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Mechanisms Underlying Induction of Long-Term Potentiation in Rat Medial and Lateral Perforant Paths In Vitro

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SUMMARY AND CONCLUSIONS

1. The mechanisms underlying the induction of long-term potentiation (LTP) in the medial and lateral perforant paths were studied by recording excitatory postsynaptic potentials (EPSPs) from rat dentate granule cells in vitro using extracellular and whole-cell recording techniques.

2. Paired stimuli (interstimulus interval, 50–1,000 ms) resulted in facilitation of the lateral and depression of the medial perforant path-evoked EPSPs, respectively. This physiological difference was used to isolate responses evoked by stimulation of a single path.

3. Tetanic stimulation induced LTP in both pathways, although the magnitude of LTP in the lateral perforant path was significantly less than that in the medial perforant path. Both forms of LTP were blocked by the *N*-methyl-D-aspartate (NMDA) receptor antagonist D-2-amino-5-phosphonovaleric acid (D-APV).

4. Buffering intracellular calcium by loading granule cells with the calcium chelator bis (O-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid prevented LTP in both pathways.

5. Pairing of low-frequency (0.25 Hz) afferent stimulation with postsynaptic depolarization induced LTP in the medial but not the lateral perforant path. However, pairing of higher-frequency stimulation (1–4 Hz) with postsynaptic depolarization did potentiate the lateral perforant path–evoked EPSP in some cells.

6. Both the medial and lateral perforant path–evoked EPSPs had two components; a fast component blocked by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione and a slower, voltage-dependent component blocked by D-APV.

7. The results indicate that the induction of LTP in both the medial and lateral perforant paths requires activation of postsynaptic NMDA receptors and a rise in intracellular calcium. The differences between LTP in the lateral and medial perforant paths may be due to differences in the density or properties of the postsynaptic NMDA receptors activated by either pathway.

INTRODUCTION

Long-term potentiation (LTP), a long-lasting increase in synaptic efficacy elicited by brief high-frequency activation of afferent fibers, has been studied in greatest detail in hippocampal CA1 pyramidal cells. It is now generally held that induction of LTP at the synapse between Schaffer collateral-commissural fibers and CA1 pyramidal cells requires activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors during postsynaptic depolarization. This permits entry of calcium (Ca^{2+}) through the NMDA receptor ionophore and a rise in the concentration of Ca^{2+} within the dendritic spine, a requisite trigger for LTP induction (for review, see Collingridge and Lester 1989; Nicoll et al. 1988). Although LTP was first described in detail for the excitatory synapses made by perforant path fibers onto granule cells of the dentate gyrus (Bliss and Gardner-Medwin 1973; Bliss and Lomo 1973), much less is known about LTP induction mechanisms at this synapse. For example, whether a rise in postsynaptic Ca^{2+} is required for LTP in dentate granule cells has not been directly tested, nor has the ability to induce LTP by pairing low-frequency afferent stimulation with postsynaptic depolarization.

A potential problem in studying LTP in the dentate gyrus is that the perforant path is not a homogenous fiber tract but can be divided into distinct medial and lateral components. The medial and lateral perforant paths originate in the medial and lateral portions of the entorhinal cortex, respectively, and contact the dendrites of granule cells in a laminar fashion; medial perforant path fibers form synapses in the middle third of the molecular layer, whereas lateral perforant path fibers occupy the outer third (Hjorth-Simonsen 1972; Hjorth-Simonsen and Jeune 1972; Steward 1976). Perhaps of greater importance than this anatomic distinction are the robust pharmacological (Kahle and Cotman 1989; Koerner and Cotman 1981) and physiological (Abraham and McNaughton 1984; McNaughton 1980; McNaughton and Barnes 1977; Tielen et al. 1981) differences between the medial and lateral perforant paths.

Despite these clear differences in the medial and lateral perforant paths, most mechanistic studies of LTP in the dentate gyrus have not reported which pathway was examined (Hanse et al. 1991; Laroche et al. 1989; Linden et al. 1988; Morris et al. 1986), have not specified any differences between LTP in the two paths (McNaughton 1982; McNaughton et al. 1978), or have focused solely on the medial perforant path (Burgard et al. 1989; Errington et al. 1987). The few studies of LTP in the lateral perforant path suggest that the mechanisms underlying its induction may differ from those responsible for induction of LTP in the medial perforant path (Abraham et al. 1985; Bramham et al. 1988, 1991). The goal of this study was to examine and compare the basic requirements for the induction of LTP in the medial and lateral perforant path inputs to granule cells of the dentate gyrus.

METHODS

Under halothane anesthesia, Sprague-Dawley rats (age 20–30 days) were decapitated and hippocampal slices (400 μm) prepared using standard techniques (Malenka et al. 1988). Slices were placed in a holding chamber for $\geq$1 h before being transferred to

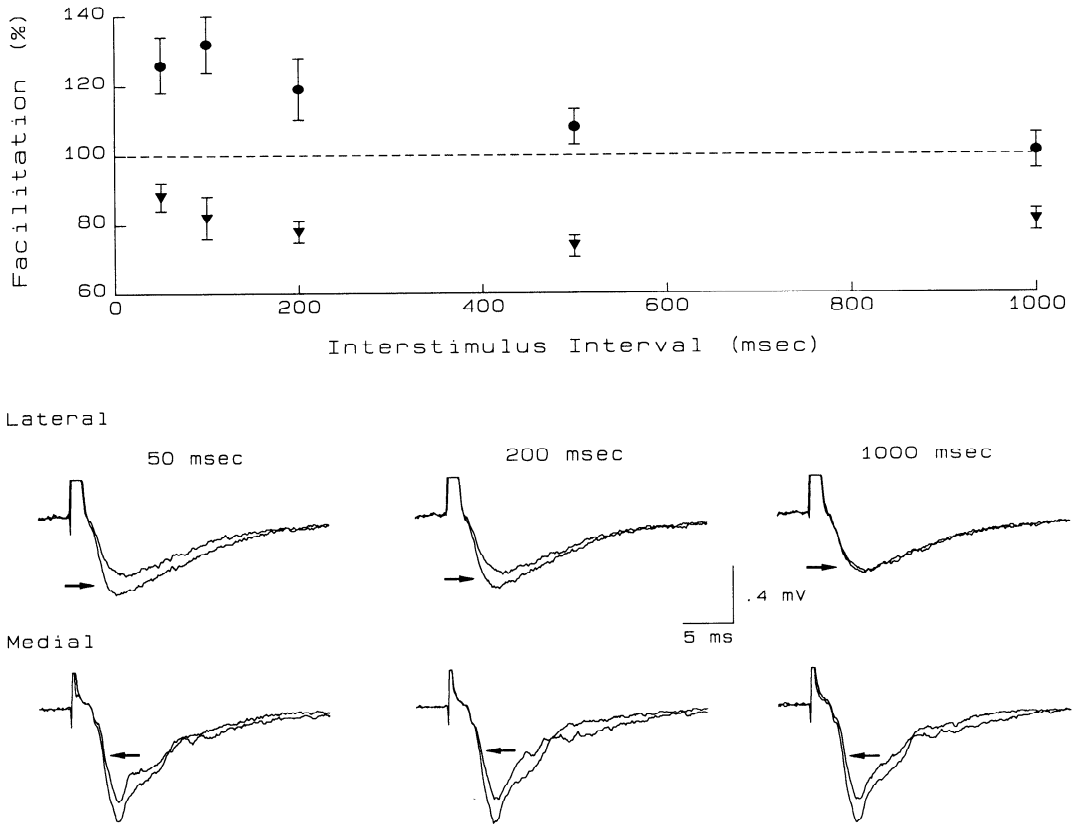

FIG. 1. Paired stimuli cause paired-pulse facilitation in the lateral perforant path and paired-pulse depression in the medial perforant path. The graph plots the initial slope of the excitatory postsynaptic potential (EPSP) (mean ± SE) evoked by the 2nd of 2 stimuli as a function of the interstimulus interval for the lateral (●) and medial (▼) perforant paths. For all experiments (n = 7), the EPSP slope of the 2nd response was calculated relative to the initial response. Data traces are superimposed responses to the 1st and 2nd stimuli at different interstimulus intervals. All traces were recorded from the same slice. Arrows: response elicited by the 2nd stimulus.

the recording chamber in which a slice was submerged beneath a continuously superfusing solution (2 ml/min) that had been saturated with 95% O_2-5% CO_2. The composition of the solution was as follows (in mM): 119 NaCl, 4 KCl, 4 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 4 $CaCl_2$, 11 glucose, and 0.1 picrotoxin. The temperature of the solution was maintained between 29 and 31°C.

Extracellular field excitatory postsynaptic potentials (EPSPs) were recorded in the molecular layer with electrodes (2–6 MΩ) filled with 3 M NaCl. "Blind" whole-cell patch-clamp recordings (Blanton et al. 1989) were made from dentate granule cells with electrodes (6–12 MΩ) filled with (in mM) 117.5 cesium gluconate, 17.5 CsCl, 8 NaCl, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 0.2 ethylene glycol-bis(β-aminioethyl ether)-*N,N,N′,N′*-tetraacetic acid, 2–4 Mg-ATP, and 0.2 guanosine triphosphate, pH = 7.2, osmolality = 290–300 mOsm. Electrodes with higher than normal resistances were used because it was found empirically that this facilitated the ability to maintain stable recordings and induce LTP. Recordings were made under current clamp using an Axoclamp 2A (Axon Instruments). During synaptic stimulation, cells were held under current clamp at a constant membrane potential ranging from −70 to −85 mV. Bridge balance was continuously maintained by monitoring a cell's response to a 100-ms hyperpolarizing current step (0.1–0.3 nA). Access resistance varied between 12 and 55 MΩ. All membrane potentials presented in the RESULTS refer to the potential recorded at the soma.

To elicit EPSPs, stimuli were applied once every 18–20 s with bipolar stainless steel electrodes placed in the middle or outer third of the molecular layer to stimulate the medial or lateral perforant paths, respectively. Simultaneous extracellular recording in the middle and outer thirds of the molecular layer confirmed that the stimulation used in these experiments activated a relatively narrow beam of fibers, because a current sink recorded at the same layer as the stimulation was converted to a current source when recording at the alternate layer <150 μm apart. The final determination of whether the medial or lateral perforant path was stimulated relied primarily on the synaptic responses to paired pulses given 50–1,000 ms apart (see RESULTS).

Data were collected and analyzed on-line (2- to 10-kHz sampling rate) using a 386-based personal computer programmed with Axobasic (Axon Instruments). To minimize contamination from voltage-dependent conductances, the initial slope of the EPSP was used as a measure of synaptic efficacy and was calculated using a least-squares regression. Averaged summary graphs of grouped experiments were obtained by *1*) normalizing each experiment according to the average value of all points on the 10-min baseline before LTP induction, *2*) aligning the points with respect to the time of LTP induction, and *3*) dividing each experiment into 40- to 60-s bins and averaging these across experiments (Malenka et al. 1989). Each point in these graphs is the mean ± SEM. A two-tailed t test was used to test for significant differences between groups.

Drugs were added to the superfusing solution immediately before application. Drugs used included: D-2-amino-5-phosphonovaleric acid (D-APV; Cambridge Research Biochemicals), bis (O-aminophenoxy) ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA; Molecular Probes), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Cambridge Research Biochemicals), and picrotoxin (Sigma).

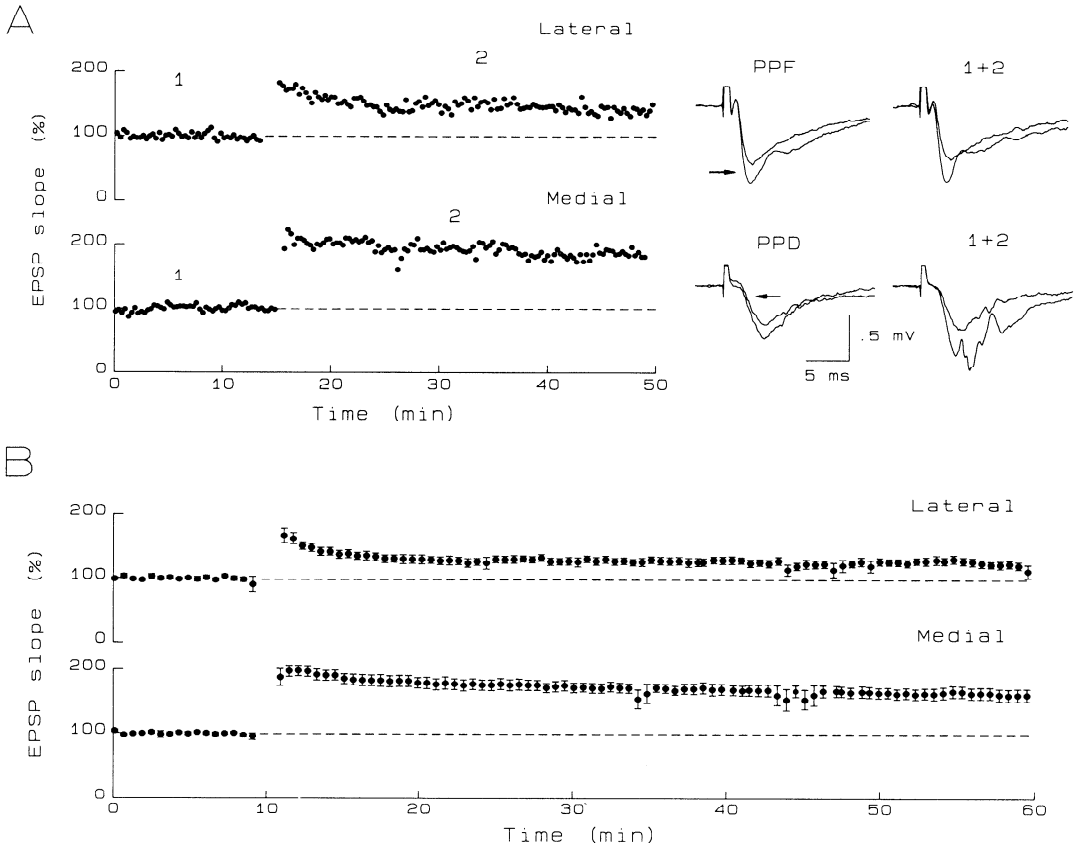

FIG. 2. Repetitive tetanic stimulation evokes long-term potentiation (LTP) in both the lateral and medial perforant paths. *A*: initial slope of the excitatory postsynaptic potential (EPSP) is plotted against time for 2 experiments in which repetitive tetani (100 Hz, 0.5 s, 3 times) were given to the lateral or medial perforant paths, resulting in LTP. The 1st column of data traces are superimposed responses to paired stimuli (100-ms interstimulus interval) and illustrate paired-pulse facilitation (PPF) in the lateral perforant path and paired-pulse depression (PPD) in the medial perforant path. Arrows: response elicited by the 2nd stimulus. The *right* column of traces were taken at the times indicated by the numbers above the graph. *B*: summary of experiments like those in *A* (lateral perforant path, $n = 16$; medial perforant path, $n = 15$).

RESULTS

Paired-pulse responses in medial and lateral perforant paths

Stimulation of an isolated medial or lateral perforant path was crucial for this study. Rather than relying solely on anatomic positioning of stimulating and recording electrodes or on quantitative differences in the waveform of the extracellular EPSP (Abraham and McNaughton 1984; McNaughton and Barnes 1977), we examined the change in the synaptic response after paired stimuli as a means of determining which pathway was stimulated. McNaughton (1980) first demonstrated that the response to the second of two stimuli (interstimulus interval, 50–1,000 ms) is facilitated in the lateral perforant path but depressed in the medial perforant path. This difference in paired-pulse responses correlated exactly with anatomic and histochemical identification of the medial and lateral perforant paths (McNaughton 1980). To verify this observation in our preparation, we recorded EPSPs simultaneously ($n = 7$ slices) from the outer and middle thirds of the molecular layer in response to stimulation electrodes placed in these layers and examined the change in synaptic efficacy in response to paired stimuli as a function of the interstimulus interval. Figure 1 shows that when the stimulating and recording electrodes were placed in the outer third of the molecular layer, paired-pulse facilitation was observed consistently. In contrast, recording an EPSP in the middle third of the molecular layer in the same slice in response to stimulation of fibers in this region resulted in paired-pulse depression. Using larger stimulus strengths or repositioning of the electrodes reduced or eliminated the difference in the paired-pulse responses.

The robust difference in the responses to paired stimuli provided physiological identification of the medial and lateral perforant paths. Thus, before each experiment, this property was used to obtain as pure a medial or lateral perforant path response as possible. Interstimulus intervals of 50–200 ms were used and electrodes positioned to maximize the magnitude of paired-pulse facilitation or paired-pulse depression when studying the lateral or medial perforant paths, respectively.

Induction of LTP in medial and lateral perforant paths

Tetanic stimulation (100 Hz, 0.25 s, given 3–4 times) reliably induced LTP in both the lateral and medial perforant paths (lateral, 14 of 16 slices; medial, 13 of 15 slices). Figure 2*A* shows individual experiments demonstrating paired-pulse facilitation and paired-pulse depression in the lateral and medial perforant paths, respectively, and the subsequent LTP after tetanic stimulation. The summary

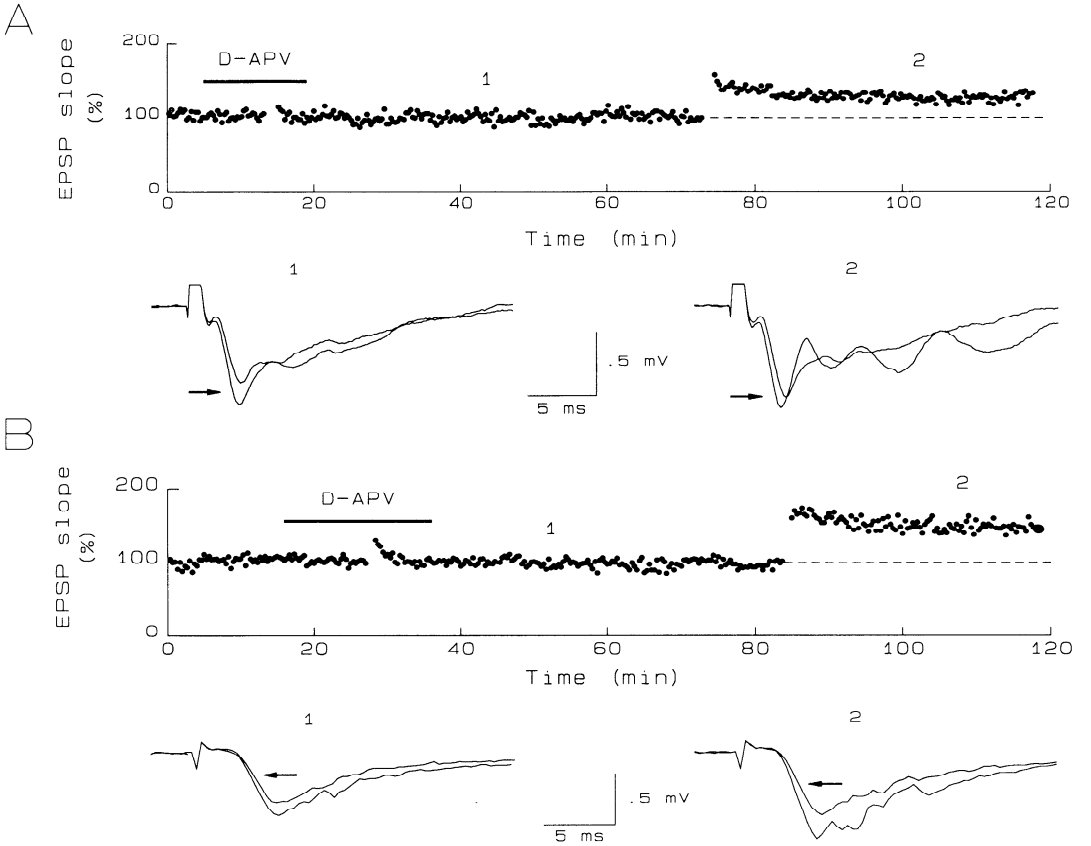

FIG. 3. D-2-amino-5-phosphonovaleric acid (D-APV) blocks long-term potentiation (LTP) in both the lateral and medial perforant paths. Excitatory postsynaptic potential (EPSP) slope is plotted against time from experiments in which tetanic stimulation (100 Hz, 0.5 s, 3 times) of the lateral perforant path (*A*) or the medial perforant path (*B*) in the presence of D-APV (25 μM) caused no long-lasting change in synaptic efficacy but did induce LTP after washout of the D-APV. Sample data traces below each graph show superimposed responses to paired stimuli (100-ms interstimulus interval) before (1) and after (2) induction of LTP. Arrows: response elicited by the 2nd stimulus.

graph (Fig. 2*B*) demonstrates that the magnitude of LTP in the lateral perforant path is much smaller than that in the medial perforant path (lateral, 28 ± 6%, mean ± SE; medial, 73 ± 10%; $P < 0.01$; measured 30 min after induction). LTP in the lateral perforant path also exhibited a significant initial decay such that 10 min after induction the EPSP slope had decayed to 45 ± 7% of its peak value. Medial perforant path LTP showed much less decay; EPSP slope 10 min after induction was 83 ± 9% of its maximum.

Role of NMDA receptors

LTP in the dentate gyrus has been reported to be blocked by the NMDA receptor antagonist D-APV (Laroche et al. 1989; Linden et al. 1988; Morris et al. 1986; Wigstrom et al. 1986), although only three studies specified that the medial perforant path was stimulated (Bramham et al. 1991; Burgard et al. 1989; Errington et al. 1987). In contrast, in the lateral perforant path LTP of the population spike, but not of the EPSP component of the field potential, was blocked by D-APV (Bramham et al. 1991). We tested the effects of D-APV on LTP in the lateral and medial perforant paths and found that it blocked the generation of LTP in both pathways without affecting basal synaptic efficacy (lateral, 10 of 10 slices; medial, 7 of 7 slices). In all experiments, after washout of D-APV the same tetanic stimulation elicited normal LTP (Fig. 3).

Role of postsynaptic Ca^{2+}

In all forms of NMDA receptor–dependent LTP studied to date, buffering postsynaptic Ca^{2+} prevents LTP (Baryani and Szente 1987; Lynch et al. 1983; Malenka et al. 1988; Sah and Nicoll 1991; Zalutsky and Nicoll 1990). To test whether a rise in postsynaptic Ca^{2+} is also required for LTP induction in the lateral and medial perforant paths, we loaded cells with the Ca^{2+} chelator BAPTA by adding 20 mM BAPTA to the whole-cell electrode solution. Figure 4*A* shows the effects of applying repetitive tetani to the lateral perforant path in a population of control cells ($n = 7$) and in a population of cells filled with BAPTA ($n = 7$). Relative to the time of breakin, the control group of cells received the LTP-inducing tetanic stimulation 20–30 min later than the cells filled with BAPTA, making it unlikely that the block of LTP was due to washout (Malinow and Tsien 1990). As shown in Fig. 4*B*, cells ($n = 7$) loaded with BAPTA also did not exhibit LTP in response to stimulation of the medial perforant path (compare with Fig. 5*B*).

Effects of pairing postsynaptic depolarization with low-frequency stimulation

In CA1 pyramidal cells, pairing postsynaptic depolarization with low-frequency afferent stimulation produces LTP (Gustafsson et al. 1987), presumably because this relieves

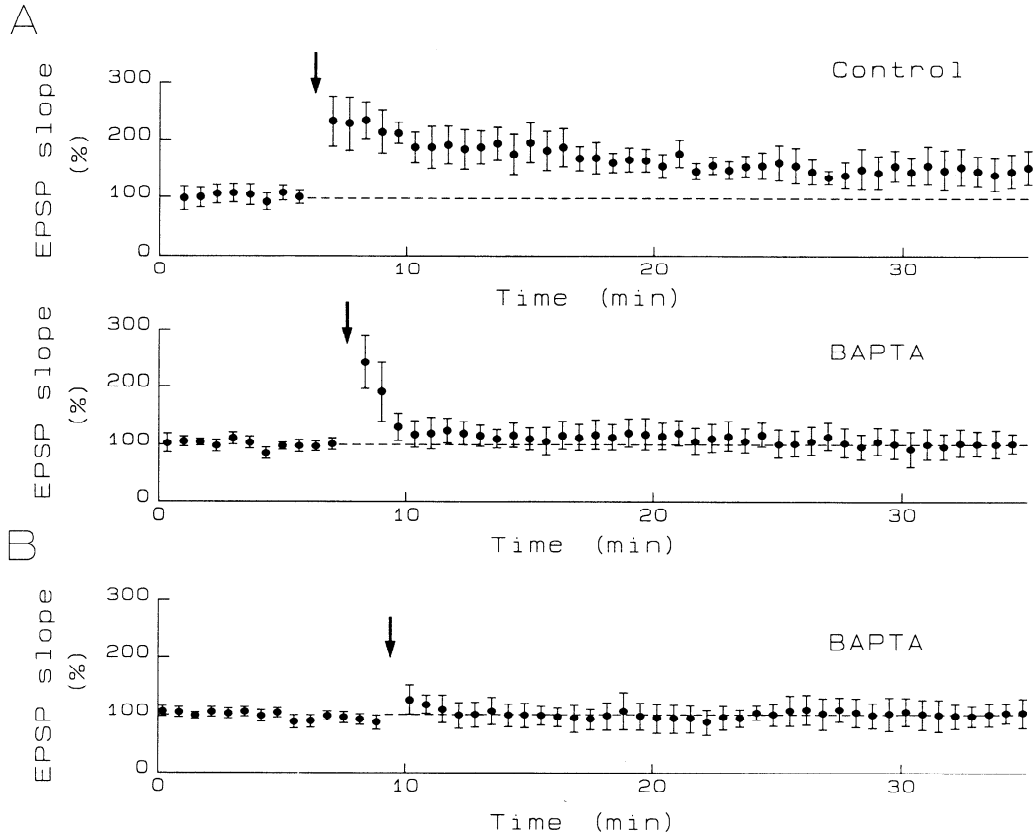

FIG. 4. Loading granule cells with the calcium chelator bis-(*O*-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) blocks long-term potentiation (LTP) in both the lateral and medial perforant paths. *A*: initial slope of the excitatory postsynaptic potential (EPSP) evoked by stimulation of the lateral perforant path is plotted against time for 7 neurons recorded with electrodes filled with the control recording solution (Control) and for 7 neurons in which 20 mM BAPTA was added to the recording solution (BAPTA). Repetitive tetani (200 Hz, 0.5 s, 3–4 times) were given at the time indicated by the arrows. *B*: initial slope of the EPSP evoked by stimulation of the medial perforant path is plotted against time for 7 neurons recorded with electrodes containing 20 mM BAPTA. Repetitive tetani were given at the time indicated by the arrow.

the Mg^{2+} block of the NMDA receptor ionophore, resulting in increased Ca^{2+} entry. However, in anterior cingulate cortex cells, this pairing protocol does not elicit LTP even though LTP induction requires activation of NMDA receptors (Sah and Nicoll 1991). It was therefore of interest to determine whether LTP could be elicited in the lateral and medial perforant paths by directly depolarizing granule cells during afferent stimulation. In the lateral perforant path (Fig. 5*A*), pairing 30–40 stimuli given at 0.25 Hz with postsynaptic depolarization caused no significant change in synaptic efficacy ($n = 7$), even though subsequent tetanic stimulation elicited LTP in these same cells. In the medial perforant path, this same pairing protocol elicited robust LTP in all cells examined ($n = 8$) (Fig. 5*B*). The inability to elicit LTP in the lateral perforant path by this pairing protocol was not due to inadequate depolarization at synaptic sites, because in each cell it was confirmed that EPSPs could be reversed and during the pairing, cells were held at a membrane potential within 10 mV of the EPSP reversal potential.

A recent study reported that in CA1 pyramidal cells, the frequency at which afferents are stimulated during postsynaptic depolarization influenced the ability to generate LTP (Colino et al. 1992). This observation raised the possibility that during postsynaptic depolarization, stimulation frequencies >0.25 Hz may be required to elicit LTP in the lateral perforant path. Figure 6 shows one of five neurons in which, after stimulation of the lateral perforant path at 0.25 Hz during postsynaptic depolarization, the same number of stimuli were given at 1 Hz, resulting in LTP. However, in nine additional cells, pairing postsynaptic depolarization with 1- to 4-Hz afferent stimulation did not affect synaptic efficacy, indicating that even at these higher stimulation frequencies, LTP was more difficult to induce in the lateral perforant path than in the medial perforant path (Abraham et al. 1985).

NMDA component of EPSPs in the medial and lateral perforant paths

The block of LTP by D-APV or by buffering postsynaptic Ca^{2+} and the ability to induce LTP by pairing postsynaptic depolarization with low-frequency afferent stimulation suggest that transmitter released from either pathway activates postsynaptic NMDA receptors. Although stimulation of an unspecified portion of the perforant path can elicit an EPSP (Lambert and Jones 1990) or excitatory postsynaptic current (EPSC) (Keller et al. 1991) with a clear NMDA receptor–mediated component, the existence of such a component in response to lateral perforant path stimulation has

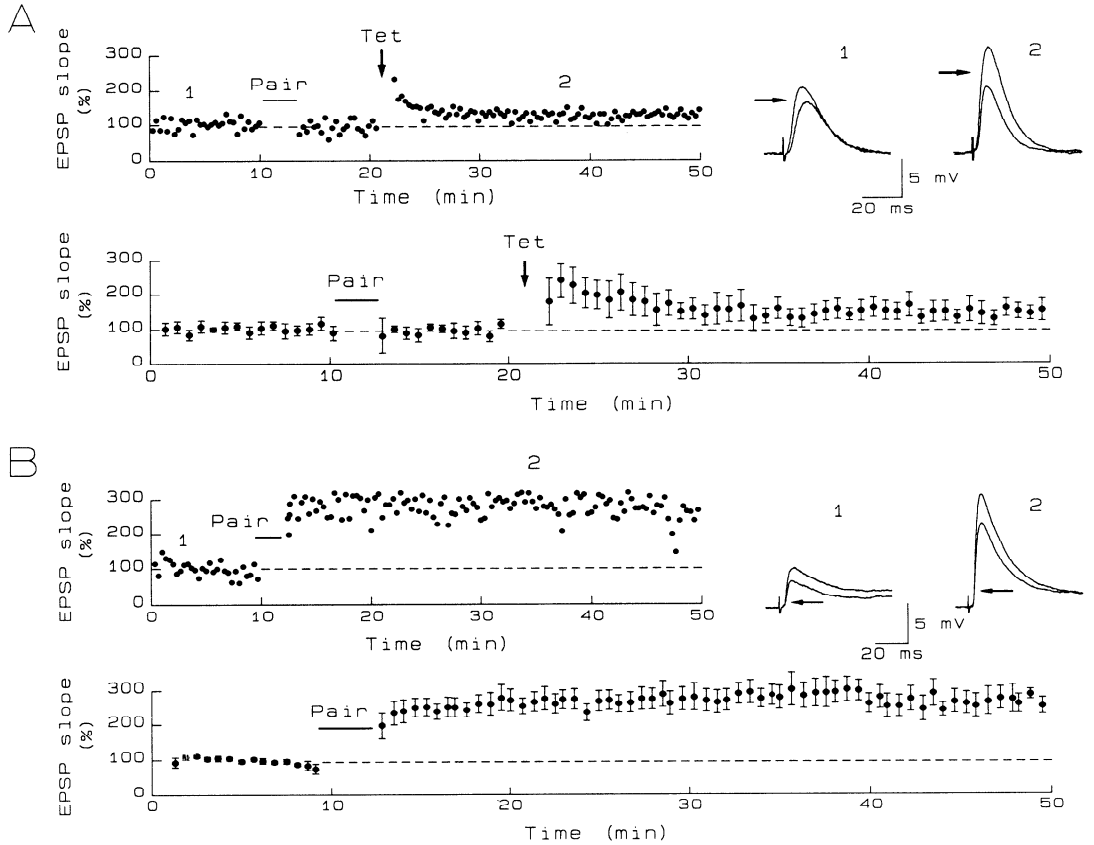

FIG. 5. Pairing of postsynaptic depolarization with low-frequency (0.25 Hz) stimulation induces long-term potentiation (LTP) in the medial perforant path but not in the lateral perforant path. For all graphs, the initial slope of the excitatory postsynaptic potential (EPSP) evoked by stimulation of the lateral perforant path (*A*) or by stimulation of the medial perforant path (*B*) is plotted against time. *Top* graphs in *A* and *B* show individual experiments, and the *bottom* graphs are summaries of 7 (*A*) or 8 (*B*) neurons. At the time marked *Pair*, cells were depolarized to within 10 mV of the EPSP reversal potential and 30–40 stimuli were given at 0.25 Hz. At the time labeled Tet, repetitive tetani (100 Hz, 0.5 s, 3 times) were given. The data traces are superimposed responses to paired stimuli (100-ms interstimulus interval) taken at the times indicated by the numbers above the graph. Arrows: response elicited by the 2nd stimulus.

been questioned (Dahl et al. 1990; Mody et al. 1988). Figure 7 shows a pharmacological dissection of the medial perforant path EPSP at different membrane potentials ($n = 4$). Depolarizing the cell to −40 mV increased the duration of the EPSP, and its additional slow tail was blocked by D-APV (25 μM). At −80 mV, the non-NMDA receptor antagonist CNQX (10–20 μM) reduced the EPSP by >90% ($n = 4$), whereas depolarizing the cell to −40 mV (still in the presence of CNQX) revealed an EPSP with slower kinetics that was completely blocked by D-APV (25 μM). These results confirm that the medial perforant path activates both NMDA and non-NMDA receptors.

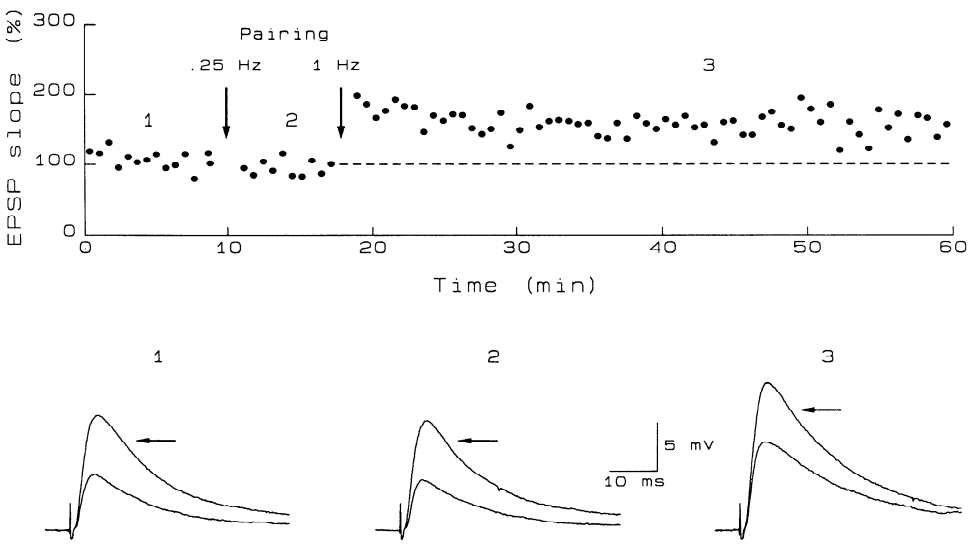

FIG. 6. Pairing postsynaptic depolarization with 1-Hz stimulation of the lateral perforant path induces long-term potentiation (LTP). The initial slope of the excitatory postsynaptic potential (EPSP) is plotted against time from an experiment in which pairing postsynaptic depolarization with lateral perforant path stimulation (30 stimuli at 0.25 Hz) caused no change in synaptic strength. However, pairing postsynaptic depolarization with higher-frequency stimulation (30 stimuli at 1 Hz) induced LTP. Data traces: superimposed responses to paired stimuli (100-ms interstimulus interval) taken at the times indicated by the numbers above the graph. Arrows: response elicited by the 2nd stimulus.

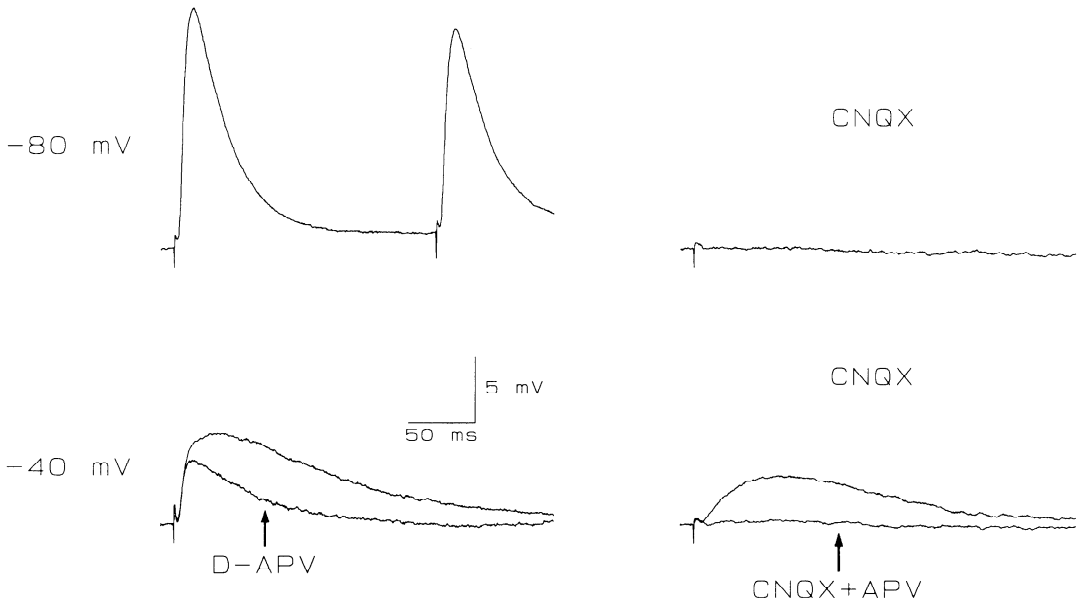

FIG. 7. Medial perforant path activates both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors. At a negative (−80 mV) membrane potential, 2 afferent stimuli (200-ms interstimulus interval) cause paired-pulse depression (*top left*), and the excitatory postsynaptic potential (EPSP) is largely abolished by the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM) (*top right*). Depolarization of the cell to −40 mV by current injection reveals a prolonged EPSP, the later component of which is blocked by the NMDA antagonist D-2-amino-5-phosphonovaleric acid (D-APV) (25 μM) (*bottom left*). At −40 mV and in the presence of CNQX, a slower EPSP is revealed and is entirely blocked by the addition of D-APV (*bottom right*).

Figure 8*A* shows an example of a similar pharmacological examination of the lateral perforant path EPSP and demonstrates a clear voltage-dependent and CNQX-insensitive component of the EPSP that was blocked by D-APV (n = 4). Because it was often difficult to obtain a stable lateral perforant path EPSP while holding the cell at −30 to −40 mV, we also examined the effects of CNQX and D-APV on the reversed EPSP generated while holding the cell at +40 mV (Fig. 8*B*) (n = 2). Again a clear NMDA receptor–mediated component of the EPSP was observed.

DISCUSSION

The purpose of this study was to examine and compare the mechanisms underlying the induction of LTP in the medial and lateral perforant paths. The location of maximal paired-pulse facilitation or paired-pulse depression was used to isolate relatively pure lateral or medial perforant path responses, respectively. It was assumed that this physiological criterion was a more reliable measure of lateral versus medial perforant path stimulation than anatomic localization or synaptic waveform characteristics, both of which would be significantly more affected by variations in slice geometry or stimulation current spread. Although our procedure does not ensure an absolutely pure medial or lateral perforant path response, it does maximize the proportion of stimulated fibers that are part of the appropriate pathway.

Tetanic stimulation reliably induced LTP in both lateral and medial perforant paths, although the magnitude of LTP was much less in the lateral than in the medial perforant path. Similar results were obtained in previous in vivo studies in which lateral perforant path LTP was both weak and difficult to elicit (Abraham et al. 1985). These observations suggested that there may be differences in the mechanisms underlying LTP induction in the two pathways. By analogy with the different types of LTP elicited in the mossy fiber and commissural inputs onto CA3 pyramidal cells (Zalutsky and Nicoll 1990), our first test for possible differences between LTP in the lateral and medial perforant paths examined the effects of the NMDA receptor antagonist D-APV. Consistent with previous studies (Bramham et al. 1991; Burgard et al. 1989; Errington et al. 1987), medial perforant–path LTP was prevented by D-APV. However, contrary to a recent report (Bramham et al. 1991), we found that LTP in the lateral perforant path was also blocked by D-APV.

A second test for differences between LTP in the medial and lateral perforant paths examined the effects of loading granule cells with the Ca^{2+} chelator BAPTA. Like the NMDA receptor–dependent LTP found in CA1 pyramidal cells (Lynch et al. 1983; Malenka et al. 1988), CA3 pyramidal cells (Zalutsky and Nicoll 1990), anterior cingulate cells (Sah and Nicoll 1991), and neocortical cells (Baranyi and Szente 1987), LTP in both the medial and lateral perforant paths was blocked by buffering postsynaptic Ca^{2+}, indicating that a rise in postsynaptic Ca^{2+} is required for LTP induction in both pathways.

Recently, two forms of NMDA receptor–dependent LTP have been distinguished. The more extensively characterized form found in CA1 pyramidal cells can be elicited by pairing postsynaptic depolarization with low-frequency afferent stimulation (Gustafsson et al. 1987), whereas in layer V or VI cells of the anterior cingulate cortex, this same pairing procedure does not induce LTP; a strong tetanus is required (Sah and Nicoll 1991). A third test for differences between lateral and medial perforant path LTP therefore

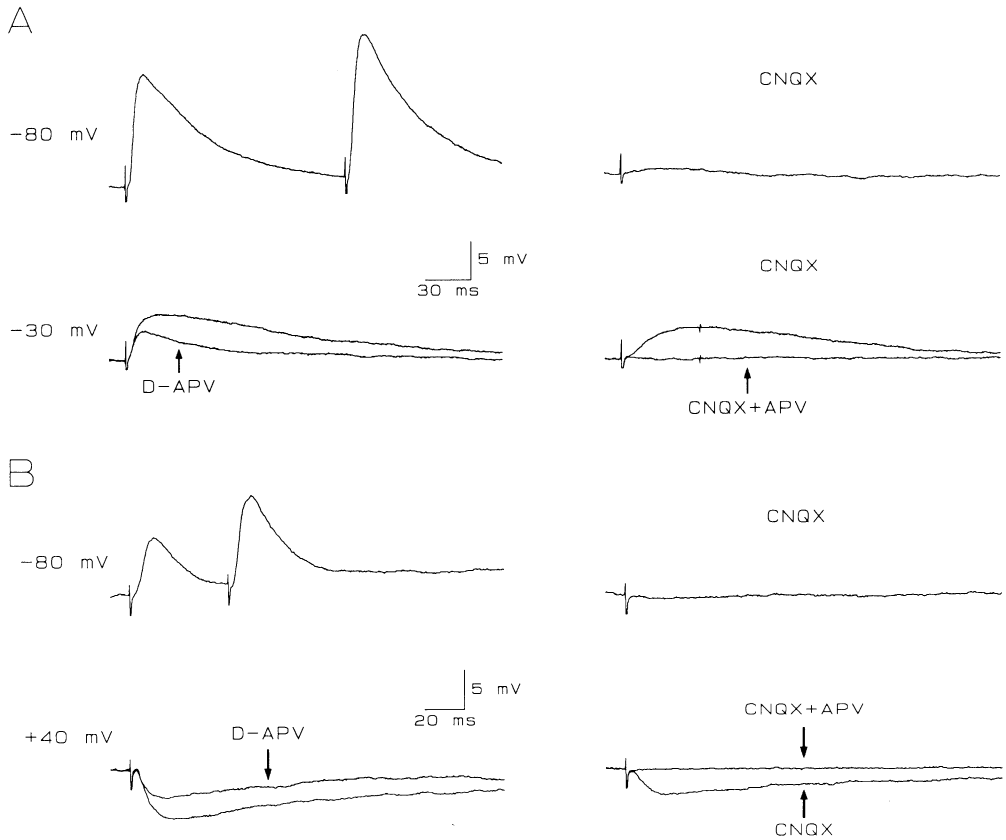

FIG. 8. Lateral perforant path activates both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors. *A*: at a negative membrane potential (−80 mV), 2 afferent stimuli (150-ms interstimulus interval) cause paired-pulse facilitation (*top left*), and the excitatory postsynaptic potential (EPSP) is largely abolished by the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM) (*top right*). Depolarization of the cell to −30 mV by current injection prolongs the EPSP, the later component of which is blocked by the NMDA antagonist D-2-amino-5-phosphonovaleric acid (D-APV) (25 μM) (*bottom left*). Depolarization to −30 mV in the presence of CNQX reveals a slower EPSP that is blocked by D-APV. *B*: EPSPs evoked by stimulation of the lateral perforant path from another cell. As in *A*, 2 stimuli (50-ms interstimulus interval) cause paired-pulse facilitation (*top left*), and the EPSP at −80 mV is abolished by CNQX (*top right*). Depolarizing the cell to +40 mV reverses and prolongs the EPSP, the late component of which is reduced by D-APV (*bottom left*). In the presence of CNQX, the EPSP at +40 mV is slow and entirely blocked by D-APV (*bottom right*).

examined which form of NMDA receptor–dependent LTP occurred in the two paths. Applying low-frequency (0.25 Hz) stimuli during postsynaptic depolarization generated robust LTP in the medial perforant path yet did not change synaptic efficacy in the lateral perforant path. Increasing the stimulation frequency of the lateral perforant path to 1–4 Hz during postsynaptic depolarization induced LTP in five cells, although in an additional nine cells LTP could not be elicited using any pairing protocol.

Two previous reports are consistent with the ability to induce LTP in the dentate gyrus by pairing postsynaptic depolarization with low-frequency afferent stimulation. McNaughton (1982) showed that lateral perforant–path LTP studied in vivo exhibited cooperativity (McNaughton et al. 1978), a property normally indicating a requirement for postsynaptic depolarization (but see Sah and Nicoll 1991). In addition, it was found that pairing a brief tetanus to a conditioning pathway with a single test stimulus could induce LTP in the dentate gyrus (Hanse et al. 1991), although the pathway stimulated was not specified.

All of the properties of LTP in the medial and lateral perforant paths described thus far are indicative of synapses at which transmitter activates both non-NMDA and NMDA receptors (Nicoll et al. 1988). We found that both medial and lateral perforant–path EPSPs contain two components that are distinguished by their sensitivity to specific non-NMDA and NMDA receptor antagonists and by their voltage dependence. Thus transmitter released from either medial or lateral perforant path fibers can activate both NMDA and non-NMDA receptors. This proposal is consistent with recent reports that unspecified perforant path–evoked EPSPs or EPSCs exhibit prominent non-NMDA and NMDA receptor–mediated components (Keller et al. 1991; Lambert and Jones 1990). However, previous studies of the lateral perforant path–evoked EPSP differ in that no NMDA component was observed (Dahl et al. 1990; Mody et al. 1988). One of these studies used extracellular recording (Dahl et al. 1990), and thus membrane potential could not be manipulated. As previously suggested (Keller et al. 1991), the inability of the intracellular study (Mody et al. 1988) to observe an NMDA component may have been due to concurrent activation of inhibitory postsynaptic potentials, which were blocked in the present study.

The only significant difference found in this study between lateral and medial perforant–path LTP was that during postsynaptic depolarization, higher-frequency stimulation was required to induce LTP in the lateral perforant path. In addition, even with higher-frequency stimulation

during postsynaptic depolarization, it was more difficult to induce LTP in the lateral perforant path. One simple hypothesis that could account for these differences is that the proportion of the evoked EPSP mediated by NMDA receptors is less in the lateral perforant path than in the medial perforant path, resulting in a smaller increase in postsynaptic Ca^{2+} during LTP induction in the lateral perforant path. Pairing higher-stimulation frequencies with postsynaptic depolarization would elicit LTP more frequently in the lateral perforant path because of the resulting greater summation of intracellular Ca^{2+} or Ca^{2+}-activated processes (Colino et al. 1992).

On the basis of the effect of APV on population spike responses evoked in Mg^{2+}-free medium, a similar proposal was made several years ago (Coan et al. 1987). A smaller NMDA receptor–mediated component of the EPSP would occur if transmitter released from lateral perforant path terminals activates a smaller number of NMDA receptors than does transmitter released from medial perforant path terminals. Quantitative autoradiography in fact demonstrates a higher density of NMDA-sensitive binding sites in the inner molecular layer when compared with the outer molecular layer (Monaghan and Cotman 1985). Alternatively, medial and lateral perforant–path synapses could contain NMDA receptors with functionally distinct properties (Monyer et al. 1992), such that their affinity for glutamate or permeability to Ca^{2+} are markedly different. A definitive test of quantitative differences in the NMDA component of synaptic transmission in the medial and lateral perforant paths will require examination of unitary synaptic events generated by the different fiber populations. Such an analysis in stellate cells of rat visual cortex found that the ratio of NMDA to non-NMDA receptor–mediated synaptic currents varied fourfold at different synapses (Stern et al. 1992).

Although we have focused primarily on the role of NMDA receptors in the induction of LTP in the medial and lateral perforant paths, additional factors also may be quite important for LTP induction in the dentate gyrus. For example, the opioid receptor antagonist naloxone blocks LTP in the lateral but not the medial perforant path (Bramham et al. 1988). Further work will be necessary to characterize completely all the differences in the mechanisms underlying LTP in the medial and lateral perforant paths and whether these differences have functional significance.

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