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Long-term plasticity in cingulate cortex requires both NMDA and metabotropic glutamate receptor activation

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Abstract

We tested whether induction of homosynaptic long-term potentiation and long-term depression of synaptic strength in posterior cingulate cortex requires NMDA and/or metabotropic glutamate (mGlu) receptor activation. In in-vitro slices of rat posterior cingulate cortex, the NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5; 15–20 μM) blocked induction of both long-term potentiation and long-term depression of mono- and polysynaptic population potentials in deep laminae. In contrast, DL-2-amino-3-phosphonopropionic acid (DL-AP3; 15–25 μM), a selective mGlu receptor antagonist, blocked homosynaptic long-term potentiation and long-term depression of monosynaptic transmission, but was ineffective in blocking the induction of either type of plasticity at polysynaptically-driven sites. The selective mGlu receptor agonist, *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), induced a marked depression of subicular-evoked monosynaptic potentials which reversed upon drug washout, but produced little depression of polysynaptic responses. We conclude that metabotropic glutamate receptor activation is necessary for the induction of long-term synaptic plasticity only at monosynaptic subiculo-cingulate terminals, while NMDA receptor activation is necessary for the induction of long-term potentiation/long-term depression of both mono- and polysynaptic pathways.

Keywords: Glutamate; Long-term depression; Long-term potentiation; Metabotropic receptor; NMDA receptor; Plasticity; Posterior cingulate cortex

1. Introduction

Long-term synaptic plasticity is thought to play an essential role in learning and memory (Dunwiddie and Lynch, 1978), as well as in the developmental shaping of cortical networks (Kaas, 1987). A number of studies have suggested that two distinct subtypes of glutamate receptors can play important roles in the induction of long-term plasticity: the NMDA receptor subtype, which gates influx of Ca^{2+} (Harris et al., 1984; Sumoto, 1987; Dudek and Bear, 1992) and the metabotropic (mGlu receptor), a class of G-protein coupled receptors which affect multiple second messenger systems (Irving et al., 1990; Behnisch et al., 1991).

Early work in hippocampus showed that homosynaptic long-term potentiation induced in area CA1 or the dentate gyrus by high-frequency stimulation (> 50 Hz), requires the activation of NMDA receptors which gate the influx of Ca^{2+} (Collingridge et al., 1983; Harris et al., 1984; Chetkovich et al., 1991). More recent work in the hippocampus has found that long-term depression of synaptic strength can be induced by prolonged low-frequency stimulation (< 5 Hz) (Dudek and Bear, 1992; Wexler and Stanton, 1993), and that induction of long-term depression also requires some increase in intracellular free $[Ca^{2+}]$ (Mulkey and Malenka, 1992). Low-frequency stimulus-induced long-term depression can be blocked by either an NMDA receptor antagonist such as D-2-amino-5-phosphonopentanoic acid (D-AP5) (Dudek and Bear, 1992; Wexler and Stanton, 1993), or an mGlu receptor blocker such as DL-2-amino-3-phosphonopropionic acid (DL-AP3) (Stanton et al., 1991; Wexler and Stanton, 1993). Activation of mGlu receptors has been found to enhance the expression of long-term potentiation (Ito and Sugiyama, 1991), and may be required for its induction (Bashir et al., 1993b; Musgrave et al., 1993). Long-term depression can also be elicited by presynaptic activity that is correlated with postsynaptic hyperpolarization (Stanton and Sejnowski, 1989; Artola et al., 1990; Stanton et al., 1994), conditions that might favor lower stimulus-induced increases in $[Ca^{2+}]$ and activation of mGlu receptors over NMDA receptors.

Most studies examining the role of stimulus frequency

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and NMDA and mGlu receptors in the induction of long-term synaptic plasticity have been conducted in the hippocampus at monosynaptic terminal fields (Zheng and Gallagher, 1992; Irving et al., 1990). There is a relative dearth of information on the neuropharmacology of mono- and polysynaptic plasticity in neocortex. This absence of data particularly needs remedying in posterior cingulate cortex, the only known region where hippocampal output converges monosynaptically with both sensory input from thalamus and neocortical feedback (Gabriel and Sparenborg, 1987; Swanson et al., 1987).

A major characteristic of cingulate cortical neuroanatomy is a distinctive and segregated laminar parcellation of thalamic, subicular and extracingulate afferents. Stimulation of the subiculo-cingulate tract elicits monosynaptic responses of deep laminar pyramids that exhibit long-term plasticity, and polysynaptic responses of superficial laminae pyramids that do not (Hedberg et al., 1993; Hedberg and Stanton, 1995). Thus, the amplitude and frequency of afferent input, coupled with activation of multiple subtypes of glutamate receptors, could allow simultaneous potentiation of postsynaptic input strength in some pathways and depression of synaptic responses in others. In the present study, we used the cingulate slice preparation to test the contribution of NMDA and/or mGlu receptors to the induction of long-term plasticity at superficial and deep layer synapses. These data should provide useful characterization of cellular mechanisms of posterior cingulate cortex plasticity needed to understand the role of cingulate cortex in learning and memory.

2. Materials and methods

2.1. Slice preparation and drug perfusion

Paracoronal slices of rat posterior cingulate cortex containing subiculum and physiologically intact segments of the subiculo-cingulate tract were cut as described previously (Hedberg et al., 1993), and preincubated for 5–10 min in an ice-cold bath of artificial cerebrospinal fluid (composition in mM: NaCl 126, KCl 5, NaHPO4 1.25, $MgCl_2$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10), which was continuously bubbled with a 95%O_2/5% CO_2 gas mixture (pH 7.2). Following preincubation, slices were floated onto the net of a continuous perfusion interface brain slice chamber (Haas et al., 1979) and warmed to 33–35°C. Bipolar stainless-steel microelectrodes (Frederick Haer Co.) with 25 μm diameter tips were then positioned in subiculo-cingulate tract fibers.

Extracellular recording electrode placement was performed under a dissecting microscope, and superficial (II/III–IV) and deep (V–VI) laminae were visually identified for electrode placement by their cytoarchitectural characteristics. At each recording site, an extracellular electrode (2 M NaCl-filled glass micropipette; 1–5 MΩ) was lowered 100–150 μm into either superficial or deep laminae of the slice and angled toward the greatest density of pyramidal somata. In some slices, two electrodes were used to obtain simultaneous superficial and deep laminae recordings.

Intracellular recordings were obtained from impalements of pyramidal neurons with somata in laminae V and VI using high-impedance glass micropipettes (80–140 MΩ; A-M Systems Inc.) filled with 2 M potassium acetate (pH = 7.2). Electrodes were connected by an Ag/AgCl half cell to an intracellular amplifier with negative capacitance feedback and an active bridge circuit (Axoclamp 2A; Axon Instruments), to permit measurement of intracellular postsynaptic potentials under current-clamp conditions. Impalements were typically made 75–150 μm below the surface of the slice and were considered acceptable if resting membrane potential was more negative than −65 mV and input resistance was greater than 30 MΩ.

DL-2-Amino-3-phosphonopropionic acid (DL-AP3; 15–25 μM; Sigma) and D-2-amino-5-phosphonopentanoic acid (D-AP5; 15–20 μM; Sigma), were used as mGlu and NMDA receptor antagonists respectively. While other mGlu receptor antagonists such as (*RS*)-α-methyl-4-carboxyphenylglycine (MCPG) have recently been described, their ability to block long-term plasticity at neocortical mGlu receptors has been called into question (Chinestra et al., 1993; Manzoni et al., 1994; Bordi and Ugolini, 1995). DL-AP3, D-AP5, and the mGlu receptor agonist *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD; 25–50 μM; Tocris Neuramin), were bath-applied by dissolving directly in artificial cerebrospinal fluid (ACSF). After baseline data were collected, a nylon valve was opened and the perfusing medium entering the chamber was shunted to a reservoir of drug-containing ACSF, which reached the chamber 3 min later.

To evaluate the pharmacologic actions of each drug on synaptic transmission, field potentials were evoked by subicular stimulation once every 30 s (0.033 Hz) during at least 30 min of drug perfusion. High- or low-frequency stimulation was followed immediately by 30–40 min of test stimuli, after which the drug was washed from the chamber by switching to drug-free ACSF inflow. Baseline responses to single-pulse stimulation were monitored for several hours after washout. Stimulus-evoked field and intracellular excitatory postsynaptic potential (EPSP) responses to stimulation were recorded on-line using a PC-DOS-based data acquisition and analysis system (Datawave Instruments).

2.2. Stimulation and recording paradigms

Baseline evoked responses were obtained by stimulating every 30 s with a 400 mA square pulse (duration 200 μs) and recording postsynaptic responses in deep laminae. To induce homosynaptic long-term potentiation, subicular inputs were tetanized using two high-frequency trains (100

Hz/2 s) separated by a 10 s intertrain interval. Test stimuli (0.033 Hz) began 30 s after high-frequency stimulation and continued for at least 20–30 min. To induce homosynaptic long-term depression, subicular inputs were exposed to 20 min of low-frequency (1 Hz; 1200 stimuli) single-pulse stimulation. Again, post-conditioning test pulses began 30 s after low-frequency stimulation and continued for 20–30 min. Protocols were repeated in the presence, and after washout, of bath-applied drug. For each of the two population action potential negativities evoked in deep laminae by subicular stimulation, peak amplitude was measured from the pre-stimulus baseline, and latency-to-peak was measured from the onset of stimulus artifact.

For intracellular recordings, changes in synaptic strength were assessed by measuring EPSP amplitude, latency and initial descending dV/dt at 30 s intervals before and after conditioning stimulus trains. 4–5 signal averaged sequential responses to test stimuli given during the preconditioning period were compared with signal averages of 4–5 sequential responses to test stimuli given 20–30 min after high or low-frequency stimulation. Statistical significance of all long-term changes in component amplitude, latency and dV/dt were evaluated using a two-tailed *t*-test for paired data.

3. Results

In both superficial (II/III–IV) and deep (V–VI) laminae of posterior cingulate cortex, single pulse stimulation of the subiculo-cingulate tract evokes characteristic field potentials. In earlier studies (Hedberg et al., 1993; Hedberg and Stanton, 1995), we used current source density analysis to examine the neurophysiologic origin of these characteristic components. In superficial laminae, the field potential is composed of a brief initial negativity at < 3 ms (probably reflecting a monosynaptic population EPSP), followed by a larger, broad negativity (NS) at 4–8 ms (probably reflecting both EPSP and a polysynaptic population action potential), and finally a secondary long-latency, long-duration, low-amplitude positivity peaking at 10–12 ms. Conversely, evoked field potentials in deep laminae show an initial short-latency, short-duration, high amplitude population spike negativity (N1), probably reflecting the monosynaptically driven discharge of deep laminae pyramids. This is followed by a second, broader negativity (N2; 2–3 ms) thought to be the di- or polysynaptically driven population discharge of laterally adjacent deep laminae pyramids (Fig. 1A).

Changes in suprathreshold stimulus intensity produced little variation in the amplitude or latency of the N1 component in deep laminae. In contrast, superficial laminae field potentials showed great variability in both latency and amplitude as a function of stimulus intensity (Hedberg et al., 1993; Hedberg and Stanton, 1995). While this study focuses on the contribution of glutamate-receptor-driven activation of deep laminae pyramidal cells, the N1 and N2 components do not reflect only pyramidal cell activity. Interneurons containing the inhibitory neurotransmitter γ-aminobutyric acid (GABA) are also driven by subicular stimulation, and GABA receptors are not blocked by any of the pharmacological agents used in this study. Nevertheless, the monosynaptically-elicited EPSP and population spike components of monosynaptic field potentials should be relatively independent of later inhibition, since the subiculo-cingulate terminal field in cingulate cortex is overwhelmingly glutamatergic.

High-frequency subicular stimulation (100 Hz/2 s/2 ×) reliably produced long-term potentiation of deep laminae synaptic potentials, expressed as both increased population spike amplitude and decreased response latency (Table 1). Superficial laminae field potentials showed rapid short-term enhancement of negativity amplitude, along with a correspondent reduction in response latency. However, this post-tetanic potentiation endured no more than a few seconds, and was followed by a transient depression which lasted less than 3 min, after which amplitude and latency returned to baseline values.

Table 1 summarizes changes from baseline population spike amplitude 20–30 min after either low-frequency or high-frequency stimulation. In superficial laminae, following high-frequency stimulation, mean negativity amplitude was significantly decreased by $-14.8 \pm 3.7\%$ (mean ± S.E.M.; $n = 19$). In contrast, as illustrated in Fig. 1A, field potentials evoked in deep laminae showed statistically significant long-term potentiation, with a mean increase in N1 population spike amplitude of $+44.7 \pm 14.5\%$ (mean ± S.E.M.; $P < 0.05$; paired *t*-test; $n = 18$) compared with pre-tetanized baselines. When subicular afferent fibers were activated by low-frequency stimulation (1 Hz/20 min), superficial laminae showed only a transient depression of dV/dt and negativity amplitude (Table 1, Lam II/III–IV). In contrast, significant sustained depression of synaptic strength was observed in deep laminae (Table 1, Lam V–VI) where N1 population spike amplitude was reduced by $-19.5 \pm 7.2\%$ ($P < 0.05$; paired *t*-test; $n = 8$) compared with pre-stimulus baseline. Long-term depression persisted for over 20 min post-stimulus.

Single neuron correlates of long-term potentiation were also observed in intracellular recordings from deep laminae pyramidal neurons ($n = 5$). When compared with pre-stimulus baseline values recorded intracellularly from deep laminae pyramidal neurons, high-frequency stimulation at intensities initially subthreshold to action potential generation elicited marked long-term potentiation of EPSP amplitudes recorded intracellularly ($+106.5 \pm 57\%$; $P < 0.05$; paired *t*-test, $n = 5$), as well as a small decrease in EPSP peak latency (Fig. 5).

Since NMDA receptor-gated Ca^{2+} influx is required for the induction of long-term potentiation at many (but not all) synapses studied to date, we tested whether the NMDA receptor antagonist D-AP5 blocks long-term potentiation in

posterior cingulate cortex. In 4 experiments with identical time courses (Fig. 1B), when 20 μM D-AP5 was bath applied (solid bar) 20 min prior to the first tetanus (100 Hz/2 s/2×; first arrow), the induction of long-term potentiation was blocked. Instead, high-frequency stimulation caused a long-term depression of N1 amplitude. Following drug washout, subsequent tetanic stimulation (second and third arrows) now elicited two episodes of additive long-term potentiation. In all 8 slices (Fig. 2B), mean amplitude change in the presence of D-AP5 was $-8.0 \pm 9.1\%$ for N1 and $-2.6 \pm 1.8\%$ for N2. Response latency did not change significantly in the present of D-AP5.

Since NMDA receptor activation may also play a role in the induction of long-term depression in hippocampus (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Wexler and Stanton, 1993), we next tested whether application of a low-frequency stimulus which normally elicits long-term depression would do so during NMDA receptor blockade. Low-frequency stimulation was given in a continuous train (1 Hz/20 min) to a different set of slices in

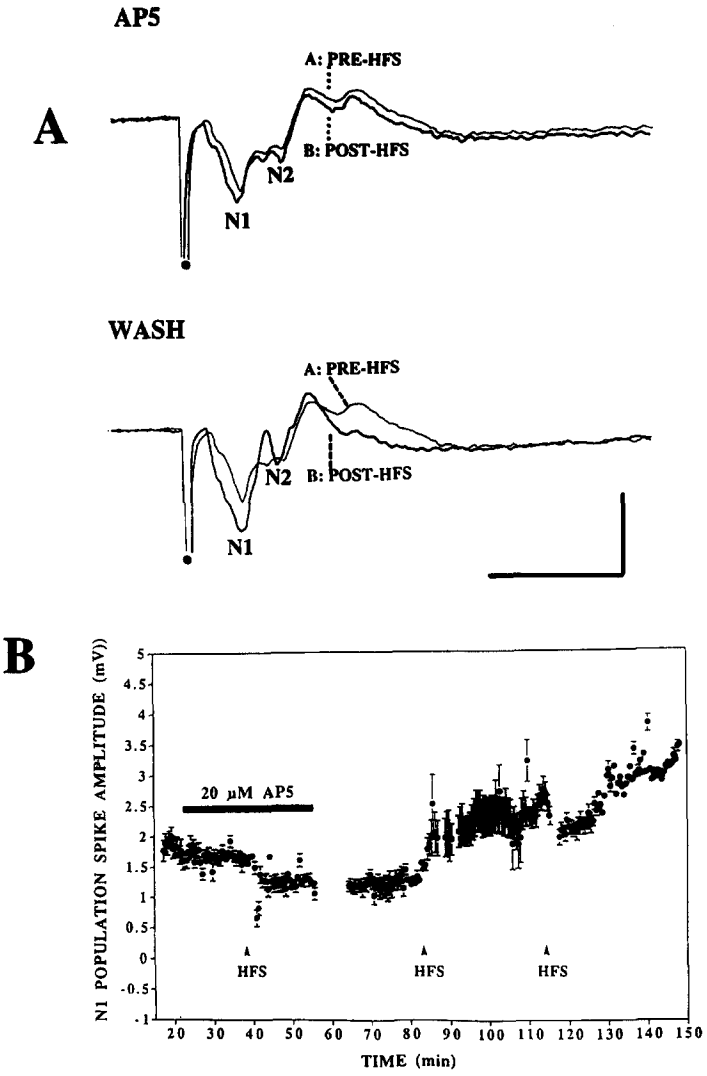

Fig. 1. Blockade of long-term potentiation of subiculo-cingulate synapses in posterior cingulate cortex by the NMDA receptor antagonist D-AP5. *Panel A:* Mean response waveform of deep laminae field potentials evoked by single-pulse subicular stimulation. Waveform (AP5) shows EFPs evoked in the presence of DL-AP5 both before and 20 min after high-frequency stimulation (HFS; 100 Hz/2s). Waveform (wash) shows potentiated field potential evoked 40 min after DL-AP5 washout, and both before and 20 min post-tetanus. Mono- and di/polysynaptic population action potentials are labeled N1 and N2, respectively. Vertical calibration is 0.4 mV and horizontal calibration is 30 ms. Black dots indicate stimulus onset. *Panel B:* Mean N1 population spike amplitude plotted over time for test stimuli applied once every 30 s (each point is the mean ± S.E.M., $n = 4$ slices). High-frequency stimuli (HFS; arrows) were given via the subiculo-cingulate tract and consisted of three 100 Hz trains lasting for 2 s each, given 10 s apart. AP5 20 μM was bath-applied continuously to the preparation during the period indicated by the solid horizontal bar. AP5 completely blocked the induction of long-term potentiation by the first HFS, converting the response to a significant long-term depression of synaptic potentials. After 30 min of drug-free washout, a second and third HFS was able to elicit significant long-term potentiation of synaptic transmission. $P < 0.05$, paired t-test comparing amplitude 30 min post-HFS with pre-HFS baselines.

Table 1
Mean long-term activity dependent changes in synaptic transmission in posterior cingulate cortex

Conditioning frequency		n	X%Δ	S.E.M.
Laminae II/III–VI (superficial)				
(NS amplitude)	100 Hz	19	−14.8	± 3.7 [a]
	1–5 Hz	6	−12.8	±12.7
Laminae V–VI (deep)				
(N1 amplitude)	100 Hz	18	44.7	±14.5 [b]
	1–5 Hz	8	−19.5	±11.5

[a,b] $P < 0.05$, paired t-test compared to pre-conditioning baseline.

the presence of 15–20 μM D-AP5. As shown in Fig. 2A, the N1 evoked 30 min after low-frequency stimulation showed no significant amplitude reduction in D-AP5 ($-0.7 \pm 9.7\%$; $n = 6$), and N2 showed a mean amplitude increase of $+22.5 \pm 11.4\%$ ($n = 4$). As with both amplitude measurements, response latencies did not change significantly. Following washout, a second administration of low-frequency stimulation yielded a clear recovery of long-term depression. Thus, D-AP5 at 15–20 μM impaired induction of both types of plasticity in posterior cingulate cortex, indicating that activation of NMDA receptors is necessary for both homosynaptic long-term potentiation and long-term depression at subiculo-cingulate synapses on deep laminae pyramids.

The polysynaptic N2 component of the deep lamina response showed a somewhat different sensitivity to NMDA receptor blockade from that of the monosynaptic

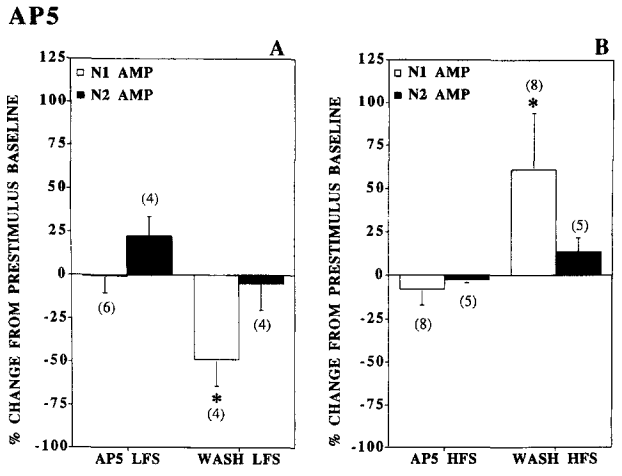

Fig. 2. Blockade of long-term potentiation and long-term depression by the NMDA receptor antagonist D-AP5. Bar graphs plot the mean ± S.E.M. % change from pre-drug baseline in amplitude of N1 (open bars) and N2 (solid bars) field potential components 30 min after conditioning stimulation (HFS or LFS), in the presence of 15–20 μM D-AP5 versus drug washout. The number of slices is indicated above each bar, and error bars are the S.E.M. AP5 completely blocked the induction of long-term depression (A) and long-term potentiation (B) of both N1 and N2 field potential components. After a 30 min drug washout, a second conditioning stimulation now elicited significant long-term depression or long-term potentiation of the N1 potential. * $P < 0.05$; paired t-test compared to pre-conditioning baselines.

N1. As shown in Fig. 2A,B, D-AP5 blocked the induction of both long-term potentiation and long-term depression of the N2 component (solid bars, AP5). However, N2 showed little or no evidence of recovery of synaptic plasticity after D-AP5 washout (solid bars, wash). While not all slices showed recovery of N1 plasticity following drug washout, in the majority which did, sustained changes in N1 population spike amplitude approximated those induced in untreated control slices. In addition, the induction of long-term potentiation ($+26.4 \pm 6.9\%$; $n = 7$) and long-term depression ($-19.4 \pm 4.3\%$; $n = 9$) of N2 amplitude in naive slices was not significantly different from that shown by N1. Accordingly, the lack of recovery of long-term synaptic plasticity following washout may not reflect an inherent inability of the N2 to exhibit long-term potentiation/depression, but may be due to a partial blockade by a residual concentration of D-AP5.

Since the NMDA receptor antagonist D-AP5 blocked the induction of long-term potentiation and depression of N1, as well as of the putatively di- or polysynaptically driven N2 population spike, we next sought to determine to what degree, if any, mGlu receptor activation might also be needed to induce long-term changes in these two response components. Previous studies in hippocampus (Stanton et al., 1991; Wexler and Stanton, 1993), neocortex (Kato, 1993) and cerebellum (Linden et al., 1991) suggest that mGlu receptors play a necessary role in the induction of long-term depression, while Bashir et al. (1993a) have supplied evidence that mGlu receptor activation enhances the induction of long-term potentiation by weak stimuli. In view of these findings, we used the selective mGlu receptor antagonist DL-AP3 (Schoepp and Johnson, 1989; Behnisch et al., 1991) to determine whether mGlu receptor blockade can also prevent the induction of long-term potentiation/depression in cingulate cortex.

As shown in Fig. 3A, bath application of DL-AP3 (15–25 μM) effectively prevented the induction of long-term depression in the N1 component of the population spike in deep laminae. N1 amplitude averaged a change of only $-4.4 \pm 6.8\%$ (open bar, $n = 8$) after low-frequency stimulation in the presence of DL-AP3. Similarly, DL-AP3 also blocked the induction of long-term potentiation (Fig. 3B), showing a mean tetanus-induced change in N1 of $-20.4 \pm 13.6\%$ (open bar; $n = 5$). When washout of DL-AP3 was followed by a second tetanus (Fig. 3B), N1 amplitude now showed significant long-term potentiation ($+30.6 \pm 8.4\%$; $P < 0.05$; paired t-test; open bar; $n = 7$). On the other hand, Fig. 3A also illustrates that, post-washout, low-frequency stimulation was not as effective in restoring induction of long-term depression of either N1 amplitude or latency (open bar, $n = 5$).

In contrast to its effect on the monosynaptically driven N1, bath application of 15–25 μM DL-AP3 was not effective in blocking long-term changes in the amplitude of the putative polysynaptic N2 population spike. Low-frequency stimulation in the presence of DL-AP3 elicited long-term

depression of N2 amplitude of $-21.2 \pm 9.5\%$ ($P < 0.05$; paired t-test; $n = 5$) which, after drug washout, could be depressed even further ($-44 \pm 13\%$; $P < 0.05$; paired t-test; $n = 5$) by a second low-frequency stimulation (Fig. 3A). Conversely, high-frequency stimulation in the presence of DL-AP3 evoked a persistent increase of $+13.8 \pm 2\%$ ($P < 0.05$; paired t-test; $n = 4$) in mean N2 amplitude, which further was potentiated $+24.2 \pm 12.8\%$ ($n = 5$) by a second tetanus after DL-AP3 washout (Fig. 3B). The increase in N2 response amplitude was associated with persistent reductions in response latency. Thus, mGlu receptor blockade was not effective in preventing the induction of long-term plasticity in the N2 component.

Since blockade of mGlu receptors differentially inhibited the induction of long-term plasticity, we tested the effects of mGlu receptor activation by bath-applying the selective mGlu agonist, *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD; 25–50 μM) to cingulate slices. ACPD was selected as, in contrast to other more recently developed compounds, it has been repeatedly shown to be a potent agonist for several subtypes of mGlu receptors at the concentrations we employed (Monaghan et al., 1989; Irving et al., 1990). As summarized in Fig. 4, ACPD produced a marked depression in the mean amplitude of both N1 and N2 population spike components, which reversed substantially upon washout. Fig. 4 also illustrates that the mean decrement in N2 amplitude produced by ACPD ($-25.3 \pm 11.3\%$; $P < 0.05$; paired t-test; $n = 4$) was considerably less than ACPD's depression of N1 amplitude ($-47.6 \pm 12.4\%$; $P < 0.05$; paired t-test; $n =$ 5); an observation consistent with persistence of long-term plasticity of the N2 component despite mGlu receptor blockade. Interestingly, both N1 and N2 population spike amplitudes exhibited a large rebound potentiation following ACPD washout. Assuming a relatively selective blockade of only mGlu receptors at the DL-AP3 concentrations used, our data are consistent with the conclusion that induction of long-term plasticity at subicular synapses in posterior cingulate cortex requires activation of both NMDA and metabotropic subtypes of glutamate receptor.

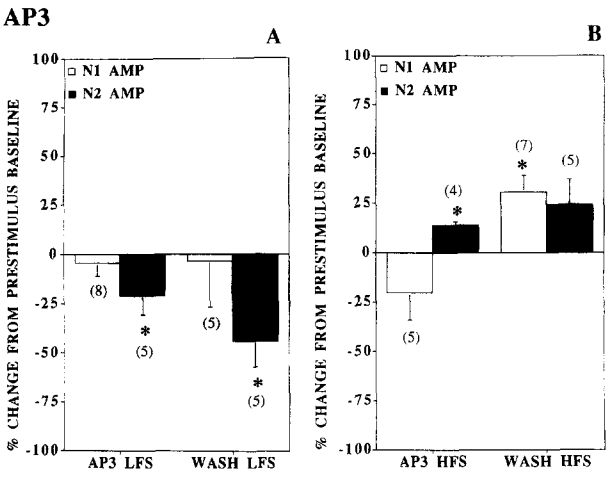

Fig. 3. Blockade of long-term potentiation and long-term depression by the metabotropic receptor antagonist DL-AP3. Bar graphs plot the mean ± S.E.M. % change from pre-drug baseline in amplitude of N1 (open bars) and N2 (solid bars) field potential components 30 min after conditioning stimulation (HFS or LFS), in the presence of 15–25 μM DL-AP3 versus drug washout. AP3 completely blocked the induction of long-term depression (A) and long-term potentiation (B) of the N1, but not the N2, field potential component. After a 30 min drug washout, there was recovery of the ability to evoke long-term potentiation of the N1 component (* $P < 0.05$, paired t-test compared to pre-HFS baselines), but no recovery of long-term depression.

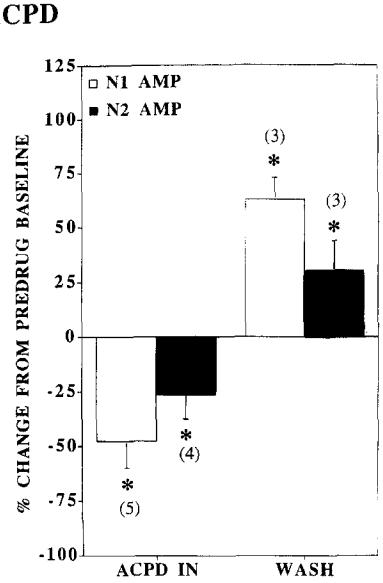

Fig. 4. Effects of the metabotropic receptor agonist *trans*-ACPD on subicular evoked field potentials in deep laminae. Bar graphs plot the mean ± S.E.M. % change from pre-drug baseline in amplitude of N1 (open bars) and N2 (solid bars) field potential components 40 min after the start of perfusion with *trans*-ACPD (50–80 μM). Post washout measurements were taken after 30 min of drug washout. * $P < 0.05$; paired t-test compared to pre-drug baselines.

To correlate field potential data with pharmacological blockade of long-term synaptic plasticity in single neurons, we examined EPSPs recorded intracellularly from deep laminae (V/VI) pyramidal cells. Intracellular impalements were identified as pyramidal and monosynaptic by: (1) positioning of the microelectrode in the pyramidal cell layer; (2) invariant < 3.0 ms latency to EPSP onset regardless of stimulus intensity; (3) presence of spike accommodation; (4) input resistances of > 40 MΩ and resting membrane potentials of 60–70 mV. Signal-averaged examples of EPSPs evoked in deep laminae pyramids by subicular stimulation shown in Fig. 5A illustrates that, while high-frequency stimulation in the presence of D-AP5 produced only a depression of EPSP amplitude, responses evoked in the same neuron by a second post-washout tetanus elicited a marked long-term potentiation of EPSP amplitude (+150% or more of pre-stimulus baseline). As summarized in Fig. 5B, bath application of D-AP5 (15–20 μM) completely blocked the induction of long-term potentiation of intracellular EPSPs by high-frequency stimula-

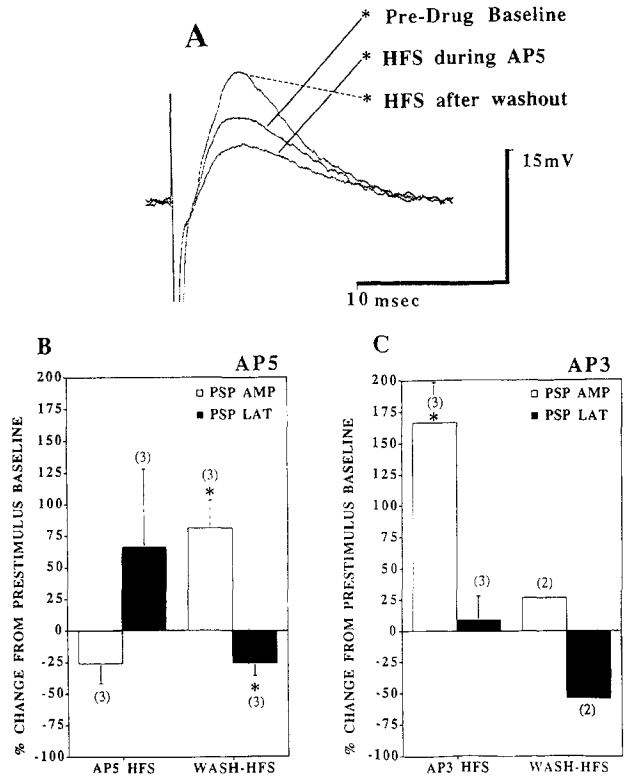

Fig. 5. Blockade of long-term potentiation of intracellular EPSPs in deep laminae pyramidal neurons by D-AP5 and DL-AP3. *Panel A:* Superposition of intracellularly recorded EPSPs from a single deep laminae pyramidal neuron (resting membrane potential = −71mV; R_N = 45 MΩ) before drug application (Pre-Drug Baseline), following HFS in presence of 20 μM D-AP5 (HFS during AP5), and following a second HFS given after 30 min of drug washout (HFS after washout). Each trace is the signal average of 5 successive traces recorded at 30 s intervals. *Panel B:* Bar graphs plot mean ± S.E.M.% changes in amplitude and latency of EPSPs recorded intracellularly from deep laminae (V–VI) pyramidal neurons with resting membrane potential < −60 mV, R_N > 25 MΩ. HFS was administered first during perfusion of 15–20 μM D-AP5, which completely blocked the induction of long-term potentiation. A second HFS given after 30 min of drug-free wash however, elicited significant long-term potentiation (* $P < 0.05$, paired *t*-test compared to pre-HFS baselines). *Panel C:* Bar graphs as in B. In contrast to AP5, the mGlu receptor antagonist DL-AP3 did not block the induction of long-term potentiation by HFS (* $P < 0.05$, paired *t*-test compared to pre-HFS baselines).

tion ($n = 3$). In contrast, a second tetanus given 30–40 min after drug washout showed significantly potentiated EPSP amplitude (+81% ±26.3%; $P < 0.05$; paired *t*-test; $n = 3$) and a corresponding decrease in latency (−26% ± 13%; paired *t*-test; $P < 0.05$; $n = 3$). The blockade of long-term potentiation of intracellular EPSPs by D-AP5 is consistent with our data showing blockade by D-AP5 of long-term plasticity in the monosynaptic N1 component of evoked field potential recordings. Paradoxically, in view of our field potential findings, application of DL-AP3 (20–25 μM) did not block long-term potentiation of intracellular EPSP amplitude (Fig. 5C, +157 ± 39.5%; $P < 0.05$; paired *t*-test; $n = 3$). In the two impalements that lasted throughout the 30–40 min of DL-AP3 washout (Fig. 5C), a second high-frequency stimulation evoked smaller additional long-term potentiation (+26%), and decreased mean latency (−51%).

4. Discussion

In slices from posterior cingulate cortex, high-frequency, monosynaptic, excitatory activation of deep laminae pyramidal neurons by subicular afferents induced robust homosynaptic long-term potentiation, reflected in both the N1 and N2 components of deep laminae field potential population spikes. Conversely, prolonged low-frequency stimulation induced homosynaptic long-term depression of both population response components. In contrast to those findings, neither high- nor low-frequency activation of superficial laminae neurons, including both pyramidal cells and local-circuit inhibitory interneurons, was able to elicit any long-term changes in synaptic strength. A similar lack of long-term plasticity in superficial laminae of neocortex has been reported in cingulate cortex (Hedberg and Stanton, 1995), visual cortex (Kirkwood et al., 1993; Aizenman et al., 1994) and somatosensory barrel cortex (Keller and Arondiadou, 1994). In each area, the presumed polysynaptic activation of superficial pyramidal neurons has been suggested to make the temporal coordination of excitation with postsynaptic depolarization necessary to induce long-term potentiation less likely.

Our pharmacological data indicate that induction of long-term potentiation at synapses responsible for eliciting the N1 monosynaptic population spike in cingulate cortex is prevented by both the ionotropic NMDA receptor blocker D-AP5, and the mGlu receptor antagonist DL-AP3. While the first finding is consistent with many studies on the NMDA dependence of most forms of long-term potentiation in hippocampus and neocortex, a necessary role for mGlu receptors in the induction of long-term potentiation is a more recent finding. Zheng and Gallagher (1992) suggested that, in the dorsolateral septal nucleus, mGlu receptors may be solely responsible for long-term potentiation induced by stimulation of hippocampal afferents: e.g., NMDA receptors could be blocked by D-AP5 with no obvious effect on the induction of long-term potentiation. Likewise, Bashir et al. (1993a) showed that mGlu receptor activation enhances long-term potentiation evoked by weak stimulation in CA1. Long-term potentiation dependent on mGlu receptor activation has also been reported at hippocampal mossy fiber synapses (Ito and Sugiyama, 1991), as well as in the apical dendritic laminae of pyriform cortex (Fejtl et al., 1994), the latter authors noting, in addition, that mGlu receptor activation by AP4 or ACPD also reduced synaptic potentials by acting presynaptically as voltage-dependent calcium channel blockers.

In our study, the induction of long-term depression of the N1 population spike component was also blocked by either D-AP5 or DL-AP3. Since DL-AP3 is a somewhat

selective mGlu receptor antagonist, it has been suggested that it blocks the induction of long-term synaptic depression by inhibiting glutamate-stimulated, mGlu receptor-mediated phosphoinositide turnover in hippocampus (Stanton et al., 1991) and in visual cortex (Dudek and Bear, 1989). A role for NMDA receptors in the induction of long-term depression has also been demonstrated using D-AP5 (Dudek and Bear, 1992; Mulkey and Malenka, 1992). More recently, Wexler and Stanton (1993) have shown that, in hippocampal area CA1, both NMDA and mGlu receptor activation are necessary for the induction of depotentiation by low-frequency stimulation (Selig et al., 1995). Deep laminae neurons of cingulate cortex appear to share with CA1 pyramidal neurons a capacity for bi-directional long-term plasticity of monosynaptic responses after high- and low-frequency stimulation.

Since no long-term potentiation or depression has been observed of field potentials reflecting the activation of polysynaptically-driven superficial laminae pyramids, the robust long-term change measured in the putatively-polysynaptically evoked N2 population spike is intriguing. Several authors have attributed their failure to potentiate superficially-recorded polysynaptic potentials to reciprocal inhibition arising from simultaneous activation of neighboring interneurons (Aizenman et al., 1994). This is also a possibility in cingulate cortex. Although GABAergic interneurons appear to be equally distributed throughout all laminae (Palacios et al., 1981; Mugnaini and Oertel, 1985), binding studies indicate an overall greater density of GABA receptor sites in superficial versus deep laminae (Hedberg and Vogt, 1988). The larger number of superficial GABAergic binding sites might enhance inhibition and timing delays accompanying otherwise initially synchronous activation of superficial excitatory synapses by ascending axonal collaterals from deep laminae pyramids. Thus, a strong and asynchronous inhibitory influence might prevent the pairing of presynaptic excitation with the postsynaptic depolarization needed to induce long-term potentiation of superficial laminae synapses.

Binding studies have shown that the density of GABA receptors on laminae V–VI pyramidal neurons is 50% of that on superficial laminae pyramids (Hedberg and Vogt, 1988). If the N2 reflects direct orthodromic activation by collaterals of laminae V–VI pyramids synapsing on the somata or basal dendrites of laterally adjacent deep laminae pyramids (Vogt, 1985), it follows that there may be both less inhibitory input to these neurons, and more synchronous excitatory input coincident with subicular stimulation frequencies. These factors could synergize to support the induction of long-term plasticity in a polysynaptically-activated neuronal population. Given cingulate cytoarchitecture, it is also possible that a deep-superficial-deep excitation loop involving laminae V–VI pyramids could shunt afferent inflow at theta frequencies past the more complex superficial 'processing' circuitry. This could elicit greater long-term potentiation of deep laminae responses: i.e., subicular input at this frequency would produce sufficient recurrent collateral excitation of monosynaptically-driven pyramids (N1) to potentiate N2.

More difficult to explain is the lack of effect that mGlu receptor blockade had on induction of long-term potentiation/depression of the N2 population spike. Since D-AP5 thoroughly blocked all long-term plasticity shown by the putatively di- or polysynaptically elicited N2 component, it could be concluded that such plasticity is exclusively NMDA-receptor mediated. This conjecture would be supported if there were relatively fewer mGlu receptors located on the recurrently activated somata and basal dendritic arbors of those deep laminae pyramidal neurons responsible for N2 generation. Consistent with this idea, ACPD infusion did produce half the depression of N2 potential amplitudes, compared with N1 (Fig. 4). Answering this question will require studies of the laminar distribution and density of NMDA and MGlu receptors.

In summary, our data indicate that: (1) In in-vitro slices of posterior cingulate cortex, long-term synaptic plasticity can be induced by subicular stimulation of deep laminae pyramidal neurons, and not in superficial laminae. (2) Long-term potentiation and depression induced monosynaptically in cingulate cortex by subicular stimulation require the activation of either NMDA or mGlu receptors, since both D-AP5 and DL-AP3 were effective in blocking their induction. (3) Long-term potentiation and long-term depression of the putatively polysynaptically activated pyramidal neurons responsible for the N2 component of the deep laminae field potential were not blocked by DL-AP3, indicating that mGlu receptors may not be required for the expression of plasticity at these synapses. (4) Bath application of ACPD depressed the N1 field potential component to a greater extent than N2 amplitude, also suggesting that fewer mGlu receptors are activated by collateral excitation. (5) Intracellular recordings from deep laminae pyramidal neurons also show blockade of long-term potentiation by D-AP5, but not DL-AP3.

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