Group I mGluR Agonist DHPG Facilitates the Induction of LTP in Rat Prelimbic Cortex In Vitro

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Morris, Shanida H., S. Knevett, E. G. Lerner, and Lynn J. Bindman. Group I mGluR agonist DHPG facilitates the induction of LTP in rat prelimbic cortex in vitro. J. Neurophysiol. 82: 1927–1933, 1999. Long-term potentiation (LTP) of synaptic transmission is a favored neural model for learning and memory. In isolated slices of rat prelimbic cortex, glutamatergic activation of metabotropic receptors (mGluRs) is required for the production of LTP at synapses on layer V neurons. Group I mGluRs are found in neocortex, and in prelimbic cortex they have been located on layer V neurons. We have now investigated whether application of the selective group I mGluR agonist, (S)-3,5-dihydroxyphenylglycine (DHPG) facilitates the induction of LTP. We recorded field potentials in layer V in response to test shocks applied to layer II and measured the population spike amplitude and slope. Intracellular recording was used to examine the correspondence between excitatory postsynaptic potentials (EPSPs) and action potentials with components of the field potential, and to further investigate the action of DHPG. Repetitive bursts of stimulation at theta frequencies (TBS) did not consistently alter the magnitude or slope of the population spike (mean response 105 ± 4%, mean ± SE of control at 30 min after TBS ended, n = 9 slices, no significant difference). When DHPG was added to the bathing medium for 10 min during continued test stimulation, the slope and amplitude of the population spike were significantly reduced, but 30 min after wash out of the DHPG, they recovered (mean response 89 ± 10% of control, n = 6 slices, no significant difference). However, when TBS was administered in conjunction with bath application of DHPG, LTP of the population spike was induced (mean response 147 ± 12% of control at 30 min after TBS ended, P = 0.004, paired t-test, n = 9 slices). We conclude that co-application of DHPG with TBS facilitates the induction of LTP of the population spike, which supports a role for group I mGluRs in the activity-dependent induction of LTP in the prelimbic cortex.

INTRODUCTION

Long-term potentiation (LTP) of synaptic transmission is a neural model of information storage. In the awake rat, LTP can persist for days in the prelimbic cortex (Doyère et al. 1993), a cortical region important for spatial memory (Floresco et al. 1997; Fritts et al. 1998). In isolated slices of rat prelimbic cortex, LTP is readily induced by repeated bursts of stimulation at theta frequencies (TBS), and metabotropic glutamate receptors (mGluRs) were shown to be crucial for the induction of the LTP (Vickery et al. 1997). Application of the selective group I and II mGluR antagonist (R,S)-α-methyl-4-carboxyphenylglycine (MCPG) during TBS significantly reduced both the incidence and magnitude of LTP of synaptic transmission at layer V neurons.

Activation of group I mGluRs stimulates the production of inositol triphosphate (IP_3) and diacylglycerol (DAG) by phospholipase C. The increase in IP_3 and DAG increase intracellular [Ca^{2+}] and activate protein kinase C (PKC), respectively. A rise in intracellular [Ca^{2+}] is critical for induction of LTP in layer V of prelimbic cortex (Hirsch and Crépel 1992) as elsewhere. Block of normal IP_3 and IP_4 metabolism or of PKC activation has been shown to prevent LTP induction in hippocampus (Behnisch and Reymann 1994; Malinow et al. 1989, respectively), although not yet in prelimbic cortex. Layer V neurons in the prelimbic cortex express group I mGluRs. Application of the selective group I mGluR agonist (S)-3-hydroxyphenyl glycine (S-3HPG) or the selective group I mGluR5 agonist, (RS)-2-chloro-5-hydrophenylglycine (CHPG) causes depolarization, increased spike firing, and a reduction in afterhyperpolarization (AHP) of layer V prelimbic neurons, even when synaptic transmission is blocked (Morris 1998; Vickery et al. 1997). Furthermore, in cultured neurons of medial frontal cortex, the selective group I mGluR agonist, (S)-3,5-dihydroxyphenylglycine (DHPG) produced a rise in intracellular [Ca^{2+}] generated by release from intracellular stores. MCPG antagonized this action of DHPG (Morris et al. 1998). Taken together, these data suggest that MCPG reduced the probability of LTP induction in prelimbic cortex by limiting group I mGluR activation during TBS and the consequent postsynaptic rise in [Ca^{2+}], in addition to any effect on PKC activation.

If group I mGluRs are involved in the synaptic induction of LTP in prelimbic cortex, their activation by selective group I mGluR agonists should facilitate the induction of LTP by coincident TBS. Indeed, DHPG has been shown to facilitate LTP production by high-frequency tetanic stimulation in the dentate gyrus of the hippocampus of anesthetized rats (Manahan-Vaughan and Reymann 1996). In experiments on slices of hippocampus from immature rats, the brief application of DHPG alone has induced LTP in the dentate gyrus (O’Leary and O’Connor 1997). In contrast, Palmer et al. (1997) found that bath application of DHPG produced synaptic depression in CA1 of the hippocampus in vitro that persisted after wash out of the drug.

We have now explored the immediate and long-term effects of bath application of DHPG on layer V responses evoked by low-frequency test shocks in the prelimbic cortex. We then compared the effect of TBS alone, in a paradigm that was not effective in consistently eliciting long-term changes, with the effect of pairing TBS in the presence of applied DHPG. A preliminary report of this data has been published (Morris et al. 1999).
To study long-term effects of DHPG and TBS, we recorded mass field potentials within layer V rather than intracellular excitatory postsynaptic potentials (EPSPs) as in our previous study (Vickery et al. 1997). Field potentials evoked by stimulation of hippocampal afferents have been recorded in layer V of prelimbic cortex in awake and anesthetized rats (Doyère et al. 1993; Jay et al. 1995), but the source and distribution of field potentials evoked in isolated slices of medial prefrontal cortex have not been analyzed. We therefore mapped the distribution of field potentials in the prelimbic cortex and compared intracellular and extracellular recordings to analyze the observed changes produced in the field response by DHPG and TBS.

Methods

Preparation and drugs

Coronal slices 400 µm thick containing the prelimbic area of medial frontal cortex were prepared from male Sprague-Dawley rats killed by cervical dislocation. Most rats were 80–100 g, which corresponds to 3–5 wk old. Details of the location of prelimbic area of cortex are given in Vickery et al. (1997). After incubation at room temperature for >1 h, slices were submerged in artificial cerebrospinal fluid solution (ACSF) containing (in mM) 125 NaCl, 3.2 KCl, 26 NaHCO3, 1.2 NaH2PO4, 2 MgCl2, 2 CaCl2, and 10 d-glucose, bubbled with 95% O2–5% CO2, maintained at 31–33°C to within 0.5°C.

Drugs were applied to submerged slices by bath perfusion at a flow rate of 2–3 ml/min through a dead space of ≈2 ml for 10 min. The selective group I mGluR agonists S-3HPG (at 200 µM) or S-3,5-DHPG (at 100 µM, Tocris Cookson) were dissolved in ACSF. In all experiments bicuculline methiodide (Sigma) was present in the ACSF at 1 µM.

Recording, stimulation, and analysis of data

Field potentials were recorded with a glass pipette of input resistance ≈5–10 MΩ, filled with ACSF. It was connected, via an Axoclamp ×1.0 headstage, to a micromanipulator mounted on a Shaublin lathe bed to position the microelectrode within 10 µm on the slice. Intracellular recordings in layer V neurons were made with glass pipettes filled with 4 M K-acetate, connected to an Axoclamp (∞0.1) headstage. When the extracellular field response was recorded immediately outside a neuron from which intracellular recordings had been made, the stimulus artefact was wider than when a headstage suitable for extracellular recording was used (Fig. 1C, bottom panel, cf. A and B).

The stimulating electrodes were two insulated nichrome wires (diameter 55 µm) twisted together with ≈100 µm between the tips. They were positioned at the border of layers I and II, with the recording electrode placed at right angles to the pial surface beneath one of them. The polarity of stimulation was adjusted to give the lower threshold response.

Test shocks were of constant voltage, 0.05 or 0.1 ms duration, applied at 15,005-s intervals, and averages of eight successive field responses were collected over 2 min for extracellular recordings to maximize signal-to-noise. The stimulus intensity was adjusted so that the control peak-to-peak amplitude of the population spike was ≈50% of maximal amplitude. The peak-to-peak amplitude of the population spike (Fig. 1B, bottom panel, d to a mV) and the population spike slope (Fig. 1B, bottom panel, c–b, mV/ms) were measured, and the larger change was used for the normalized data. Individual intracellular EPSPs and responses to intracellular current injection were collected (see Vickery et al. 1997 for details). Data were stored digitally using a Pclamp analysis program (Axon instruments). For intracellular responses, the initial EPSP slope over 1 ms from its onset was measured. Responses were normalized to the mean during a 20-min control period to pool data from several slices within each experimental protocol.

A conditioning TBS train consisted of 4 shocks at 100 Hz, repeated 10 times at 5 Hz, with the shock duration doubled, to 0.1 or 0.2 ms. Two or five TBS trains were given at 1-min intervals. The changes in the population spike were classified as LTP if the response was ≥120% of the normalized control at 30 min after the end of the

![FIG. 1. A: distribution of field responses with depth in gray matter in prelimbic cortex. Top trace: negative waves at 470 µm (33% of the distance between the pial surface and white matter, which was 1,420 µm in this slice). Axon volley peak marked by vertical dotted line i, and onset of postsynaptic negative wave by dotted line ii. Middle trace: at 620 µm (43%), axon volley followed by positive and negative postsynaptic waves. Bottom trace: at 820 µm (57%, assumed to be layer V) onset of positive postsynaptic wave marked by dotted line i, and peak by line iii. The peak of the ensuing negative wave is marked by line iv. Calibration bars 0.5 mV, 2 ms. B, top panel: superimposed traces in normal artificial cerebrospinal fluid (ACSF) and in ACSF containing 20 µM 6-cyano-7-nitroquinazoline-2,3-dione (CNQX). The artefact, axon volley, and the population spike are labeled. The CNQX abolished the postsynaptic waves of the field potential but left the axon volley intact. Bottom panel: the population spike is enlarged. Horizontal lines a and d indicate where peak-to-peak amplitude was measured. Lines b and c show example of region over which slope of population spike was measured. C: comparison of intracellular response with field response recorded immediately outside neuron. Top trace: intracellularly recorded postsynaptic potential in response to single shock recorded from layer V neuron. Bottom trace: average of 8 successive field responses recorded 10 µm outside neuron at same stimulus strength. The extracellular headstage causes large artefact in extracellular recording (onset marked by dotted line i). Vertical line ii shows onset of intracellular excitatory postsynaptic potential (EPSP), which overlaps axon volley in field response. Action potential threshold marked by line iii, coincident in this cell with the peak extracellular negative wave.

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conditioning or drug application, as long-term depression (LTD) if it was ≤80% of control, and as no change if it was between the two criteria. On the pooled data, a repeated measures, two-sample ANOVA was applied, using the SPSS software package. Data for the last 16 min of control were compared with data from 16 to 32 min after the end of the conditioning period or drug application.

RESULTS

Distribution of field potentials

The field potentials recorded at different depths of prelimbic cortex measured from the medial, pial surface can be seen in Fig. 1A. The distribution of current sinks and sources was similar to that seen in the hippocampus and neocortex in spite of the different cellular and axonal organization of each of the three cortical regions. This is presumably because the mass field potential is dominated by the current flow in the large pyramidal cells of layer V and VI. Recording from a position corresponding to layer II/III, at 33% of the depth of the gray matter in this slice (Fig. 1A, top trace) the field response comprised a small axonal volley (marked with dotted line i) followed by a large initial negative wave (onset labeled ii). The axon volley varied in amplitude among experiments and was comprised a small axonal volley (marked with dotted line i) followed by a large initial negative wave (onset labeled ii). The postsynaptic response had a small initial positive component followed by the negative wave. Within layer V, at 57% of the depth of the gray matter (Fig. 1A, middle trace) the postsynaptic response comprised an initial positive wave (peak labeled iii) followed by a negative wave (iv). The positive wave has the same onset latency as the postsynaptic negative waveform seen in superficial layers. Deeper in the gray matter corresponding to layer VI, the positive wave was smaller and monophasic (not illustrated).

Comparison of layer V EPSPs and field responses

The field recordings used in the DHPG and LTP experiments were confined to layer V to correspond to the previous intracellular study of LTP and the action of mGluRs (Vickery et al. 1997), and because responses in layer V reflect the output from prelimbic cortex. The layer V field potential shown in Fig. 1A (bottom trace) is typical. To interpret the origin of the layer V field potential, we compared intracellular and extracellular responses (Fig. 1C, n = 5 slices). While the intracellularly recorded EPSP has a time course comparable with the firstpostsynaptic component of the field potential, its onset can overlap with the axon volley (Fig. 1C). Confirmation that the axon volley overlaps the onset of the postsynaptic positive wave of the field potential comes from the field recordings in CNQX (Fig. 1B). Presumably the backfiring of afferent axons by the stimulus in layers II/III initiates an axon volley that is still traveling down to layer V while the transmitter-induced postsynaptic depolarization is occurring at superficial dendrites. The intracellularly recorded action potential had a similar latency to the population spike recorded immediately outside the cell (Fig. 1C), although in more depolarized neurons action potentials could be elicited before the onset of the population spike (not illustrated).

In preliminary experiments we measured the amplitude and slope of the positive wave of the field potential, corresponding mainly to the field EPSP, and the amplitude and slope of the negative wave, corresponding largely to the population spike. However, we found that the largest and most reliable changes following TBS or DHPG application were in the peak-to-peak amplitude and/or slope (measured points indicated in Fig. 1B, bottom panel) of the layer V population spike.

Immediate and long-term effects of bath application of group I mGluR agonists

Selective activation of group I mGluRs by bath application of 200 μM S-3HPG or 100 μM DHPG for 10 min decreased the amplitude and/or slope of the population spike (Fig. 2A). In preliminary experiments with S-3HPG the amplitude was decreased to 80.6 ± 9.7% (mean ± SE) of control (n = 3). In

![Figure 2](http://jn.physiology.org/doi/10.1152/jn.00063.2017)

**Fig. 2.** Effects of (RS)-3,5-dihydroxyphenylglycine (DHPG). A: depressant effect of DHPG on superimposed layer V field responses. In the control trace (thin black line) the axon volley is followed by postsynaptic positive and negative waves. After 10 min application of 100 μM DHPG, the field response (thick black line) was decreased. There was partial recovery 20 min after wash out of DHPG (gray line). B: excitatory effect of DHPG on layer V neuron. Three traces superimposed, showing spike firing in response to intracellular injection of 0.8-nA current pulse. Control (thin black line) shows 4 spikes are elicited by current pulse, exposure to DHPG (thick black line labeled with arrow) produced reduction in AHP in spite of 3 mV depolarization and increased firing of 5 spikes. After 10 min wash out (thin line), response is nearly back to control levels (4 spikes and 1 mV of remaining depolarization). Calibration bars 30 ms, 10 mV.
DHPG the mean decrease in amplitude and/or slope of the population spike was to 73 ± 8% of control at 10–12 min after application (P = 0.03, 2-tailed paired t-test, n = 6).

Thirty minutes after wash out of S-3HPG, the mean population spike amplitude was 78.8 ± 26.6% (SE) of control. There was one persisting increase, one persisting decrease at 30 min after wash out, and one with no effect. After wash out of DHPG, the mean decrease in population spike amplitude and/or slope at 30 min was 11.0 ± 10.1% of control (no significant difference). Two of six slices exhibited a persistent depression of >20%, and the rest showed no effect (Fig. 4A). Pooling data from both group I agonists gave a mean decrease in population spike amplitude and/or slope at 30 min to 86 ± 10.2% (n = 9) that was also not significantly different from control.

Intracellular recording in layer V neurons (n = 3) revealed that DHPG elicited membrane depolarization, an increase in the number of spikes evoked by a current pulse of fixed size and reduction in the amplitude of the AHP seen following a train of spikes (Fig. 2B). Pooled data for the AHP amplitude was obtained during the application of steady hyperpolarizing current, to counteract the membrane depolarization and to evoke the same number of spikes on a current pulse of fixed strength. The mean reduction in AHP amplitude in DHPG was to 27.1 ± 17% of control (P = 0.051, 2-tailed paired t-test, n = 3). Wash out produced partial recovery to 87.7 ± 10% of control.

S-3HPG and DHPG also produced a reduction in the EPSP slope. The decrease was to 53.8 ± 10% of control (P = 0.031, 2-tailed paired t-test, n = 4), but this may be the result of membrane depolarization at the synapses. Current injected at the soma may not efficiently clamp the membrane potential in the dendrites. On wash out of the S-3HPG or DHPG, the membrane potential and the spike amplitude recovered to control values, although in two of four of the cells the EPSP remained depressed. The membrane depolarization produced by bath application of group I agonists is a possible explanation for the reduction in the field response, but it is also known that DHPG reduces synaptic transmission in hippocampus, probably via activation of presynaptic group I mGluRs (Gereau and Conn 1995).

**TBS protocol subthreshold for inducing consistent long-lasting changes in field responses**

We needed to define the parameters of conditioning stimulation that would be subthreshold for inducing consistent long-term changes in the absence of group I agonist but that would enable us to uncover any contribution of group I mGluRs when applied together. Trial experiments were carried out using two trains of TBS (n = 4). No changes that persisted longer than 2 min after the end of TBS were produced. When S-3HPG application was combined with two TBS trains (n = 5), inconsistent effects at 30 min after the TBS trains were produced, namely one LTD, one LTD, and three no effects. These were similar to the effects of application of S-3HPG alone, which resulted in one persistent increase, one persistent decrease, and one no effect. Hence the protocol of two TBS trains was inadequate to alter the response to group I mGluR agonist.

We then tried five trains of TBS (n = 9). Mixed effects were now produced by the conditioning trains on the population spike amplitude and/or slope. At 30 min there was one slice with LTD, three with LTP, and five slices with no long-term effects, but including two short-term depressions that returned to control levels by 30 min after TBS. The mean change in the amplitude and/or slope of the population spike 30–32 min after the completion of TBS was not significantly different from control (105 ± 4%, P = 0.97, 2-tailed paired t-test, n = 9, Fig. 4B). We found that five TBS trains was a suitable paradigm for testing the modulatory effect of group I mGluRs.

**Co-application of five trains of TBS and of DHPG**

Bath application of 100 µM DHPG combined with five TBS trains was tested on nine slices obtained from five rats. DHPG reduced the size of the field response in the 6 min before TBS in each preparation. The co-application of TBS and DHPG led to LTD of the population spike in seven of the nine slices. An individual experiment is illustrated in Fig. 3. In six of the slices, both the amplitude and the slope of the population spike were potentiated but in the seventh, only the slope was potentiated. The pooled data, illustrated in Fig. 4C, revealed a mean change in the population spike at 30–32 min after the completion of TBS to 147 ± 12% of control (P < 0.005, 2-tailed paired t-test, n = 9). The significance of the changes was confirmed using ANOVA; comparing the last 16 min of control
DISCUSSION

Our demonstration that excitation of group I mGluRs facilitates the induction of LTP in the prelimbic cortex supports the hypothesis that mGluRs are normally involved in this process (Vickery et al. 1997). Activation of group II and III mGluRs is more likely to have depressant actions, because they lead to down regulation of cAMP (Pin and DuVoisin 1995). Activation of group I mGluRs leads to a rise in postsynaptic [Ca\(^{2+}\)] and increased PKC activity, both of which are crucial for LTP induction. Moreover, in prelimbic cortex, the group II agonist (2S,1’R,2’R,3’R)-2-(2,3’-dicarboxycyclopropyl)-glycine (DCGIV) has been shown to facilitate the induction of LTD, not LTP (Otani et al. 1998).

Application of a group I mGluR agonist may not have the same efficacy at the group I mGluRs as glutamate. Another is that neuronally released glutamate may activate other mGluR subtypes. However, previous work has shown that the selective but broad spectrum mGluR antagonist MCPG blocks LTD. This demonstrates that excitation of mGluRs by endogenous release of glutamate does play a role in LTD induction.

Although there are studies of the distribution of the group I receptors mGluR1 and mGluR5 in cerebral cortex (Fotuhi et al. 1993; Romano et al. 1995), the published data do not describe their location in prelimbic cortex. Vickery et al. (1997) and Morris (1998) showed, using S-3HPG, or the mGluR5-specific agonist CHPG, in conjunction with block of synaptic transmission by baclofen, that there were group I mGluRs located on layer V cells. Because the excitatory effects observed were considerably less dramatic in the presence of baclofen than in control conditions, the mGluRs present on layer V cells probably represent only a fraction of the mGluRs mediating excitatory effects within the slice. Activation of mGluRs in other layers during the TBS trains may be important in LTD induction.

The level of dendritic Ca\(^{2+}\) attained during conditioning was hypothesized (Lisman 1989) to be a critical factor in determining whether LTP or LTD or no effect would result. There is indirect evidence to support the hypothesis (Artola et al. 1990; Cummings et al. 1996). It is therefore likely that the rise in [Ca\(^{2+}\)] produced by group I mGluR activation in vivo, or following exogenous DHPG application, could modulate the incidence of LTP or LTD, depending on the presence and efficacy of other factors altering the dendritic [Ca\(^{2+}\)]. For example, in the hippocampal area CA1, Palmer et al. (1997) found a 10-min bath application of DHPG resulted in a small but significant LTD. The fact that DHPG in their experiments led to a persistent change could be due to the reduced [Mg\(^{2+}\)] of 1.0 mM, which they used, which would allow more Ca\(^{2+}\) entry via N-methyl-D-aspartate (NMDA) channels during the depolarization produced by DHPG. Indeed they increased the incidence and size of the LTD by complete removal of Mg\(^{2+}\) from the bathing medium. We found, using 2 mM Mg\(^{2+}\), that there was transient depression in all slices. Although in some slices there was a persistent depression, the pooled data did not show significant LTD. Our intracellular recordings suggest the long-lasting depression is not due to persistent depolarization in the neurons.

![Diagram](http://jn.physiology.org/)

FIG. 4. Pooled normalized field responses plotted against time, showing effect of bath application of DHPG (A) effect of 5 TBS trains (B) and combination of DHPG application and 5 TBS trains (C). Each point represents the mean of averages of 8 successive field responses ±SE, normalized to the mean of all the responses in the control period. A: graph shows effect of bath application of DHPG at 100 μM for 10 min to 6 slices at time shown by horizontal black bar. The reduction in field response disappears after replacing DHPG with CSF. B: 5 TBS trains applied at 1 min intervals between vertical lines to 9 slices. Pooled response shows no significant effect. C: DHPG applied to 9 slices for 10 min (horizontal black bar). After 6 min a decreased pooled response is apparent, and 5 TBS trains were applied between vertical lines. After wash out of DHPG, LTD of the pooled response is revealed.

before DHPG was co-applied with TBS with the period 16–32 min after the end of TBS, the potentiation is significant at P = 0.010. For similar periods in the experiments with five trains of TBS alone, there was no significant change (P = 0.97). Thus activation of group I mGluRs can facilitate induction of activity-dependent LTP.

There was, however, no significant difference in the non-normalized control measures for the two sample populations tested with TBS alone or with TBS in the presence of DHPG (P = 0.25, 2-tailed unpaired t-test, n = 9, 9). Differences in the slices or size of responses is not likely therefore to account for the facilitatory effect of DHPG. No correlation was found between the magnitude of the depressant effect of DHPG immediately before the first train of TBS and the magnitude of the response at 30 min post TBS (R\(^2\) = 0.176).
The facilitatory role of co-application of DHPG with electrical stimulation in prelimbic cortex has been to shift the balance of long-term changes from a mixture of LTP, LTD, and no effect to a more reliable production of LTP. This is consistent with the rise in dendritic [Ca\(^{2+}\)] produced by selective group I mGluR activation, which is prevented by the selective group I mGluR antagonist 4-carboxyphenylglycine in prelimbic neurons (Morris et al. 1998) and/or the rise in PKC activation that would also occur.

It is interesting that there was no correlation between the depressant effect of DHPG on the field response and the magnitude of the LTP produced by the TBS trains applied in DHPG. The action of the mGluR agonist in facilitating LTP is therefore unlikely to be on the electrical activity during induction. This observation is in accord with the lack of significant effect of MCPG on the electrical excitation during TBS, although the drug reduced the incidence of LTP induction (Vickery et al. 1997). We infer that the membrane depolarization elicited by DHPG is not the mechanism by which the group I mGluR activation promotes LTP induction by TBS. Intriguing results of Manahan-Vaughan and Reymann (1996) show that DHPG applied to the hippocampus in vivo 5 min after the conditioning trains was nevertheless facilitatory for LTP induction. The effect of the DHPG is therefore likely to be on the biochemical cascades triggered by electrical activity during conditioning.

Field potential recordings are widely used in the hippocampus because the organized laminar structure of afferent fibers and the single layer of postsynaptic pyramidal cells enables the clear interpretation of field EPSPs and population spikes. The cellular organization of prelimbic cortex is not as clearly laminar, and there are additional layers of pyramidal cells with apical dendrites in layer II/III and VI. The distribution of afferent axons is also different from hippocampus, for example the hippocampal afferents to prelimbic cortex (Jay and Witter 1991) run from the underlying white matter, roughly parallel with apical dendrites, toward the pial surface. Nevertheless the recording of field potentials offers advantages over intracellular recording in terms of stability for long periods of time. Field potential recordings have been employed to good effect in the study of normal and abnormal synaptic transmission in the prefrontal cortex of the rat studied by means of anterograde transport of phaseolus vulgaris-leucoagglutinin.

Our in vitro analysis of field potential distribution following stimulation of layer I/II is the first to be made in prelimbic cortex. It is not surprising, in view of the course of the afferent axons that there can be temporal overlap of the axon volley in layer V with the positive wave of the postsynaptic field EPSP (fEPSP). Although action potential firing occurs during the population spike, there is also some overlap of action potentials in layer V with the positive wave of the fEPSP. It is not clear whether the enhanced population spike seen in our field potential recordings of LTP represents a decrease in the threshold for action potential firing (E-S potentiation) or a change in synaptic transmission. We could not detect an increase in the fEPSP in any of the three LTPs induced by TBS alone: the enhanced population spike was seen when there was no preceding increase in the first postsynaptic, positive wave. This is unexpected, because intracellular recordings (Vickery et al. 1997) showed an enhanced initial slope of EPSPs in the majority of TBS-induced LTPs. We suspect therefore that the lack of change in the first positive wave of the field response is the result of overlap of enhanced sinks and sources, or overlap of spike firing and source currents. However, we cannot exclude E-S potentiation as the underlying mechanism of the LTP induced by co-application of DHPG and TBS. Whatever the underlying mechanism, long-lasting enhancement of the population spike in layer V is of functional importance in that it reflects increased firing of output neurons to other synapses in the cortex and other regions of the brain.

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