-frequency LTP

The goal of the present study was to examine the induction requirements for LTP obtained by pairing depolarization with single afferent pulses. We found that: 1) LTP of maximal magnitude (>40%) was induced when depolarization ($V_m \geq -40$ mV, 40–60 s) was paired with four to six single, synaptically initiated action potentials (LTP_{AP}); 2) LTP_{AP} was NMDAR-independent; 3) pairing mild depolarization ($V_m = -50$ mV, 60 s) with six single subthreshold EPSCs resulted in LTP of submaximal magnitude (<30%; LTP_{EPSC}); 4) LTP_{EPSC} was purely NMDAR-mediated.

In agreement with previous reports (Kelso et al., 1986; Malinow and Miller, 1986; Gustafsson et al., 1987; Debanne et al., 1996; Magee and Johnston, 1997; Sjöström et al., 2001) we found that background depolarization enables high- and low-frequency LTP to be induced under conditions where those same afferent stimuli, when applied alone, did not induce any significant postsynaptic modification. In both types of induction, robust LTP was obtained only when the synaptically initiated depolarization, combined with the background somatic depolarization, was above the threshold for action potential. Our results further demonstrate that in the presence of prolonged depolarization, the number of afferent pulses required for LTP can be reduced to as low as four given at 0.1 Hz. Depolarizing the postsynaptic cell above the threshold for action potential is sufficient for the induction of maximal LTP. NMDAR-mediated component of LTP was obtained by pairing small depolarization with six single subthreshold EPSCs. Collectively, these results demonstrate that postsynaptic depolarization regulates the induc-

tion and magnitude of LTP during low-frequency afferent stimulation.

NMDARs and low-frequency LTP

NMDAR, often described as a Hebbian molecule, serves as a coincident detector allowing Ca^{2+} influx only when glutamate is present and the postsynaptic membrane is sufficiently depolarized (Bliss and Collingridge, 1993). Functional and calcium imaging studies have demonstrated that NMDARs are active and generate Ca^{2+} signals at potentials close to resting (Mayer et al., 1984; Regehr and Tank, 1994; Swadlow and Hicks, 1997; Mainen et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002). However, at potentials close to resting, LTP has been difficult to obtain and it was suggested that at that level of postsynaptic activity, LTD is more readily induced (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Ngezahayo et al., 2000; Shouval et al., 2002).

The present study demonstrates that pairing mild depolarization with subthreshold EPSCs results in the induction of purely NMDAR-dependent LTP (LTP_{EPSC}). This component is of relatively small magnitude (<30%) and can be prevented independently by APV and 7-Cl-KYN. The induction parameters of LTP_{EPSC} suggest that while membrane depolarization is required for NMDAR plasticity, clear separation of the NMDAR-dependent component of LTP can be obtained by keeping the synaptic stimulation subthreshold for the generation of somatic action potentials. These findings are in agreement with previous reports and demonstrate that somatic spiking is not necessary for NMDAR-mediated strengthening of the active synapses (Kelso et al., 1986; Golding et al., 2002).

Depolarizing the somatic V_m has been used routinely in plasticity studies for recruitment of synaptic NMDARs blocked at more hyperpolarized potentials (Kelso et al., 1986; Gustafsson et al., 1987; Stricker et al., 1996; Sjöström et al., 2001). Our observation that increasing membrane depolarization from −60 to −50 mV led to substantial increase of the NMDAR-mediated component of LTP is consistent with the recruitment of NMDARs relatively inactive at close to resting potentials. The mechanism of electrotonic recruitment of distal NMDARs along the somatodendritic axis was beyond the scope of the study. Accumulating evidence indicates however that enhancement of sodium and calcium channel activity and inactivation of potassium currents contribute to the electrogenesis necessary for activation of synaptic NMDARs (Stuart and Sakmann, 1995; Sjöström et al., 2001; Stuart and Hausser, 2001).

A potentially important finding of the present study is that we failed to observe LTD during our subthreshold and suprathreshold induction conditions. It has been demonstrated that LTD is produced in response to “weaker” prolonged low-frequency trains that insufficiently depolarize the post-synaptic membrane and fail to activate NMDARs (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Furthermore, it was proposed that clamping the postsynaptic cell between −60 and −52.5 mV would be sufficient to induce LTD while with more positive potentials LTP would be obtained (Shouval et al., 2002). LTD induction by pairing at even more depolarized potentials has also been reported (Feldman et al., 1998; Ngezahayo et al., 2000). The discrepancies between some of the above studies and our results are most likely due to the different induction parameters used. Specifically, the duration of depolarization used for LTP induction here is smaller to that used by most studies. Another potentially important difference is that we induce LTP by limited number of pairings. Pairing prolonged depolarization with four to six single afferent pulses (0.1 Hz) is sufficient for the development of input specific LTP. The degree of depolarization in the presence of afferent stimulation however, seems to be the most important factor for the induction and magnitude of LTP.

VDCCs (voltage-dependent calcium channels) and low-frequency NMDAR-independent LTP

The synaptic modifications associated with LTP are confined to the excitatory glutamatergic synapses located in dendrites and dendritic spines of CA1 pyramidal cells (Yuste and Denk, 1995; Malenka and Nicoll, 1999; Sabatini et al., 2002). The necessary signal for LTP is Ca^{2+}, which may enter the spine through NMDARs, VDCCs, Ca^{2+} permeable AMPA channels (Yuste et al., 1999) or be released from intracellular stores (Emptage et al., 1999).

In contrast to the input specific NMDAR-dependent LTP, NMDAR-independent LTP can be input specific (homosynaptic), not input specific (heterosynaptic) or a combination of both (Brown et al., 1990; Kullmann et al., 1992; Bliss and Collingridge, 1993; Hanse and Gustafsson, 1995; Chen et al., 1998). During induction, the input specificity of LTP may be affected by the intensity and type of afferent stimulation and the properties of postsynaptic Ca^{2+} signals. Since for the input specificity of LTP afferent stimulation is required, potentiation obtained without afferent stimulation is not input specific (Kullmann et al., 1992; Chen et al., 1998).

When LTP is obtained by tetanic stimulation, the rate and intensity of stimulation determine the form of LTP through activation of different postsynaptic mechanisms of plasticity. NMDAR-dependent LTP usually requires lower frequency (≤30 Hz) afferent stimulation (Grover and Teyler, 1990, 1992; Morgan and Teyler, 2001). Increasing the intensity of afferent stimulation increases the contribution of Ca^{2+} influx via VDCCs. Thus, very high-frequency (≥200 Hz) tetanization or high-intensity θ-burst stimulation induces potentiation by Ca^{2+} entry through L-type VDCCs (Grover and Teyler, 1990, 1992; Hanse and Gustafsson, 1995; Morgan and Teyler, 2001). Pairing studies have confirmed that VDCC-dependent LTP requires strong postsynaptic depolarization (Kullmann et al., 1992; Chen et al., 1998; Stricker et al., 1999). Our results are in agreement with these studies and further clarify the induction requirements for both types of LTP. To our knowledge, this study presents the first report of NMDAR-independent LTP induced by single afferent pulses. Thus, NMDAR-dependent LTP requires pairing of depolarization ($V_m \leq -50$ mV) with subthreshold synaptic stimulation

whereas NMDAR-independent LTP can be obtained by pairing depolarization ($V_m \geq -40$ mV) with suprathreshold stimulation. We have estimated the voltage requirements for LTP through separation of voltage clamp depolarization from that induced by afferent stimulation. This was achieved by reducing the frequency and duration of afferent stimulation. Furthermore, the low-frequency afferent rate allowed us to monitor the postsynaptic response during induction and conclude that NMDAR antagonism is ineffective when the afferent pulses discharge the depolarized pyramidal cell. Thus, the appearance of somatic action potentials during induction is a potential indicator for the occurrence, magnitude and mechanism of LTP. Taken together these findings suggest that NMDAR-independent LTP requires stronger depolarization and the generation of somatic action potentials, conditions most likely occurring during high-frequency tetanization (Grover and Teyler, 1990; Hanse and Gustafsson, 1995; Grover, 1998).

In the present study, low-frequency NMDAR-dependent and NMDAR-independent LTP were induced by single postsynaptic manipulation (depolarization). LTP obtained by postsynaptic current injection is a controlled way of LTP induction. Reducing the rate and number of afferent pulses circumvents a number of confounding mechanisms including altered transmitter release, temporal and spatial integration of EPSCs, buildup of synaptic and extrasynaptic glutamate, and saturation of uptake systems. Most of these mechanisms may affect the input specificity of LTP by recruitment of new release sites. In the present study, we minimized the possibility of heterosynaptic spread of potentiation by two additional measures. First, the number and intensity of afferent pulses were kept to minimal levels and second, potassium- and chloride-mediated inhibitory systems were kept intact. Thus, regardless of the mechanism, our results suggest that the locus of expression of synaptic modification for NMDAR-dependent LTP and NMDAR-independent LTP is postsynaptic and confined to the same set of activated inputs.

Previous studies have suggested that VDCC-dependent LTP and NMDAR-dependent LTP require distinct transduction cascades (Grover and Teyler, 1990; Cavus and Teyler, 1996). Pharmacological treatments with APV during high-frequency tetanization decrease the magnitude of potentiation suggesting that this LTP is a compound potentiation consisting of VDCC-mediated and NMDAR-mediated components (Grover and Teyler, 1990; Cavus and Teyler, 1996; Grover, 1998). In the present study, NMDAR antagonists had no effect on the magnitude of low-frequency NMDAR-independent LTP. This finding indicates that during supramaximal induction conditions postsynaptic Ca^{2+} levels are above the threshold for activation of NMDAR-coupled biochemical pathways. If this is the case, this result would suggest a cross-talk between Ca^{2+} influx through VDCCs and NMDAR-dependent mechanisms of plasticity. This interpretation is consistent with a common expression mechanism of NMDAR-independent and NMDAR-dependent LTP. Indeed, recent evidence suggests that the expression and maintenance of NMDAR-independent LTP appear to require enhancement of postsynaptic AMPAR function (Grover, 1998; Stricker et al., 1999).

Sodium channels, action potentials and low-frequency LTP

Distal and proximal dendrites of pyramidal neurons contain voltage-gated sodium channels, which main function seems to be the integration of excitatory synaptic input (Magee and Johnston, 1995; Golding and Spruston, 1998; Magee, 2000). Somatic depolarization, similar to that used in the present study, has been the tool for investigating the role of sodium channels in plasticity and in shaping the integrative properties of pyramidal neurons (Deisz et al., 1991; Magee and Johnston, 1995, 1997; Stuart and Sakmann, 1995; Markram et al., 1997; Andreasen and Lambert, 1999; Golding et al., 2002). Thus, activation of axosomatic sodium channels by depolarizing current injection amplifies dendritic excitatory postsynaptic potentials (EPSPs) while TTX prevents the depolarization-induced amplification and reduces the size of large EPSPs (Stuart and Sakmann, 1995; Andreasen and Lambert, 1999).

The contribution of sodium channels to synaptic plasticity has been established by demonstrating that activation of dendritic sodium channels provides the electrogenesis required for calcium influx through NMDARs and VDCCs during LTP (Magee and Johnston, 1997; Markram et al., 1997; Magee, 2000; Golding et al., 2002). Although in the present study we have examined the role of sodium channels in LTP indirectly and mostly in respect to the generation of somatic action potential, several observations were consistent with contribution of these channels to plasticity. First, the amount of potentiation was generally proportional to the degree of membrane depolarization. Second, potentiation reached its maximum only when the depolarization was paired with suprathreshold synaptic responses, conditions presumably reflecting full activation of axosomatic and dendritic sodium channels. Third, LTP induced by strong depolarization and somatic action potentials was least affected by NMDAR antagonists. These findings are consistent with other plasticity studies demonstrating the contribution of sodium channels to the generation of dendritic spikes and the spread of back-propagating action potentials (Jaffe et al., 1992; Magee and Johnston, 1995, 1997; Markram et al., 1997; Golding et al., 2002). Our results also confirm however, that depolarization and postsynaptic action potentials without afferent stimulation, does not lead to input specific, Hebbian plasticity (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Conti and Lisman, 2002).

The main source of postsynaptic depolarization under physiological conditions arises from the temporal and spatial integration of dendritic EPSPs (Cash and Yuste, 1998, 1999; Magee and Cook, 2000). Activation of unitary synaptic contacts however, seems to be too weak for plasticity induction and more than one presynaptic cell must fire simultaneously for the postsynaptic membrane to be depolarized sufficiently (Debanne et al., 1996). This is the essence of the 'cooperativity' requirement and is best supported by the fact that stronger LTP is obtained when the

number of discharging presynaptic fibers is increased (McNaughton et al., 1978; Lee, 1983; Debanne et al., 1996).

The back-propagating somatic action potential is another mechanism capable of providing the dendritic depolarization necessary for LTP (Stuart and Sakmann, 1994; Stuart et al., 1997). Action potentials initiated at the soma could function as an enabling signal for LTP by invading the distal dendrites and activating NMDARs and VDCCs (Magee and Johnston, 1997; Markram et al., 1997). Thus, pairing subthreshold EPSPs with postsynaptic action potentials induced by somatic current injection resulted in persistent changes in EPSP amplitude (Magee and Johnston, 1997; Markram et al., 1997). The compound depolarization from properly timed EPSPs and postsynaptic burst firing appears to be equivalent to the background depolarization used here and in other pairing studies. Indeed, it has been shown that postsynaptic bursting and complex spike bursting, which are common in the intact hippocampus, enable LTP under conditions where single postsynaptic spikes were ineffective (Thomas et al., 1998; Pike et al., 1999). Thus, the prolonged somatic depolarization may be a laboratory equivalent of the electrogenesis associated with pyramidal cell bursting in intact animals during exploratory and learning behaviors (Buzsáki, 1989; Buzsáki et al., 1996). This is consistent with the observation that in the presence of background depolarization, neither presynaptic nor postsynaptic bursting is necessary and LTP can be elicited by single synaptically evoked action potentials.

In summary, our results demonstrate the effects of membrane voltage on the induction, magnitude and type of LTP in CA1 pyramidal cells. They suggest that purely NMDAR-dependent LTP is obtained by pairing small membrane depolarization with subthreshold EPSCs. In the depolarized cell, synaptically evoked low-frequency somatic action potentials lead to robust LTP, of a form that is insensitive to NMDAR antagonists. Finally, in the presence of dendritic depolarization, NMDAR-mediated plasticity mechanisms can function independently from the neuron output regions and the somatic action potential.

Acknowledgements—This work was supported by the Canadian Institute of Health Research and the National Alliance for Research on Depression and Schizophrenia. We thank Dr. T. P. Hicks and Y. Gorfinkel for help with the manuscript and C. Metivier for technical assistance.

REFERENCES

Andreasen M, Lambert JD (1999) Somatic amplification of distally generated subthreshold EPSPs in rat hippocampal pyramidal neurones. J Physiol 519:85–100.

Azouz R, Jensen MS, Yaari Y (1996) Ionic basis of spike afterdepolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. J Physiol 492:211–223.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39.

Bliss TV, Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232:331–356.

Brown TH, Kairiss EW, Keenan CL (1990) Hebbian synapses: biophysical mechanisms and algorithms. Annu Rev Neurosci 13:475–511.

Buzsáki G (1989) Two stage model of memory trace formation: a role for "noisy" brain states. Neuroscience 31:551–570.

Buzsáki G, Penttonen M, Nadasdy Z, Bragin A (1996) Pattern and inhibition-dependent invasion of pyramidal cell dendrites by fast spikes in the hippocampus *in vivo*. Proc Natl Acad Sci USA 93: 9921–9925.

Cash S, Yuste R (1998) Input summation by cultured pyramidal neurons is linear and position-independent. J Neurosci 18:10–15.

Cash S, Yuste R (1999) Linear summation of excitatory inputs by CA1 pyramidal neurons. Neuron 22:383–394.

Cavus I, Teyler TJ (1996) Two forms of long-term potentiation in area CA1 activate different signal transduction cascades. J Neurophysiol 76:3038–3047.

Chen HX, Hanse E, Pananceau M, Gustafsson B (1998) Distinct expressions for synaptic potentiation induced by calcium through voltage-gated calcium and *N*-methyl-D-aspartate receptor channels in the hippocampal CA1 region. Neuroscience 86:415–422.

Conti R, Lisman J (2002) A large sustained Ca^{2+} elevation occurs in unstimulated spines during the LTP pairing protocol but does not change synaptic strength. Hippocampus 12:667–679.

Cummings JA, Mulkey RM, Nicoll RA, Malenka RC (1996) Ca^{2+} signaling requirements for long-term depression in the hippocampus. Neuron 16:825–833.

Debanne D, Gähwiler BH, Thompson SM (1996) Cooperative interactions in the induction of long-term potentiation and depression of synaptic excitation between hippocampal CA3-CA1 cell pairs *in vitro*. Proc Natl Acad Sci USA 93:11225–11230.

Deisz RA, Fortin G, Zieglgansberger W (1991) Voltage dependence of excitatory postsynaptic potentials of rat neocortical neurons. J Neurophysiol 65:371–382.

Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci USA 89:4363–4367.

Emptage N, Bliss TV, Fine A (1999) Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. Neuron 22:115–124.

Feldman DE, Nicoll RA, Malenka RC, Isaac JT (1998) Long-term depression at thalamocortical synapses in developing rat somatosensory cortex. Neuron 21:347–357.

Goda Y, Stevens CF (1996) Long-term depression properties in a simple system. Neuron 16:103–111.

Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. Neuron 21:1189–1200.

Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. Nature 418:326–331.

Grover LM (1998) Evidence for postsynaptic induction and expression of NMDA receptor independent LTP. J Neurophysiol 79:1167–1182.

Grover LM, Teyler TJ (1990) Two components of long-term potentiation induced by different patterns of afferent activation. Nature 347:477–479.

Grover LM, Teyler TJ (1992) *N*-methyl-D-aspartate receptor-independent long-term potentiation in area CA1 of rat hippocampus: input-specific induction and preclusion in a non-tetanized pathway. Neuroscience 49:7–11.

Gustafsson B, Wigström H, Abraham WC, Huang YY (1987) Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. J Neurosci 7:774–780.

Hanse E, Gustafsson B (1995) Long-term potentiation in the hippocampal CA1 region in the presence of *N*-methyl-D-aspartate receptor antagonists. Neuroscience 67:531–539.

Häusser M, Spruston N, Stuart GJ (2000) Diversity and dynamics of dendritic signaling. Science 290:739–744.

Hebb DO (1949) The organization of behaviour. New York: Wiley.

Izumi Y, Clifford DB, Zorumski CF (1990) Glycine antagonists block the induction of long-term potentiation in CA1 of rat hippocampal slices. Neurosci Lett 112:251–256.

Jaffe DB, Johnston D, Lasser-Ross N, Lisman JE, Miyakawa H, Ross WN (1992) The spread of Na^+ spikes determines the pattern of dendritic Ca^{2+} entry into hippocampal neurons. Nature 357:244–246.

Jensen MS, Azouz R, Yaari Y (1996) Spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. J Physiol 492:199–210.

Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529–531.

Kelso SR, Ganong AH, Brown TH (1986) Hebbian synapses in hippocampus. Proc Natl Acad Sci USA 83:5326–5330.

Kemp JA, Foster AC, Leeson PD, Priestley T, Tridgett R, Iversen LL, Woodruff GN (1988) 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. Proc Natl Acad Sci USA 85:6547–6550.

Kemp JA, Priestley T (1991) Effects of (+)-HA-966 and 7-chlorokynurenic acid on the kinetics of *N*-methyl-D-aspartate receptor agonist responses in rat cultured cortical neurons. Mol Pharmacol 39:666–670.

Kerr DS, Abraham WC (1995) Cooperative interactions among afferents govern the induction of homosynaptic long-term depression in the hippocampus. Proc Natl Acad Sci USA 92:11637–11641.

Kleckner NW, Dingledine R (1988) Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. Science 241:835–837.

Kovalchuk Y, Eilers J, Lisman J, Konnerth A (2000) NMDA receptor-mediated subthreshold $Ca^{(2+)}$ signals in spines of hippocampal neurons. J Neurosci 20:1791–1799.

Kullmann DM, Perkel DJ, Manabe T, Nicoll RA (1992) Ca^{2+} entry via postsynaptic voltage-sensitive Ca^{2+} channels can transiently potentiate excitatory synaptic transmission in the hippocampus. Neuron 9:1175–1183.

Lee KS (1983) Cooperativity among afferents for the induction of long-term potentiation in the CA1 region of the hippocampus. J Neurosci 3:1369–1372.

Lerma J, Zukin RS, Bennett MV (1990) Glycine decreases desensitization of *N*-methyl-D-aspartate (NMDA) receptors expressed in *Xenopus*-oocytes and is required for NMDA responses. Proc Natl Acad Sci USA 87:2354–2358.

Lynch G, Larson J, Kelso S, Barrionuevo G, Schottler F (1983) Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature 305:719–721.

Magee JC (2000) Dendritic integration of excitatory synaptic input. Nat Rev Neurosci 1:181–190.

Magee JC, Cook EP (2000) Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. Nat Neurosci 3:895–903.

Magee JC, Johnston D (1995) Synaptic activation of voltage-gated channels in the dendrites of hippocampal pyramidal neurons. Science 268:301–304.

Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. Science 275: 209–213.

Mainen ZF, Malinow R, Svoboda K (1999) Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. Nature 399:151–155.

Malenka RC, Kauer JA, Zucker RS, Nicoll RA (1988) Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science 242:81–84.

Malenka RC, Nicoll RA (1999) Long-term potentiation: a decade of progress? Science 285:1870–1874.

Malinow R, Miller JP (1986) Postsynaptic hyperpolarization during conditioning reversibly blocks induction of long-term potentiation. Nature 320:529–530.

Markram H, Lübke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275:213–215.

Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. Nature 309:261–263.

McNaughton BL, Douglas RM, Goddard GV (1978) Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. Brain Res 157:277–293.

Morgan SL, Teyler TJ (2001) Electrical stimuli patterned after the theta-rhythm induce multiple forms of LTP. J Neurophysiol 86: 1289–1296.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Ngezahayo A, Schachner M, Artola A (2000) Synaptic activity modulates the induction of bidirectional synaptic changes in adult mouse hippocampus. J Neurosci 20:2451–2458.

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462–465.

Parsons CG, Zong X, Lux HD (1993) Whole cell and single channel analysis of the kinetics of glycine-sensitive *N*-methyl-D-aspartate receptor desensitization. Br J Pharmacol 109:213–221.

Pike FG, Meredith RM, Olding AW, Paulsen O (1999) Rapid report: postsynaptic bursting is essential for "Hebbian" induction of associative long-term potentiation at excitatory synapses in rat hippocampus. J Physiol 518:571–576.

Regehr W, Tank D (1994) Dendritic calcium dynamics. Curr Opin Neurobiol 4:373–382.

Sabatini BL, Oertner TG, Svoboda K (2002) The life cycle of $Ca^{(2+)}$ ions in dendritic spines. Neuron 33:439–452.

Scharfman HE, Sarvey JM (1985) Postsynaptic firing during repetitive stimulation is required for long-term potentiation in hippocampus. Brain Res 331:267–274.

Shouval HZ, Bear MF, Cooper LN (2002) A unified model of NMDA receptor-dependent bidirectional synaptic plasticity. Proc Natl Acad Sci USA 99:10831–10836.

Sjöström PJ, Turrigiano GG, Nelson SB (2001) Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. Neuron 32:1149–1164.

Stricker C, Cowan AI, Field AC, Redman SJ (1999) Analysis of NMDA-independent long-term potentiation induced at CA3-CA1 synapses in rat hippocampus *in vitro*. J Physiol 520:513–525.

Stricker C, Field AC, Redman SJ (1996) Changes in quantal parameters of EPSCs in rat CA1 neurones in vitro after the induction of long-term potentiation. J Physiol 490:443–454.

Stuart G, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. Nature 367:69–72.

Stuart G, Sakmann B (1995) Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons. Neuron 15: 1065–1076.

Stuart G, Spruston N, Sakmann B, Hausser M (1997) Action potential initiation and backpropagation in neurons of the mammalian CNS. Trends Neurosci 20:125–131.

Stuart GJ, Hausser M (2001) Dendritic coincidence detection of EPSPs and action potentials. Nat Neurosci 4:63–71.

Swadlow HA, Hicks TP (1997) Subthreshold receptive fields and baseline excitability of "silent" S1 callosal neurons in awake rabbits: contributions of AMPA/kainate and NMDA receptors. Exp Brain Res 115:403–409.

Thomas MJ, Watabe AM, Moody TD, Makhinson M, O'Dell TJ (1998) Postsynaptic complex spike bursting enables the induction of LTP by theta frequency synaptic stimulation. J Neurosci 18:7118–7126.

Yuste R, Denk W (1995) Dendritic spines as basic units of neuronal integration. Nature 375:682–684.

Yuste R, Majewska A, Cash SS, Denk W (1999) Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors and optical quantal analysis. J Neurosci 19:1976–1987.

Yuste R, Tank DW (1996) Dendritic integration in mammalian neurons, a century after Cajal. Neuron 16:701–716.

(Accepted 19 March 2004)