Effect of the Group I Metabotropic Glutamate Agonist DHPG
on the Visual Cortex

XIAO-TAO JIN, CHRISTOPHER J. BEAVER, QINGHUA JI, AND NIGEL W. DAW
Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Connecticut 06520

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Jin, Xiao-Tao, Christopher J. Beaver, Qinhua Ji, and Nigel W. Daw. Effect of the group I metabotropic glutamate agonist DHPG on the visual cortex. J Neurophysiol 86: 1622–1631, 2001. Metabotropic glutamate receptors have a variety of effects in visual cortex that depend on the age of the animal, the layer of the cortex, and the group of the receptor. Here we describe these effects for group I receptors, using both in vivo and in vitro preparations. The metabotropic group I glutamate receptor agonist 3,5 dihydroxyphenylglycine (DHPG) potentiates the responses to N-methyl-D-aspartate (NMDA) and \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) in slices of rat visual cortex. It also increases, initially, the visual response in the cat visual cortex. Both these effects are largest at 3–4 wk of age and decline to insignificance by 10 wk of age. Both are also largest in lower layers of cortex, which explains why the facilitatory effects found with the general metabotropic glutamate agonist 1S,3R aminocyclopentane-1,3-dicarboxylic acid (ACPD) are observed only in lower layers. Prolonged application of DHPG in the cat visual cortex, after the initial excitatory effect, produces depression. We also found that DHPG facilitates the NMDA response in fast-spiking cells, which are inhibitory, providing a partial explanation for this. Thus there are multiple effects of group I metabotropic glutamate receptors, which vary with layer and age in visual cortex.

INTRODUCTION

We have found that the metabotropic glutamate agonist, 1S,3R-aminocyclopentane-1,3-dicarboxylic acid (ACPD) has both depressive and facilitatory effects in visual cortex and that the facilitatory effects are found only in lower layers (Reid and Daw 1997; Wang and Daw 1996; Wang et al. 1998). There are eight mGluRs, divided into three groups: group I (mGluRs 1 and 5), group II (mGluRs 2 and 3), and group III (mGluRs 4, 6, 7, and 8) (see Conn and Pin 1997; Nakanishi 1994). ACPD is primarily effective at Group I and II mGluRs (Conn and Pin 1997). Experiments show that group II receptors have depressive effects in visual cortex, both in vivo and in vitro (Beaver et al. 1999; Flavin et al. 2000). Our hypothesis therefore is that the facilitatory effects of ACPD are due to its action on group I receptors (Daw and Reid 1996; Daw et al. 1999).

There are developmental as well as laminar variations in the distribution of mGluRs in the visual cortex (Reid and Romano 2001; Reid et al. 1995, 1997). Levels of mGluR5 receptor decrease by a factor of 10 between birth and adult, with a rearrangement of the laminar distribution, while mGluR1 \( \alpha \) decreases by a factor of 2 with no change in laminar distribution. The overall level of mGluRs 2/3 does not vary much, but there is a decrease with age in layer 4. Thus we decided to investigate how the action of the agonist 3,5-dihydroxyphenylglycine (DHPG), which is specific to group I mGluRs (Gereau and Conn 1995; Ito et al. 1992; Schoepp et al. 1994), varies with both layer and age. For comparison with previous work, we concentrated on the effects in cat visual cortex in vivo, where visual responses and spontaneous activity can be measured, and in slices of rat visual cortex in vitro, where pre- and postsynaptic effects can be distinguished.

METHODS

Recordings from slices

Three 4-wk-, five 7-wk-, and eight 10-wk-old rats were used in this study. Coronal slices from rat visual cortex were cut at 400 \( \mu \)m thick on a vibratome and incubated at room temperature in a chamber containing artificial cerebrospinal fluid (ACSF) comprising (in mM) 128 NaCl, 2.5 KCl, 2 MgSO\(_4\), 2 CaCl\(_2\), 1.25 NaH\(_2\)PO\(_4\), 10 glucose, and 26 NaHCO\(_3\) at pH 7.3 and gassed with a mixture of 95% O\(_2\)-5% CO\(_2\).

For recordings, a slice was transferred to a perfusion chamber, held between two pieces of netting (Jin and Daw 1998), and perfused with ACSF. The solution was heated to 33–35°C in its reservoir and again by a heat exchanger next to the perfusion chamber. The flow was 3 ml/min. Patch electrodes (3–8 M\( \Omega \)) were pulled and filled with (in mM) 125 K-methanesulfonate, 5 NaCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 10 EGTA, 10 HEPES, 5 K-ATP, and 1 GTP tris salt with or without guanosine 5\'-O-(2-thiodiphosphate) (GDP-\( \beta \)-S, 1 mM) at pH 7.3. Recordings were made “blind” (Blanton et al. 1989), using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). The electrode was advanced through the tissue under positive pressure in voltage clamp, with a 10-nV pulse applied every 10–20 ms, until a small increase in resistance was seen. Pressure was then released and suction applied until the seal resistance exceeded 1 G\( \Omega \). Further suction ruptured the cell membrane to give recordings in the whole cell configuration.

The recording electrode was placed at a particular distance between pial surface and white matter, to record from cells in a particular layer. Measuring from the pia, the boundaries are at 10% for layers 1/2, 32–36% for 3/4, 47–52% for 4/5, and 72–75% for 5/6, independent of whether one is in monocular or binocular parts of visual cortex (Reid and Juraska 1991). A multiple-barrel iontophoretic electrode was inserted to the same depth in the tissue near the cell body. The iontophoretic electrode contained 20 mM \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and 55 mM DHPG (pH 7.4–7.8) in different barrels.
10 nA, ejecting current 100–150 nA for DHPG, and 10 nA above threshold for both NMDA and AMPA). To test the effect of DHPG on the cell’s NMDA or AMPA response, NMDA or AMPA was first applied three times, 5 s each time, as a control, in current clamp. DHPG was then applied for 15–30 s, and NMDA or AMPA retested. In these experiments, tetrodotoxin (TTX, 0.5 mM) was used in the bath solution to block synaptic transmission. In other experiments, kynurenic acid (1 mM) was added to the bath to block AMPA and NMDA receptors, and the effect of DHPG on action potential frequency elicited by current injection was tested. To test the specificity of the effects, we used the mGluR1 antagonist (S)-α-amino-4-carboxy-2-methylbenzeneacetic acid (LY367385, 20 μM) together with the mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP, 2 μM) in the bath.

Data were collected and analyzed with pCLAMP software (Axon Instruments, Foster City, CA). Results were quantified by measuring the depolarization from NMDA or AMPA in the presence of DHPG, divided by the area under the depolarization from NMDA or AMPA alone. Results are presented as means ± SD, and significance evaluated by Student’s t-test. The spike frequency was calculated from the first interspike interval, with a minimum of two trials for each current intensity.

NMDA, kynurenic acid, and TTX were obtained from Sigma Chemical (St. Louis, MO). AMPA was obtained from Research Biochemical (Natick, MA); DHPG was obtained from Tocris Cookson (St. Louis, MO).

**Whole animal recording**

Light-reared animals (n = 18) were kept from birth in colony rooms with 12:12 light:dark cycles. Kittens were housed with their mother until 6–8 wk of age at which time they were housed singly in cages.

**SURGERY.** Animals were sedated with acepromazine (0.1 mg/kg) and given a preanesthetic dose of atropine (0.04 mg/kg). Anesthesia was induced with halothane (4%) in a mixture of 67% nitrous oxide, 33% oxygen and maintained with 0.5–1.0% halothane afterward. After a tracheotomy and cannulation of the femoral vein, the skull was opened up over the lateral gyrus, and a small hole made in the dura for

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**Fig. 1.** A and B: effect of 3,5 dihydroxyphenylglycine (DHPG) on the response to N-methyl-D-aspartate (NMDA) recorded in 2 layer VI cells from rat visual cortex, one with normal intracellular solution (A) and one with guanosine 5′-O-(2-thiodiphosphate) (GDP-β-S) in the recording pipette (B). With normal intracellular solution, the NMDA response was potentiated by a factor of 1.8 by DHPG. With GDP-β-S in the recording pipette, the NMDA response in the presence of DHPG was 1.1 times control. Recovery records (3rd line) taken 60 s later. Histograms underneath give a summary of the effects found. C and D: effect of DHPG on the response to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) in 2 layer VI cells from rat visual cortex, with normal intracellular solution (C) and with GDP-β-S in the pipette (D). With normal intracellular solution, the AMPA response was potentiated by a factor of 1.97 by DHPG. With GDP-β-S in the recording pipette, the AMPA response in the presence of DHPG was 1.13 times control. Recovery records taken 60 s later. Histograms underneath give a summary of the effects found.
insertion of the electrode. All wound margins were treated with lidocaine (2%). The eyes were covered with contact lenses of zero power, and curvature appropriate to focus the retina on a tangent screen at 57 in. After surgery the animal was paralyzed by intravenous infusion of pancuronium bromide at 0.6–1.5 mg/h (Elkins-Sinn, Cherry Hill, NJ). Heart rate and end-tidal \( \text{CO}_2 \) were monitored continuously, and \( \text{CO}_2 \) was maintained at 3.8–4.5% by adjusting the respirator.

**ELECTRODES AND RECORDING.** Single-unit recordings were made with a carbon-fiber-in-glass microelectrode with six side barrels for iontophoresis. After isolation of a unit, the receptive field was mapped on a tangent screen with a hand-held projector to determine the preferred orientation, optimal size, and velocity of the stimulus. Subsequently, computer-generated stimuli were used to stimulate the cell through the dominant eye with the preferred parameters. The stimulus rested at first for 1 s outside of the receptive field, then swept across the receptive field, resting for 1 s on the far side, and swept back, resting 1 s more. This procedure was repeated three to five times, depending on the velocity of the stimulus, for one group of records every minute. Amplified spikes were discriminated with a voltage window and monitored for amplitude and waveform on a storage oscilloscope. The computer was also used to store spike discharge times and to construct a peristimulus time histogram on-line as spikes came in. The data were stored on a hard disk for subsequent off-line analysis using custom-written ASYST programs (Asyst Software, Rochester, NY).

Control groups of records were taken until six consecutive groups showed a set of stable responses at which time DHPG (Tocris, St. Louis, MO), at a concentration of 100 mM and pH 7.8–8.0, was iontophoresed for 3 min (Medical Systems, Greenvale, NY). Ejection currents ranging from \(-20\) to \(-90\) nA were used. Retaining currents of \(+5\) to \(+10\) nA were used to prevent leakage of the DHPG between drug applications. As the cell recovered, records were taken every minute until six consecutive groups showed a consistent set of responses. Data were used for analysis only if the response after recovery was at least 70%, but no more than 130%, of the response before drug application.

**DATA ANALYSIS.** Firing rates were averaged over the peak of the visual response. Visual responses were expressed as average firing rate during the response minus the spontaneous activity. Spontaneous activity was measured during the second before the stimulus started to move. The firing rate for the preferred direction was taken in the case of cells with a preferred direction, and the firing rates in both directions were analyzed for cells responding to both forward and reverse movement. The effect of DHPG on the visual response was estimated by taking the firing rate during the first 2 min and the last 2 min of a 4-min drug application as a percentage of control. Control firing rates were calculated by averaging the firing rates during the control and recovery periods.

We used a concentration for the drug in the pipette (25 mM) roughly 3 log units higher than the EC\(_{50}\) for DHPG (2–6 \( \mu \)M) in vitro (Conn and Pin 1997), to obtain a concentration at the level of the EC\(_{50}\) of the drug at receptors in vivo (Fox et al. 1989). An ejection current of \(-40\) nA was selected for comparisons between animals after initially testing a range of ejection currents (\(-20\) to \(-90\) nA), as it produced a reliable effect in a majority of cells. The efficacy of the drug within an experiment was also judged by comparing responses within a penetration. That is, if a cell within a particular layer did not show an effect, cells in other layers within the same penetration typically did.

**LESIONS AND HISTOLOGY.** At least two lesions were made in each penetration through the recording electrode, using 3.5–4.0 \( \mu \)A DC for 10 s. On completion of the experiment, the animal was deeply anesthetized with 4% halothane and perfused through the heart with 0.066 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The lateral gyrus was removed and allowed to sink in a 30% sucrose/4% paraformaldehyde solution. Frozen sections (60 \( \mu \)m) were cut and stained with methylene blue-azure. The electrode tracks were reconstructed, and cells were assigned to layers according to layering criteria described by Kelly and Van Essen (1974). Cells were assigned to the borders of layers when the histology indicated that their inclusion in layers 2 and 3, 4, 5, or 6 was ambiguous.

These methods fulfill the guidelines of the American Physiological Society and were approved by the Yale Animal Care and Use Committee.

**RESULTS**

The effect of DHPG on the response to glutamate agonists was measured in cells recorded from slices of rat visual cortex. The voltage response to NMDA was potentiated by DHPG in cells from lower layers of cortex (Fig. 1A). This was a repeatable phenomenon, which went away within a minute of the application of DHPG, as seen in three applications of DHPG following a series of applications of NMDA (Fig. 2). The extent of the potentiation was calculated from the area under the response (time-voltage integral) to NMDA together with DHPG, divided by the area under the response to NMDA alone, and was \( 2.1 \pm 0.9 \) in eight cells recorded, representing a significant increase (mean \( \pm \) SD, \( P < 0.01 \), \( t \)-test). To test whether this potentiation was a presynaptic or postsynaptic effect, GDP-\( \beta \)-S was placed into the recording pipette. This blocks the activity of G-proteins in the postsynaptic cell, and consequently any effect of metabotropic glutamate receptors in the postsynaptic cell, but does not affect any metabotropic responses in presynaptic cells (Eckstein et al. 1979). With this treatment, the effect of DHPG on NMDA was abolished (Fig. 1B), giving an insignificant increase in the NMDA response (1.06 \( \pm \) 0.18, \( n = 7 \)). The effect was also abolished by the specific mGluR1 antagonist, LY367385 at 20 \( \mu \)M plus the specific mGluR5 antagonist, MPEP at 2 \( \mu \)M in the bath (0.9 \( \pm \)

![Fig. 2. Repeated applications of NMDA, combined in 3 cases with an application of DHPG.](http://jn.physiology.org/Downloaded from jn.physiology.org)
0.4, \( n = 5 \)). No direct effect on NMDA receptors as reported by Contractor et al. (1998) was seen.

The response to AMPA was also potentiated by DHPG (Fig. 1C). The AMPA response in the presence of DHPG was \( 1.62 \pm 0.2 \) in 10 cells recorded, which represents a significant increase \( (P < 0.001) \). Again, little increase was seen in six cells with GDP-\( \beta \)-S placed in the recording pipette \( (1.13 \pm 0.15 \); see Fig. 1D). Thus DHPG potentiated the responses to both NMDA and AMPA through an effect on metabotropic glutamate receptors located on the postsynaptic cell.

A facilitatory effect was also seen when the postsynaptic cell was depolarized by a current pulse. An increase in firing of action potentials was produced by DHPG, from 20 to 42 Hz in the example shown (Fig. 3A). Increases were not seen with GDP-\( \beta \)-S in the recording pipette (Fig. 3B). The firing rate was \( 204 \pm 30\% \) compared with control \( (n = 6; P < 0.001) \) with normal intracellular solution, and \( 96 \pm 17\% \) compared with control \( (n = 5) \) with GDP-\( \beta \)-S (Fig. 3C).

These postsynaptic facilitatory effects found in recordings from slices would predict that DHPG should increase the visual response and/or spontaneous activity seen in recordings from animals in vivo. Generally speaking, this was the initial effect seen in responses observed in cat visual cortex (Fig. 4), primarily at young ages in lower layers. The visual response in particular was increased. However, longer applications of DHPG produced a different effect, which will be described and discussed below.

### Developmental changes in the effects of DHPG

Recordings from both slices of rat visual cortex in vitro and responses from cat visual cortex in vivo showed that these effects of DHPG declined with age. Recording from lower layers in rat visual cortex in vitro, the potentiation of the NMDA response by DHPG was \( 2.16 \pm 0.85 \) at 3–4 wk of age, \( 1.6 \pm 0.18 \) at 5–7 wk of age, and \( 1.2 \pm 0.1 \) at 8–10 wk of age.
This was a significant decrease with age ($F = 3.792; P < 0.05$; 1-way ANOVA).

A more extensive series of recordings was made in cat visual cortex, with samples of cells from all layers at a variety of ages (Fig. 6). The principal effect was seen on the visual response at 3–4 wk of age. An increase in firing of about 50% was seen in layers 2, 3, and 4, and a rather larger increase in lower layers at this age (Fig. 6, bottom left). On average, there was little change in spontaneous activity at any age in any layer (Fig. 6, top), and little change in visual response at 6–11 wk or 22 wk in any layer (Fig. 6, middle and right bottom).

Laminar differences in the effects of DHPG

It is apparent from Fig. 6 that the largest effects of DHPG in cat visual cortex are seen with cells recorded in lower layers from young animals. The largest effect was also seen in lower layers in slices of rat visual cortex (Fig. 7). The average potentiation of the NMDA response by DHPG was $1.48 \pm 0.71$ in layers 2/3 (6 cells; $P = 0.157$); $1.36 \pm 0.51$ in layer 4 (8 cells; $P = 0.09$); $2.17 \pm 0.54$ in layer 5 (6 cells; $P < 0.005$); and $2.29 \pm 0.97$ in layer 6 (8 cells; $P < 0.01$). Thus the effect of DHPG depends on both age and the layer in which the cell is recorded.

Combination of effects seen with longer applications of DHPG

When DHPG is applied to the visual cortex of the cat in vivo, the initial effect, seen after 1–2 min of iontophoresis, is facilitatory (Fig. 8, 2nd trace). However, the longer term effect, seen after 3–4 min, is depressive (Fig. 8, 3rd trace). This was generally true for cells in all layers of cortex at 3–4 wk of age, when significant effects of DHPG are most noticeable. The visual response is increased by a factor varying from 30% to more than 100% during the first 2 min (Fig. 9, first trace), then decreased to 30–70% of baseline after 2 min of application (Fig. 9, 3rd trace). Significant differences between control and drug responses (1-tailed $t$-test) were observed in layers 2/3 ($P < 0.01$), layer 4 ($P < 0.001$), and layer 5 ($P < 0.05$) for the last 2 min of DHPG application.

Does the facilitation or depression of the visual response in vivo have any effect on the receptive field properties? An analysis of the direction selectivity of cells during the first 2
It is possible, however, that activation of group I mGluRs may modify other receptive field properties not measured in this series of experiments.

A number of hypotheses may account for the result that there is an initial facilitatory effect followed by a depressive effect. Three that we investigated are: 1) that DHPG acts on both mGluR1 and mGluR5, and that one of these receptors has a facilitatory effect, followed by the other with an inhibitory effect; 2) that DHPG has a facilitatory effect on the glutamate system, followed by a facilitatory effect on the GABA system that inhibits the cell being recorded; and 3) that the mGluRs on postsynaptic cells could be desensitized by prolonged application of DHPG. The first two hypotheses depend either on a spatial separation of the two effects, with the facilitatory effect produced close to the cell recorded, and the depressive effect produced at some distance, so that the iontophoresis of DHPG reaches the facilitatory site first, or on a concentration difference, with the facilitatory effect activated at a lower concentration than the depressive effect.

To test the first hypothesis, we compared the effect of DHPG, which is an agonist for both mGluR1 and mGluR5, with the effect of CHPG, which is an agonist for mGluR5 but not mGluR1 (Brabet et al. 1995; Doherty et al. 1997). The initial effect of both drugs during the first 2 min of application was facilitatory (Fig. 10, top middle traces), and the later effect of both drugs was depressive (Fig. 10, bottom middle traces). Similar effects with CHPG and DHPG were seen in six other cells. Thus both facilitatory and depressive effects are produced by mGluR5, and the difference cannot be accounted for by a difference in effect between mGluR1 and mGluR5.

It is hard to test the second hypothesis in cats in vivo due to difficulties of interpretation when more than one drug is iontophoresed at the same time. However, we did show in slices of rat visual cortex that the NMDA response of fast-spiking cells is potentiated by DHPG (Fig. 11), as well as the NMDA response of regular spiking cells. Fast-spiking cells are stellate, while regular spiking cells are pyramidal; thus fast-spiking cells are inhibitory (Connors et al. 1982). The conclusion is that DHPG potentiates the NMDA response in both glutamate cells and GABA cells. This provides one possible explanation for our result in cat visual cortex that the initial effect of DHPG
is facilitatory, because DHPG will first reach receptors on the cell being recorded, and the later effect is depressive, because DHPG will diffuse to reach surrounding GABA cells as the iontophoresis continues, and facilitation of the GABA cell response will inhibit the cell being recorded.

It is also hard to test the third hypothesis in cats in vivo due to difficulties in distinguishing pre- from postsynaptic drug effects on the visual response. We therefore tested it in slices of rat visual cortex in the presence of TTX. Prolonged application of DHPG for 3–4 min potentiated the NMDA response throughout the period of application (Fig. 12). Indeed, the NMDA response was potentiated more toward the end of the DHPG application than it was at the start. In five cells tested, the average potentiation was 1.78 ± 0.39 (P < 0.05, 1-tailed t-test) after 30–40 s of DHPG application, and 2.1 ± 0.45 (P < 0.01) after 3–4 min of DHPG application. There were no signs of inhibition of the NMDA response at any stage of DHPG application.

**DISCUSSION**

We have found that DHPG produces facilitatory effects on cells in cat visual cortex in vivo and rat visual cortex in vitro. The facilitatory effects found earlier with ACPD in these preparations (Reid and Daw 1997; Wang et al. 1998) are presumably due to its action on group I metabotropic glutamate receptors, because the effects found with specific group II agonists are inhibitory (Beaver et al. 1999; Flavin et al. 2000), and ACPD is not very effective on group III receptors (Conn and Pin 1997). The laminar distribution of the effects of DHPG supports this hypothesis. Excitatory effects of ACPD were found only in lower layers of cortex (Reid and Daw 1997). We find that the excitatory effects of DHPG are most prominent in lower layers in both cat visual cortex in vivo, and in rat visual cortex in vitro.

The effect of DHPG seen in lower layers of cat visual cortex declines between 3–4 wk and 6–11 wk of age. This agrees with the observation that ibotenate-stimulated phosphoinositide turnover peaks at 30 days in the cat, declining after that over the next few weeks (Dudek and Bear 1989). The effect that we saw in slices of rat visual cortex declined between 3–4 and 8–10 wk of age. Measurement of glutamate-stimulated phosphoinositide turnover in the rat declined between 1 and 5 wk of age, preceding the change that we saw (Dudek et al. 1989). A similar time course has been found in the rat hypothalamus (Sortino et al. 1991). These developmental changes must be at least partly due to the decline in mGluR5 and

**FIG. 10.** The effects of 4-min applications of 25 mM DHPG at −40 nA (left column) and 30 mM CHPG at −40 nA (right column) on the same layer 6 cell of a 23-day-old animal. The cell responded best to a bar of light that was moving along the 135/315° axis (0° taken as vertical) at a velocity of 3°/s. **Top graphs:** control responses. **Top middle:** responses during the 1st 2 min of drug application. **Bottom middle:** responses during the last 2 min of drug application. **Bottom:** responses during the recovery period taken 5–11 min after the drug application was halted. Bars above the figures indicate the direction of movement of the stimulus.
mGluR1α between birth and several weeks of age, as measured by antibody staining of preparations from cat visual cortex (Reid et al. 1997), although only the decline in mGluR5 would appear to be large enough to account for the phosphoinositide changes seen.

In the rat visual cortex in vitro we studied primarily potentiation of the NMDA response. Similar poten tiation of the NMDA response have been seen in hippocampus (Fitzjohn et al. 1996), striatum (Pisani et al. 1997), spinal cord (Jones and Headley 1995; Ugolini et al. 1999), and subthalamic nucleus (Awad et al. 2000) with evidence that it is due to group I metabotropic glutamate receptors. However, a study of mouse cultured cortical neurons showed an inhibition of the NMDA response by DHPG (Yu et al. 1997). Yu et al. suggest that the discrepancy may be due to a different NMDA receptor composition in different cell types. Alternatively, second-messenger coupling and ion channels affected may vary after culturing and with selection of particular cells from the culture. In agreement with our result, the NMDA receptor channel current is reduced in the hippocampus of mGluR5 mutant mice (Lu et al. 1997).

There are other facilitatory and excitatory effects of group I mGluR receptors. This includes depolarization of the cell, abolition of spike frequency adaptation, and abolition of $I_{\text{AHP}}$ (Abdul-Ghani et al. 1996; Awad et al. 2000; Burke and Hablitz 1996; Davies et al. 1995; Gereau and Conn 1995; Godwin et al. 1996; Ito et al. 1992). We observed an increase in the frequency of firing of the cell to a depolarizing current consistent with these effects, but did not do a comprehensive study varying age and laminar location.

Iontophoresis of DHPG had a dual effect on neurons recorded in vivo from visual cortex. It increased the visual response in the first 1–2 min of DHPG application, then reduced the response in the last 3–4 min. There are several possible explanations for this. First, in the visual cortex, a neuron receives both excitatory input from a presynaptic glutamatergic neuron and inhibitory input from GABAergic neurons that are nearby. Iontophoresis of DHPG is likely to activate first the neuron that is recorded. Prolonged iontophoresis of DHPG, however, could cause it to diffuse to nearby inhibitory interneurons. Under these conditions, DHPG would have a dual effect: that is, excitation by potentiation of responses in the neuron recorded followed by inhibition due to potentiation of responses in inhibitory interneurons. This hypothesis is supported by data from the rat frontal cortex, where the frequency of spontaneous IPSCs recorded from layer 2/3 pyramidal neurons is increased by activation of group I metabotropic glutamate receptors (Chu and Hablitz 1998). This is believed to be due to increased firing of the inhibitory neurons that release GABA. Our slice work also demonstrated that DHPG potentiated the NMDA response in fast-spiking neurons. A recent study using single-cell RT-PCR techniques has demonstrated that group I metabotropic glutamate receptors are expressed in hippocampal GABAergic interneurons including fast-spiking neurons (van Hooft et al. 2000).

A second possible explanation is that postsynaptic group I mGluRs could be desensitized. We recorded NMDA responses in slices and observed the effect of prolonged DHPG application on the response to NMDA. The potentiation of NMDA responses at the end of 3–4 min of DHPG was, if anything, larger than the potentiation observed in the first 30–40 s of DHPG application. So this explanation seems unlikely. A third
possible explanation is that presynaptic group I mGluRs facilitate release of glutamate; then there is a switch to inhibition of release as a result of desensitization of group I mGluRs. This has been previously demonstrated in the rat hippocampus and in cerebral cortex synaptosomes (Herrero et al. 1998; Rodriguez-Moreno et al. 1998). To test this hypothesis, we recorded excitatory postsynaptic potentials (EPSPs) in slices and observed the effect of DHPG on EPSPs and found no significant effect. So this explanation also seems unlikely. A fourth possible explanation is that the depression observed in the last 2 min of DHPG application in vivo is due to direct effects of DHPG on NMDA receptors at high concentrations (Contractor et al. 1998). However, our slice work together with a recent study (Kreiger et al. 2000) does not support this explanation because no effect of DHPG on the NMDA response was seen when mGluR-mediated effects were blocked by either GDP-β-S or LY367385 plus MPEP.

Are the effects that we have seen due to mGluR1, mGluR5, or both? Cells tested with both CHPG and DHPG in the cat in vivo showed the same result, suggesting that mGluR5 is the primary receptor involved. Both mGluR1 and mGluR5 show a decline with age in cat visual cortex, and the magnitude of the decline in mGluR5 fits the magnitude of the decline in the response to DHPG better, but the laminar distribution of mGluR1 fits the laminar distribution of the DHPG response better (Reid et al. 1997). Electron microscopy shows both mGluR1 and mGluR5 located postsynaptically in dendrites, and some mGluR1 located presynaptically (Romano et al. 1995), but the physiological effects that we found in vitro were all postsynaptic. The NMDA response is reduced in mGluR5 mutant mice (Lu et al. 1997), while the point has not been thoroughly tested in mGluR1 mutant mice (Aiba et al. 1994a,b; Conquet et al. 1994). Thus it seems almost certain that mGluR5 is involved, and likely that mGluR1 also contributes to the effects described.

In summary, we have shown that DHPG potentiates the NMDA response in slices of rat visual cortex and facilitates the visual response of cells in cat visual cortex, followed by depression. The effects decrease with age, being maximal at 3–4 wk of age, and are most prominent in lower layers of cortex. This accounts for the excitatory actions of APDC, which are also found in lower layers of visual cortex. While the excitatory actions of APDC can be accounted for by the action of group I mGluRs, DHPG also has inhibitory effects. These inhibitory effects are due at least partly to facilitation of GABA interneurons by DHPG, although other causes may also contribute. Thus there are multiple modulatory effects of mGluRs in visual cortex, which vary with layer and age. These variations need to be taken into account in any experiments designed to study the role of mGluRs in long-term potentiation or long-term depression in the visual cortex.

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