Injection of MK-801 Affects Ocular Dominance Shifts More Than Visual Activity


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INTRODUCTION

Permanent irreversible changes occur in the visual cortex when the visual input is restricted early in life (Wiesel 1982). The clearest example is the effect of monocular deprivation (MD). After MD the cortex becomes effectively disconnected from the deprived eye. N-Methyl-D-aspartate (NMDA) receptors are believed to play an important role in this process. The prime evidence for this conclusion is that infusion of the NMDA antagonist 2-amino-5-phosphonovaleric acid (APV) into the visual cortex reduces the ocular dominance shift (Bear et al. 1990; Kleinschmidt et al. 1987). A similar result was obtained with infusion of the NMDA channel blocker (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801), also directly into the visual cortex (Rauschecker et al. 1990).

Do NMDA antagonists affect ocular dominance shifts simply because of a reduction in visually driven activity in the visual cortex? Ocular dominance shifts are a sensory-driven phenomenon, and the sensory signals are carried to the visual cortex by electrical activity. A substantial reduction in activity might therefore prevent ocular dominance shifts, just as infusion of tetrodotoxin (TTX) into the eyes blocks ocular dominance segregation during normal development (Stryker and Harris 1986) and infusion of TTX into the visual cortex prevents ocular dominance shifts from MD (Reiter et al. 1987). Compared with TTX, APV produces a less dramatic decrease in activity. Iontophoresis of APV reduces activity in the visual cortex, and this effect is greater in young animals (Tsumoto et al. 1987). APV is effective in all layers before 3 wk of age, but the effect becomes restricted primarily to layers II and III after 6 wk of age (Fox et al. 1989). Infusion of 50 mM APV into the visual cortex at 1 μl/h (the procedure used by Kleinschmidt et al. 1987) leads to a substantial depression of activity in all layers, even in adult animals (Miller et al. 1989).

As a control for their experiments on ocular dominance changes, Bear et al. (1990) recorded from cells in the cortex of their animals 2 days after starting infusion of 50 mM APV. They found that the percentage of visually driven cells in the area from which they obtained ocular dominance histograms was normal, although the response was depressed. They did not quantify the extent of the depression. Moreover, the animals tested for the effect of APV on activity in the visual cortex were different from those tested for the effect of APV on ocular dominance shifts.

We therefore decided to measure the effect of an NMDA antagonist on ocular dominance shifts and also the effect of the same NMDA antagonist at the same dose on visual activity in the same animals. We injected animals intramuscularly with MK-801, which crosses the blood–brain barrier, kept one eye closed for several days, recorded an ocular dominance histogram, and then tested the effect of the same dose of MK-801 on the activity of a single cell in the visual cortex. Results in these animals were compared with control animals injected with vehicle solution, but otherwise treated identically. A brief summary of the first series of experiments was included in a review article (Daw 1994). As a control for whether the MK-801 had a specific effect in visual cortex, we then observed the effect of MK-801 on dose-response
TABLE 1.  Conditions for monocular deprivation

<table>
<thead>
<tr>
<th>Animal</th>
<th>Eyelid Suture (Days of Age)</th>
<th>Treatment</th>
<th>Deprivation (Days of Age)</th>
<th>Recording (Days of Age)</th>
<th>Weighted Ocular Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>198A</td>
<td>34</td>
<td>MK-801</td>
<td>35–39</td>
<td>42</td>
<td>0.58</td>
</tr>
<tr>
<td>198B</td>
<td>35</td>
<td>MK-801</td>
<td>35–39</td>
<td>43</td>
<td>0.51</td>
</tr>
<tr>
<td>5117</td>
<td>35</td>
<td>MK-801</td>
<td>36–40</td>
<td>41</td>
<td>0.70</td>
</tr>
<tr>
<td>204A</td>
<td>35</td>
<td>Saline</td>
<td>36–40</td>
<td>43</td>
<td>0.68</td>
</tr>
<tr>
<td>204B</td>
<td>35</td>
<td>Saline</td>
<td>36–40</td>
<td>44</td>
<td>0.95</td>
</tr>
<tr>
<td>4383</td>
<td>27</td>
<td>Saline</td>
<td>30–37</td>
<td>38</td>
<td>0.75</td>
</tr>
<tr>
<td>4382</td>
<td>27</td>
<td>MK-801</td>
<td>30–37</td>
<td>40</td>
<td>0.70</td>
</tr>
<tr>
<td>4385</td>
<td>36</td>
<td>MK-801</td>
<td>36–43</td>
<td>44</td>
<td>0.70</td>
</tr>
<tr>
<td>4387</td>
<td>37</td>
<td>Saline</td>
<td>38–45</td>
<td>46</td>
<td>0.89</td>
</tr>
<tr>
<td>4386</td>
<td>38</td>
<td>MK-801</td>
<td>39–46</td>
<td>47</td>
<td>0.47</td>
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<td>4374</td>
<td>29</td>
<td>MK-801</td>
<td>30–37</td>
<td>38</td>
<td>0.70</td>
</tr>
<tr>
<td>4372</td>
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<td>Saline</td>
<td>32–39</td>
<td>40</td>
<td>0.97</td>
</tr>
<tr>
<td>4376</td>
<td>30</td>
<td>Saline</td>
<td>30–37</td>
<td>38</td>
<td>0.83</td>
</tr>
</tbody>
</table>

curves plotted from iontophoresis of NMDA or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA).

Methods

General procedure

Treatment was started at 4–5½ wk of age (Table 1). The eyelids of the right eye were sutured closed. The animal was then exposed to light for 8 h/day for 5 days in the first series of experiments and for 8 days in the second. It was kept in the dark with its mother at all other times until the day of recording.

Seven animals were treated with MK-801, and six were treated with an equal volume of saline. The first injection in the morning was given by using infrared light and a viewer, or, occasionally, brief room light to find the animal. The lights were turned on 10–15 min after the injection, when the MK-801 started to have an effect. The dose was ~0.1 mg/kg im. It was adjusted so that the animal was slightly ataxic but awake and playful as much as the control littersmates. On three occasions the dose was slightly too large, and the animal was sedated for ~30 min, but the next dose was then adjusted to make the animal ataxic but not sleepy. In general, small increases in the dose were necessary over the days of treatment to get a constant effect. A second injection was given 4 h after the lights were turned on. Four hours after the second injection, the lights were turned off.

MD

Eyelids were sutured together under anesthesia with ketamine (20–30 mg/kg im) and xylazine (0.5 mg/kg im). One drop of proparacaine hydrochloride (Alcaine) was dropped onto the cornea. The lid margins were cut with a drop of 10% phenylephrine hydrochloride (Neo-synephrine), and the pupils were dilated with a drop or two of 0.01% atropine. A tungsten electrode (Hubel 1957) or a carbon fiber microelectrode (Armstrong-James and Millar 1979) was inserted into the cortex for recordings from single cells. The left cortex, ipsilateral to the open eye and contralateral to the sutured eye, was chosen because there is a slight dominance in the cortex by the contralateral eye. Therefore, ocular dominance changes are more noticeable when the cortex ipsilateral to the open eye is recorded (Daw et al. 1992).

Receptive fields were characterized with a light projector moved by hand. The preferred width and length of stimulus, preferred orientation, and velocity and direction of movement were all determined with moving stimuli. If the cell responded to all directions of movement, as judged by listening to the audio monitor, it was called omnidirectional (see Daw and Ariel 1980). If the cell responded to movement along one axis, but substantially less along the perpendicular axis, it was called bidirectional. If the cell responded to movement in one direction, but substantially less to the opposite direction, it was called unidirectional. The width of tuning for a moving bar was judged by moving the stimulus through the receptive field and repeating this with angles further and further away from the preferred direction of movement until the response was no longer audible. Finally, the responses in the two eyes were compared by using the preferred stimulus. Two observers independently assigned an ocular dominance according to the seven-point scale introduced by Hubel and Wiesel (1962).

Animal were sedated with acepromazine, 0.1 mg/kg im (Fermenta Animal Health, Kansas City, MO), and given a preanesthetic dose of atropine, 0.04 mg/kg im. Anesthesia was induced with 4% halothane in a mixture of 67% nitrous oxide-33% oxygen and maintained with 0.5–0.9% halothane. After tracheotomy and insertion of an intravenous cannula into the femoral vein, the skull was opened over the lateral gyrus, and a small hole was made in the dura for insertion of the electrode. All wound margins were treated with local anesthetic (Lidocaine). After surgery, the animal was paralyzed by intravenous infusion of pancuronium bromide at 0.6–1.5 mg/l (Elkins-Sinn, Cherry Hill, NJ). Body temperature was maintained at 37.5°C with a heating pad controlled by a rectal thermometer. Heart rate and end tidal CO₂ were monitored continuously, and CO₂ was maintained at 3.5–4.2% by adjusting the respirator.

Recordings for determination of ocular dominance

The lid margins of the closed eye were separated, and both eyes were focused on a tangent screen at 57 in. by lenses of zero power and appropriate curvature. The nictitating membrane was withdrawn by a drop of 10% phenylephrine hydrochloride (Neosynephrine), and the pupils were dilated with a drop or two of 0.01% atropine. A tungsten electrode (Hubel 1957) or a carbon fiber microelectrode (Armstrong-James and Millar 1979) was inserted into the cortex for recordings from single cells. The left cortex, ipsilateral to the open eye and contralateral to the sutured eye, was chosen because there is a slight dominance in the cortex by the contralateral eye. Therefore, ocular dominance changes are more noticeable when the cortex ipsilateral to the open eye is recorded (Daw et al. 1992).

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About four penetrations were made in each cortex, spaced 0.5–1 mm apart between AP0 and P4. In six animals (3 experimental and 3 control in the second series), the electrode was inserted in a parasagittal plane, angled at 15–20° to the vertical, to sample as many layers and columns as possible. In the other seven animals, the electrode was inserted in a coronal plane, again angled at 15–20° to the vertical, to sample as many layers and columns as possible. Two or three lesions (3 μA DC for 10 s) were made in each penetration to determine the layers for the cells recorded.
Recordings for determination of the effect of MK-801 on activity

After determining the ocular dominance of a sample of cells, a computer-controlled bar of light was set up to stimulate the cell in the dominant eye with the preferred orientation, direction of movement, velocity, length, and width of the stimulus. The bar of light remained stationary on one side of the receptive field for 1 s and then swept across the receptive field, remained stationary for 1 s on the far side, swept back, remained stationary for 1 s more, and was then turned off. This procedure was repeated four times every minute; the average of these four responses constituted one group of records. Spikes were discriminated through a voltage window and monitored for amplitude and time course on a storage oscilloscope. The computer was also used to store spike discharge times and to construct a peri-stimulus time histogram (PSTH) on-line as the spikes came in. The first PSTH was displayed in red, and each subsequent PSTH was displayed in green, updated each minute, so that the effect of the injection of MK-801 could be monitored as it occurred. Spike discharge times together with details of the stimulation parameters were stored on hard disk for subsequent off-line analysis with custom-written ASYST programs (Asyst Software, Rochester, NY).

Histology

On completion of the physiological recordings, the animal was deeply anesthetized with 4% halothane and then perfused through the heart with 100 ml of Lactated Ringer, followed by 350–450 ml of 4% paraformaldehyde. The lateral gyrus was removed and allowed to sink in a 30% sucrose solution containing 4% paraformaldehyde. Frozen sections were cut at 60 µm and then stained with thionin (Nissl stain). Lesions were 50–100 µm in diameter. The electrode tracks were reconstructed, and the layer in which each cell was recorded was determined according to the layering criteria described by Kelly and Van Essen (1974).

Analysis of data

For analysis of the effect of MK-801 on the activity of a cell, firing rates, expressed as spikes/s, were averaged over the entire response duration for one direction of stimulus presentation (typically 1–2 s). Visual responses were expressed as average firing rate while the stimulus was in the receptive field minus spontaneous activity. If the cell was unidirectional, the firing rate was measured while the stimulus was moving in the preferred direction. If the cell was bidirectional, the firing rate was considered to be the average of the firing rates in both directions.

Weighted ocular dominance (WOD) was calculated from the ocular dominance histograms according to the formula

\[
\frac{1}{6N_i} + \frac{2}{6N_i} + \frac{3}{6N_i} + \frac{4}{6N_i} + \frac{5}{6N_i} + \frac{6}{N_i}
\]

where \(N_i\) is the number of cells in ocular dominance group \(i\) (Kasamatsu et al. 1981).

Effect of MK-801 on dose response curves for NMDA or AMPA

Fourteen animals were recorded, aged 37–43 days of age. A cell was isolated, and the preferred parameters for stimulation were established. Visual response was measured, and then the responses to iontophoresis of NMDA or AMPA were measured. For iontophoresis, the cycle was NMDA on for 15 s, off for 57 s, AMPA on for 15 s, off for 57 s, and so on, varying the iontophoretic current to accumulate a series of firing rates between 0 and 50 Hz. In an ideal experiment, we recorded visual and iontophoretic responses for two or three cells, then injected MK-801, and then held the cell recording two or three visual responses and an iontophoretic response once an hour. Four hours after the first injection of MK-801, a second injection of MK-801 was given, and we continued to record for another 4–6 h. Because of the difficulty of holding a cell for 10 h, we did not always obtain this ideal result, in which case a new cell would be isolated and recorded with the same procedure. Visual responses, iontophoretic responses, and spontaneous activity were calculated off-line by our ASYST program and a straight line fitted to the linear portion of the dose-response curve by Slide Write (Advanced Graphics Software, Carlsbad, CA).

RESULTS

Effect of MK-801 on ocular dominance shifts

In the first series of experiments, we compared ocular dominance histograms from three animals treated with MK-801 and two animals treated with saline. All animals were deprived for a total of 40 h, 8 h/day over 5 days and then kept in the dark until recorded. Cells in the control animals were driven by the open eye more often than cells in the animals treated with MK-801 (Fig. 1). Weighted ocular dominances for the three animals treated with MK-801 were 0.58, 0.51, and 0.70 compared with weighted ocular dominances of 0.68 and 0.71 for the two control animals. In other words, treatment with MK-801 reduced the ocular dominance shift, but not greatly.

However, the ocular dominance shift was not very large in the control animals, so it was not possible to reduce it substantially. This may have been due to the shortness of the period of deprivation (40 h) or it may have been due to the 2–3 days that the animals were kept in the dark between deprivation and recording. Consequently, we decided to do a second series of animals in which the length of deprivation was increased to 64 h (8 h/day over 8 days) and the time between deprivation and recording was kept as short as possible.

Ocular dominance histograms from four animals treated with MK-801 were compared with ocular dominance histograms from four animals treated with saline. The histograms from the control animals were substantially more shifted than in the first series of experiments (Fig. 2A). Treatment with MK-801 caused a large decrease in this shift (Fig. 2B). Weighted ocular dominances in the control animals were 0.95, 0.89, 0.97, and 0.83, whereas weighted ocular dominances in the treated animals were 0.75, 0.70, 0.47, and 0.70. Considering each animal as one observation, these numbers are significantly different (two-tailed t-test, \(P < 0.05\)). Three animals (4385, 4387, and 4386) were a few days older than the others, but all animals were deprived during the most susceptible part of the critical period, which is 4–6 wk of age (Hubel and Wiesel 1970; Olson and Freeman 1980), and there was no difference between the older animals and younger ones that received the same treatment.

In the first series of experiments we noticed that the most substantial reduction in ocular dominance occurred in upper layers. Consequently, we arranged penetrations in the second series of experiments to record mostly from upper layers. The histological reconstructions of the penetrations showed...
that >85% of the cells came from layers II, III, and IV. The difference between ocular dominance histograms recorded from MK-801 treated animals and ocular dominance histograms recorded from control animals was seen in all layers (Fig. 3). Weighted ocular dominances were 0.90 in layers II/III, 0.92 in layer IV, and 0.91 in layers V/VI for control animals, and 0.66 in layers II/III, 0.67 in layer IV, and 0.67 in layers V/VI for MK-801-treated animals. However, the sample from layers V and VI was not large enough to make it a meaningful result in those layers.

Receptive fields of cells in the animals treated with MK-801 were slightly less specific than those in the control animals. The percentage of omnidirectional cells was 9% in MK-801 animals compared with 4% in controls, the percentage of bidirectional cells was 43% in MK-801 animals compared with 36% in controls, and the percentage of unidirectional cells was 48% in MK-801 animals compared with 60% in controls. A similar result was obtained in the first series (see Daw 1994). However, the angle over which the cell responded was not significantly different: 86 ± 24° for MK-801 animals compared with 88 ± 29° for controls. In the 13 cells where we had quantitative records, the firing rate in animals treated with MK-801 (9.3 ± 3.2 spikes/s, means ± SD) was not significantly different from the firing rate in control animals (6.3 ± 3.2 spikes/s); if anything, the firing rate in the treated animals was higher.

**Effect of MK-801 on activity of single cells in the cortex**

Thirteen cells were analyzed for the effect of MK-801 on their activity. The dose of MK-801 given was the average
of the doses given during the rearing paradigm for animals treated with MK-801 during rearing. The dose given to control animals was the same as that given to treated littermates during rearing. Two cells were recorded in the first series of experiments, and 11 cells were recorded in the second. Eight were recorded from MK-801–treated animals, and five were recorded from control animals. Details of the age of the animal, MK-801 dose, and the layer of the cell recorded are given in Table 2.

In seven cells, there was little or no change in the visual response after the injection of MK-801. Some of these seven cells had a fairly constant, or even increased, response (Fig. 4A). In other cases, the response of the cell varied considerably over time, and any change was not statistically significant (Fig. 4B).

In three cells, there was a decrease in the response, correlated with the injection of MK-801. In one case the response decreased to \(~25\%\) of control after 30 min and recovered to 60% after 60 min (198A unit A), in one case the response decreased to 60% after 20 min and recovered to 80% after 45 min (198B unit A), and in one case the response decreased to \(~50\%\) and recovered after 10 min (4373 unit B). The largest of these decreases is illustrated in Fig. 4C.

In two cells, there was a long-term decrease in the response, which did not recover over the period of recording. It is difficult to know how to interpret this result because the response of a cell quite frequently decays over time, particularly when the cell is recorded for a period of \(\approx 1\) h. Consequently the decrease in these two cases could have been due to small shifts in the distance of the electrode from the cell, as a result of which amplitude of the action potential fell below the threshold of the window used to discriminate the action potential from noise.
NMDA RECEPTORS AFFECT PLASTICITY MORE THAN ACTIVITY

FIG. 3. Ocular dominance histograms from the 2nd series of animals analyzed by layer. More than 50% of the cells were in group 7 in all layers in the control animals, and <20% of the cells were in group 7 in all layers in MK-801–treated animals.

TABLE 2. Effect of MK-801 on activity of cells in animals tested for ocular dominance shifts

<table>
<thead>
<tr>
<th>Cell</th>
<th>MK-801 Dose, mg/kg</th>
<th>Layer</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>198A unit A</td>
<td>42</td>
<td>II/III</td>
<td>Decrease over 30 min</td>
</tr>
<tr>
<td>198B unit A</td>
<td>43</td>
<td>II/III</td>
<td>Decrease over 20 min</td>
</tr>
<tr>
<td>4383 unit A</td>
<td>38</td>
<td>III/IV</td>
<td>Decrease, but unstable</td>
</tr>
<tr>
<td>4382 unit A</td>
<td>40</td>
<td>IV</td>
<td>Little change</td>
</tr>
<tr>
<td>4385 unit C</td>
<td>44</td>
<td>Unknown</td>
<td>Little change</td>
</tr>
<tr>
<td>4387 unit A</td>
<td>46</td>
<td>IV</td>
<td>Long-term decrease</td>
</tr>
<tr>
<td>4386 unit A</td>
<td>47</td>
<td>Unknown</td>
<td>Little change</td>
</tr>
<tr>
<td>4374 unit A</td>
<td>38</td>
<td>IV</td>
<td>Little change</td>
</tr>
<tr>
<td>4372 unit A</td>
<td>40</td>
<td>IV</td>
<td>Long-term decrease</td>
</tr>
<tr>
<td>4373 unit A</td>
<td>41</td>
<td>IV</td>
<td>Little change</td>
</tr>
<tr>
<td>4373 unit B</td>
<td>41</td>
<td>II</td>
<td>Decrease over 10 min</td>
</tr>
<tr>
<td>4376 unit A</td>
<td>38</td>
<td>II/III</td>
<td>Little change</td>
</tr>
<tr>
<td>4377 unit A</td>
<td>40</td>
<td>III</td>
<td>Little change</td>
</tr>
</tbody>
</table>

Effect of MK-801 on responses of cells to NMDA and AMPA

We obtained results in 10 animals for the effect of MK-801 on the cortical responses to NMDA and AMPA as well as on the visual response. An example is shown in Fig. 5. The cell responded to movement in both directions for a bar of light moved at 4°/s. The visual response 4.5 h after the first injection of MK-801 and 30 min after the second was close to the response before injection of MK-801 (Fig. 5, top). AMPA and NMDA, both iontophoresed at 20 nA, gave substantial responses before injection of MK-801 (Fig. 5, left); 4.5 h later, after two injections of MK-801, the currents required were higher, but AMPA iontophoresed at 33 nA gave a substantial response, whereas NMDA iontophoresed at 35 nA gave very little response (Fig. 5, right).

Dose-response curves were measured for most cells. Results from a layer II/III cell recorded from a 42-day-old
60–70 nA; 3.5 h after the injection of MK-801, the AMPA response was again similar, and the NMDA response showed substantial recovery.

Dose-response curves were measured for 40 cells in 10 animals. In general, the slope of the line fitted to the NMDA dose-response curve was equal to the slope of the line fitted to the AMPA curve before MK-801 was injected (ratio \(0.98 \pm 0.64, n = 12\)). Cells recorded after the first injection of MK-801 had an NMDA dose-response curve that was much flatter in relation to the AMPA curve (ratio \(0.39 \pm 0.37, n = 11\)). For cells recorded after the second injection of MK-801, the NMDA curve was reduced further (ratio \(0.28 \pm 0.27, n = 15\)). Recovery was seen in three cases, and not in three others. However, it was rarely possible to hold a cell for the 10 h required for two injections of MK-801 and recovery after the second injection.

One of the few cases where we were able to hold a cell for a long period is illustrated in Fig. 7. In this animal, four cells were recorded, and the fourth was held for 11 h. Immediately before the first injection of MK-801, the NMDA and AMPA curves had similar slopes. For 3 h after the first injection of MK-801, the slope of the NMDA curve dropped to \(1/4\) the slope of the AMPA curve (Fig. 7A). Then the NMDA response recovered, just before the second injection of MK-801. After the second injection of MK-801, the slope of the NMDA curve dropped to \(<1/10\) the slope of the AMPA curve. Finally, 8 h after the first injection of MK-801, and 4 h after the second injection, the response to NMDA again recovered, in part. The visual response during this time was relatively constant (Fig. 7B).

Visual responses were compared before and after the first injection of MK-801 for eight cells (Table 3). In general there was little change, some increases and some decreases, but no overall change. This was true for doses of MK-801 in the range of those used in animals tested for ocular dominance changes (0.12 ± 0.15 mg/kg) and for rather higher doses (0.2 mg/kg). Visual responses were also compared after the second injection of MK-801 for seven cells (Table 3). There was little change at doses used in the ocular dominance experiments but a substantial decrease in two of three cases where a rather higher dose was used.

**DISCUSSION**

MK-801 produced a significant decrease in the ocular dominance shift that normally occurs after MD. This occurred with doses that did not have a substantial effect on spontaneous activity or visually evoked activity of most cortical neurons. The decrease in the ocular dominance shift occurred in all layers and was most obvious in layers II, III, and IV.

The injection of MK-801 reduced the ocular dominance shift but did not abolish it altogether. Weighted ocular dominance in the MK-801–treated animals was \(0.597 \pm 0.096\) in the first series and \(0.655 \pm 0.016\) in the second series. This compares with 0.43 in normal animals (Daw et al. 1992). Bear et al. (1990) and Rauschecker et al. (1990) also found that the ocular dominance shift was not completely abolished by their injections of APV and MK-801 directly into the cortex. Of the many treatments that affect ocular dominance shifts (see Daw 1994 for a list), only complete
NMDA receptors affect plasticity more than activity

**FIG. 5.** Visual response, response to iontophoresis of $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and response to iontophoresis of N-methyl-D-aspartate (NMDA), measured in a layer II/III cell before injection of MK-801 and 30 min after the 2nd injection of MK-801.

Abolition of activity in the cortex with TTX was proved to lead to a complete abolition of the ocular dominance shift (Reiter et al. 1986).

We should emphasize that one could almost certainly use a higher dose of NMDA antagonist and affect both visual responses and ocular dominance shifts. The effect of NMDA antagonists on visual responses decreases with age in layers IV, V, and VI, but there is a distinct effect on visual responses in layers II and III at all ages (Fox et al. 1989; Miller et al. 1989; Tsumoto et al. 1987). We agree with all of these authors that NMDA receptor blockers can decrease visual responses, but we found that the dose is critical. We found that higher doses of MK-801 (0.2 mg/kg) affected activity of visual cortex cells, but the doses used in our ocular dominance experiments (0.1–0.15 mg/kg) did not. Thus our conclusion is limited; there is a dose of MK-801 that affects ocular dominance shifts without affecting visual responses, but we would not expect this to occur with all doses.

Bear et al. (1990) found a substantial loss in selectivity for orientation and direction, recording cells in animals with APV infused into the visual cortex. We found a much smaller loss of selectivity. This may have been due to the age of the animals. Treatment in the animals used by Bear et al. started at 3–5 wk of age, compared with 30–39 days for our animals. The critical period for direction selectivity ends earlier than the critical period for ocular dominance changes (Berman and Daw 1977; Daw and Wyatt 1976), and so probably
FIG. 6. Dose-response curves for iontophoretic injection of NMDA and AMPA on a visual cortex cell before and after injection of MK-801. △ and ——: data for response to AMPA with line fitted; + and ——: data for responses to NMDA and line fitted to them.

does the critical period for orientation changes (Chapman and Stryker 1993; Kim and Bonhoeffer 1993). Consequently interventions early in the critical period should affect orientation, direction, and ocular dominance, whereas interventions late in the critical period should affect primarily ocular dominance. Alternatively, the difference could have been due to the difference in drugs (APV vs. MK-801) and routes of administration.

Bear et al. (1990) also found a reduction in response quality in their animals infused with APV. Cells with a brisk and reliable response were given a high response quality, whereas cells with a sluggish response were given a low response quality. We did not assess response quality in the neurons recorded to construct the ocular dominance histograms because we were primarily concerned with getting as large a sample as possible. Our impression, however, was that cells from both MK-801–treated animals and control animals responded equally vigorously. In addition, we did have a quantitative measure of the visual response in those neurons where we assessed the effect of injection of MK-801 on the visual response; in those neurons there was little difference between control and treated animals. Thus we did not see a decline in response quality after treatment with MK-801. This also could be due to a difference in the age of the animals.

We found that the ocular dominance shift was reduced in layer IV as well as in layers II and III. This contrasts with the NMDA contribution to the visual response at the same age, which is small in layer IV but substantial in layers II and III (the NMDA contribution to the visual response is defined as reduction of the visual response by iontophoresis of APV near the cell body) (Fox et al. 1989).

Our procedure of injecting MK-801 im could affect activity in the retina and lateral geniculate nucleus as well as activity in the visual cortex. If we had found that the activity measured from single cells in the visual cortex was significantly reduced, we would not have known whether this was due to the effect of MK-801 in retina, lateral geniculate, or cortex. However, we found that there was little effect of MK-801 on activity in cortex, so possible effects on retina and lateral geniculate are not of much concern.

Could we have obtained the reduction in ocular dominance shift because MK-801 had an overall anesthetic effect? An overall anesthetic effect could be manifested in various ways. The animals might change their behavior so that they exhibit reduced activity or more sleep. More sleep would, of course, result in more time spent with the eyes closed. We monitored the dose so that no change in behavior occurred, other than a mild ataxia, and lowered the dose immediately after the three occasions on which we noticed some sleepiness. In addition, the signals reaching the visual cortex from nonvisual sources, such as the locus coeruleus, raphe nuclei, basal forebrain, or dopaminergic nuclei, might change. Any change in signals reaching the visual cortex from nonvisual sources would probably have shown up as a change in spontaneous or visual activity after the injections of MK-801, if it were significant.

One of the questions posed by these results is how is it possible for MK-801 to affect ocular dominance plasticity without affecting visual responses? If visual activity is instructive for ocular dominance plasticity and yet is unaffected by MK-801, why does ocular dominance plasticity not proceed normally? One possibility is that MK-801 acts to block a permissive process, for example, by acting on a nonvisual input to the cortex. The intralaminar input, for example, is an ascending nonsensory input that activates cortical NMDA receptors (Fox and Armstrong-James 1986) and was implicated in ocular dominance plasticity (Singer and Rauschecker 1982). Another possibility is that MK-801 does affect NMDA receptors engaged by visual inputs but that the receptors are only blocked by MK-801 when the visual inputs cause particularly sustained or strong levels of depolarization. It is known that MK-801 blocks NMDA receptors in an activity-dependent manner (McDonald et al.
NMDA RECEPTORS AFFECT PLASTICITY MORE THAN ACTIVITY

A

Relative slope of curves

![Graph of relative slope of curves with data points for Cell A and Cell D before and after MK-801 injections.](image)

**FIG. 7.** A: slope of NMDA dose-response curve relative to slope of AMPA dose-response curve before and after 2 injections of MK-801 spaced 4 h apart. B: visual responses and spontaneous activity for cells recorded from the same animal over the same period of time.

B

Activity of cells

![Graph of spikes per second for different cells with data points for SA and VR cells before and after MK-801 injections.](image)

1981), so it is conceivable that, at a particular dose of MK-801, heightened levels of visual activity are blocked but normal visual processing is not.

How else could long-term MK-801 treatment cause a change in plasticity without causing much change in the visual response of cortical cells? Activation of the NMDA receptor has two effects, depolarization of the neuron and entry of calcium into the cell (Dingledine 1983). The visual response is affected primarily by depolarization, whereas plasticity could be affected by both depolarization and/or calcium entry. Indeed, some forms of long-term potentiation (LTP) depend on calcium entry but not activation of NMDA receptors, suggesting that calcium entry is the more crucial property (Komatsu 1991; see also Nicoll and Malenka 1995). Moreover, calcium can activate various second effector enzymes such as calcium/calmodulin-dependent protein kinase II and protein kinase C, which play a role in LTP (Malenka et al. 1989; Malinow et al. 1989) and may play a role in plasticity in the visual cortex. We suggest that our MK-801 treatment blocked calcium entry enough to affect plasticity, and the depolarization block was not enough to affect the response of the cells significantly.

Could MK-801 have a minor effect on depolarization while it has a significant effect on plasticity through calcium
entry? This is certainly possible. The relevant factor may be calcium concentrations in individual spines rather than the overall activity in the cell as a whole (Zador et al. 1990). It is difficult to detect a change in activity of <5-10%. A relatively minor change in activity if this magnitude could nevertheless be accompanied by a large change in the amount of calcium entering the cell at the dendritic spine caused by the nonlinearities in the relationship between depolarization and intraspinal calcium concentration.

In summary, we have shown that an intramuscular dose of MK-801 that produces mild ataxia without sleepiness reduces ocular dominance plasticity substantially and has a substantial effect on the responses of cells in the visual cortex to NMDA, whereas the visual responses of the cells are not affected very much. Because the effect on the visual response was minor, we suggest that the effect on plasticity occurred primarily through calcium entering the cell at sub-threshold levels of depolarization.

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Table 3. Effect of MK-801 on activity of cells in animals used for iontophoresis

<table>
<thead>
<tr>
<th>Cell</th>
<th>Dose, mg/kg</th>
<th>Layer Before MK-801</th>
<th>Response Before MK-801</th>
<th>Response After MK-801</th>
<th>Change, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>819 Mar D</td>
<td>0.12</td>
<td>II/III</td>
<td>12.3 ± 1.9 (12)</td>
<td>14.7 ± 3.2 (20)</td>
<td>+20</td>
</tr>
<tr>
<td>806 Apr D</td>
<td>0.12</td>
<td>II/III</td>
<td>13.2 ± 1.7 (12)</td>
<td>13.3 ± 3.3 (18)</td>
<td>+1</td>
</tr>
<tr>
<td>702 Dec B</td>
<td>0.15</td>
<td>II/III</td>
<td>7.3 ± 2.0 (7)</td>
<td>6.7 ± 1.2 (5)</td>
<td>-8</td>
</tr>
<tr>
<td>827 Apr D</td>
<td>0.15</td>
<td>III/IV</td>
<td>31.7 ± 4.1 (18)</td>
<td>28.1 ± 9.2 (18)</td>
<td>-11</td>
</tr>
<tr>
<td>619 Jul B</td>
<td>0.2</td>
<td>IV</td>
<td>11.3 ± 5.4 (13)</td>
<td>7.1 ± 1.3 (16)</td>
<td>-37</td>
</tr>
<tr>
<td>622 Jul F</td>
<td>0.2</td>
<td>II/III</td>
<td>9.8 ± 5.7 (16)</td>
<td>9.8 ± 2.3 (13)</td>
<td>0</td>
</tr>
<tr>
<td>624 Jul B</td>
<td>0.2</td>
<td>II/III?</td>
<td>12.6 ± 1.4 (6)</td>
<td>9.5 ± 2.0 (16)</td>
<td>-25</td>
</tr>
<tr>
<td>625 Jul B</td>
<td>0.2</td>
<td>II/III?</td>
<td>14.6 ± 1.6 (12)</td>
<td>19.7 ± 2.8 (20)</td>
<td>+35</td>
</tr>
</tbody>
</table>

Effect of second dose

| 819 Mar E| 0.12        | V                   | 20.6 ± 3.4 (19)        | 22.7 ± 3.7 (8)        | +10       |
| 806 Apr D| 0.12        | II/III              | 18.7 ± 2.5 (12)        | 17.6 ± 6.3 (16)       | -6        |
| 702 Dec C| 0.15        | II/III              | 12.8 ± 3.2 (19)        | 20.1 ± 6.3 (8)        | +57       |
| 827 Apr E| 0.15        | IV                  | 10.6 ± 1.8 (18)        | 10.9 ± 2.6 (18)       | +3        |
| 622 Jul F| 0.2         | II/III              | 9.8 ± 2.3 (13)         | 0 (12)                | -100      |
| 624 Jul C| 0.2         | II/III?             | 7.0 ± 0.9 (6)          | 1.8 ± 0.5 (16)        | -74       |
| 625 Jul D| 0.2         | II/III?             | 24.0 ± 3.8 (12)        | 22.7 ± 2.4 (15)       | -5        |

Effect of first dose

Values are means ± SD, number of subjects within parentheses.


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