Inhibition of nitric oxide synthase does not prevent ocular dominance plasticity in kitten visual cortex

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1. The neural messenger molecule nitric oxide (NO) has been shown to be involved in several forms of plasticity including hippocampal long-term potentiation. We examined the effects of chronic intracortical infusion of inhibitors of NO synthase (NOS) activity on the plasticity of visual cortical responses following monocular lid suture during the critical period.

2. Single unit recordings (618 cells) made in both the NOS inhibitor-treated (30 mM Nω-methyl-l-arginine (L-NMMA), or 22 or 2 mM nitro-l-arginine (L-NOArg) and saline-treated control hemispheres of barbiturate-anesthetized, critical-period kittens (n = 8) revealed a profound shift in favour of the non-deprived eye. Shifts were of similar magnitude in hemispheres in which NOS was inhibited and in saline control hemispheres.

3. Subsequent analysis of NOS activity in the same cortical tissue in which recordings had been made showed a pronounced decrease in NOS activity in inhibitor-treated hemispheres. In the region in which all the single unit recordings were made (< 3 mm from the infusion cannula), 22 mM L-NOArg resulted in a reduction of NOS activity to 5.55 ± 0.33% of control hemisphere NOS activity levels. L-NMMA (30 mM) also produced clear, but smaller, inhibition of NOS activity.

4. These findings demonstrate that NOS activity is not essential for ocular dominance plasticity in visual cortex.

During a period in the early postnatal life of the cat, the visual cortex exhibits great plasticity in response to manipulations of visual experience. Brief occlusion of vision in one eye during this period causes the initially binocular visual cortex to respond almost exclusively to stimulation of the unoccluded eye (Olson & Freeman, 1975; Slatz & Stryker, 1978; Wiesel, 1982; Hensch, Craig, Ruthazer, Fagiolini, Gillespie & Stryker, 1993). Anatomically, cortical territory receiving input from the deprived eye shrinks and individual deprived geniculo-cortical axon arbors show rapid decreases in arbor complexity (Slatz & Stryker, 1978; Wiesel, 1982; Antonini & Stryker, 1993a). Simple atrophy of inactive inputs cannot account for ocular dominance plasticity because even much longer periods of binocular deprivation or binocular retinal activity blockade do not cause an appreciable decrease in cortical responsiveness or thalamo-cortical arbor coverage (Wiesel, 1982; Stryker & Harris, 1986; Antonini & Stryker, 1993b). Activity of the postsynaptic cortical cell is both permissive and instructive for ocular dominance plasticity. Blockade of cortical activity reversibly prevents ocular dominance plasticity (Reiter, Wattman & Stryker, 1986). Chronic application of the Nω-methyl-d-aspartate (NMDA) receptor antagonist amino-phosphonovaleic acid (APV) greatly attenuates the ocular dominance shift in response to monocular deprivation (Bear, Kleinschmidt, Gu & Singer, 1990), although the chronic application of APV in vivo has additional effects on responsiveness that complicate interpretation (Miller, Chapman & Stryker, 1989). In addition, selectively inhibiting postsynaptic cortical cells

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by chronically infusing the GABA<sub>A</sub> receptor antagonist muscimol into the cortex effectively reverses the direction of both the electrophysiological and anatomical expressions of ocular dominance plasticity (Hata & Stryker, 1994).

A modified Hebbian model based on the competitive strengthening or weakening of inputs that are, respectively, correlated or anti-correlated in their firing pattern with the postynaptic neuron is consistent with all these observations (Miller, Kelleher & Stryker, 1998). With this model, the rapid changes in the structure of preynaptic axon arbors would occur only when the postynaptic target cell detected changes in their degree of mutual correlation. This requires the existence of a retrograde signal by which the postynaptic cell informs preynaptic axons whether to grow or shrink.

The freely diffusible gaseous neurotransmitter nitric oxide (NO) has been proposed as an intercellular messenger for some cellular models of associative synaptic plasticity such as hippocampal long-term potentiation (LTP) and cerebellar long-term depression (LTD) (reviewed in Schuman & Madison, 1994; Garthwaite & Boulton, 1995). NO synthase inhibitors or hemoglobin, which absorbs ambient NO, can greatly decrease the probability of generating certain forms of LTD in the CA1 field of the hippocampus (OTelli, Hawkins, Kandel & Araujo, 1991; but see Cummings, Nicola & Malenka, 1994; reviewed in Schuman & Madison, 1994). The paired application of exogenous sources of NO and electrical stimulation can augment CA1 responses and has been shown to increase the frequency of quantal release of cultured hippocampal neurons (OTelli et al. 1991; Zhou, Small, Kandel & Hawkins, 1993).

The ready diffusibility, membrane permeability and short half-life of NO, as well as the regulation of the neuronal forms of NO synthase (NOS) by intracellular calcium (for reviews,isto, Palmer & Higgins, 1991; Dawson & Snyder, 1994) make NO a suitable candidate for the retrograde signal predicted by the Hebbian model. NOS is present in a subset of cells throughout the neocortex (Breedt, Glusman, Forshli, Dawson & Snyder, 1991) and has been demonstrated to underlie enhanced glutamate release in cortical synaptosomes following NMDA receptor stimulation (Montague, Garay, Wynn, Marchase & Pfeiffer, 1994, but see Kirkwood & Bear, 1994, their Fig. 5b). Moreover, the distribution of NOS in visual cortex can be altered by manipulation of visual inputs (Aoki, Feinstein, Labin & Go, 1995). The properties of NO and the results cited above prompted us to consider NO as a possible retrograde signal in visual cortical plasticity.

In the present study, we have taken advantage of the availability of potent, selective inhibitors of NOS together with sensitive assays for NOS activity to determine whether NO is the intercellular messenger responsible for ocular dominance plasticity.

METHODS

Microinjection implantation and monocular deprivation

Normal kittens ranging in age from 24 to 30 days (see Table 1) were anaesthetized using halothane (0·5–2%) and nitrous oxide. Under sterile surgical conditions, two stainless steel micropipettes (model 2001 or 2002, Alar Corp., Palo Alto, CA, USA) attached to intravascular 30 gauge cannulas were stereotaxically implanted to deliver drug and control solutions bilaterally into primary visual cortex 2 mm below the cortical surface and 2 mm lateral to the midline at Bregma–Clarke AP 0.0. As drug and saline control solutions were prepared under identical conditions and encoded prior to surgery, it was possible in six animals (cats 3–8) to perform the surgery and all following procedures without knowing which hemisphere received which drug in two animals (cats 1 and 2), in which only one cannula was implanted, the experiment was not performed blind. Drug infusion solutions were prepared by warming sterile saline to just below 37°C and adjusting pH as close to 7·4 as possible while storing in one of the following agents to achieve the following concentrations: 0·04 M Na<sub>2</sub>arginine (L-NMA), or 2 or 22 M L-α-amino-γ-oxide (NG-030) (both from Sigma).

After 2 days of infusion, allowing the affected area of cortex to attain a stable size, the right eye of each animal was enucleated under anesthetic conditions using the same anaesthetic protocol as above. Animals were then returned to their littersmates to experience monocular vision for a period of 4–5 days.

Electrophysiology

Animals were premedicated with atropine (0·1 mg kg<sup>−1</sup>, s.c.) to control secretions and were prepared for physiological recording by inserting an intravenous cannula (60 gauge polyethylene tubing) into the femoral vein under halothane anaesthesia (0·5–2%). Anesthesia was subsequently maintained by intravenous administration of pentobarbitone sodium, delivered to effect a stable state of muscle relaxation in a series of boluses of about 1 mg kg<sup>−1</sup> to a total surgical dose of 20–40 mg kg<sup>−1</sup> in the first 1–2 h. A tracheal cannula was inserted, following which animals breathed 2·1% O<sub>2</sub>–N<sub>2</sub> mixture. Animals were then placed in a stereotaxic head holder and on a feedback–controlled heating pad to maintain core temperature at 38°C. The anesthetized eyelid was opened and the pupils of both eyes were dilated with a single application of atropine sulphate ophthalmic solution (1%). The stimulating electrodes were retracted with a single application of phenylephrine hydrochloride ophthalmic solution (10%). A cortical opening exposing the visual cortex up to about 8 mm anterior to the cannula in both hemispheres was drilled. Just prior to dura retraction and for the remainder of the experiment, animals were paralyzed by intravenous infusion of gallamine triethiodide (10 mg kg<sup>−1</sup>) in 5% dextrose Ringer solution (1–2 ml kg<sup>−1</sup> h<sup>−1</sup>) and artificially ventilated with a 2·1% O<sub>2</sub>–N<sub>2</sub> mixture at a rate and volume appropriate to maintain end-tidal expired CO<sub>2</sub> at 4±0.2%. Contact lenses were selected to protect and focus both eyes on a tangent screen and the optic discs were refocused and plotted to ensure that both eyes could be stimulated on the screen.

An adequate level of anesthesia was maintained by giving additional halothane as determined by continuous monitoring of slow waves and spindles in posterior temporal supraserual EEG recordings (bandpass filtered to 0–30 Hz) on a 10 s continuously waveforming display. End-tidal CO<sub>2</sub> levels and EEG (heart rate within the range of 180–200 beats min<sup>−1</sup>) were also continuously monitored for stability as a fail-safe indicator that the animal was
at an appropriate anesthetic level. Adherence to the above criteria rendered the animals completely aseptic to a fine pinch of the toe and to corneal touch prior to administration of paralytic drugs. Single unit recordings were then made by advancing a tungsten microelectrode (1-3 MG impedance) down the medial bank of the lateral gyrus in 100 μm increments with a stepping motor microdrive. The receptive field centre, orientation preference, ocular dominance, responsiveness and habituation of all units were determined by stimulating with a hand-held slit lamp. At least thirty units were characterized in each hemisphere. These protocols, including all surgery and experimental procedures, were approved by the University of California at San Francisco Committee on Animal Research.

For comparative analysis of the ocular dominance distribution of each hemisphere, the contralateral bias index (CBI) of Bexner et al. (1989) was adopted:

\[
\text{CBI} = 100 \times \frac{(1-T)+(2/3)(O-6)+(1/3)(3-O)+O/2}{12}
\]

where embedded numbers equal the number of units in each ocular dominance group and \(T\) equals the total number of visually responsive units. This index has a value of 100 for a collection of neurons that responds exclusively to the contralateral eye, 0 for exclusively ipsilateral responses, and intermediate values for binocular responses. The 'shift index' used in this paper is defined as the CBI/100 for the hemisphere contralateral to the open eye and 1-100 = CBI for the hemisphere ipsilateral to the open eye. This permits a characterization of each hemisphere's ocular dominance distribution relative to the open eye. Although the two hemispheres are not perfectly equally driven by both eyes in normal animals, it is acceptable to compare the two hemispheres in this manner because (1) the distribution in normal kittens (CBI = 50) is very close to symmetrical (CBI = 50), and (2) our blind procedures results in the experimental hemisphere and the deprived eye assessing independently.

### Tissue collection for NO assay

Immediately after finishing recording, animals were given a lethal dose of pentobarbitone sodium as monitored by EEG and were perfused transcardially with about 600 mL of ice-cold oxygenated phosphate-buffered saline. The cruciometry for recording was then expanded and the cannulae were removed. A 10 mm long segment of the lateral gyri of both hemispheres was excised by making a lateral incision at the lateral sulcus, a central incision at the splenial sulcus, a posterior incision at the callosal insertion point and an anterior incision 10 mm anterior to the callosal insertion point incision. The 10 mm segments from both hemispheres were then placed in individual slicing moulds with a series of 1 mm square sawed sections of the cortex quickly embedded in 4% agarose in saline. Slices 1 mm thick were then taken in a cranial-to-caudal sequence, alternating between hemispheres to assure identical treatment of experimental and control hemispheres, and frozen on dry ice for storage at -80°C.

**ASSAY FOR NO ACTIVITY**

NO activity is a homogenate from each 1 mm slice was quantified as described in Bredt & Snyder (1989) by measuring the conversion of \(^{14}\)C-L-arginine to \(^{14}\)C-L-NOcitrulline (New England Nuclear, Boston, MA, USA). In brief, following a 1 h incubation at 37°C, the homogenate was added to a 100 μL mixture containing 25 μM \([^{14}C]\)-arginine, incubated at 37°C for 15 min, and 3 μL of l-arginine and 1 μM l-NOcitrulline. After incubation, the mixture was added to the reaction. The \(^{14}\)C-L-NOcitrulline was quantitated by liquid scintillation counting of the reaction solution elute from 0.5 mL columns of Dowex AG50W-X8 (Sigma). Assays were carried under blind to prior treatment of the tissue. Each measurement was normalized to the total protein per sample. Although this assay does not measure absolute NO activity, it does provide a quantitative measurement of relative enzyme activity in different tissue samples.

### RESULTS

Infusion of inhibitors of NO synthesis fails to prevent ocular dominance plasticity

Single unit electrophysiological recordings from 618 neurons in eight kittens (referred to as cat 1 to cat 8) approximately 1 month old were made following about 1 week of continuous infusion at 0.5 or 1 μL h⁻¹ of one of the following: 30 μM L-NAME, 2 μM l-NOArg or 22 μM L-NOArg (see Table 1). The visual cortex in the other hemisphere of all kittens, except for cat 1 and cat 2, was infused in an identical manner except that sterile saline control solution was substituted for the drug. The labels on the pairs of vials of NO inhibitor and control solutions were coded before surgery so that the investigators did not know which was the experimental and which was the control hemisphere until after the experiment was completed. Animals were monocularly deprived by suturing the right eyelid shut 2 days after the infusion cannulae were

### Table 1. Experimental conditions and corresponding ocular dominance shifts

<table>
<thead>
<tr>
<th>Animal</th>
<th>Infusion drug</th>
<th>Infusion onset</th>
<th>MD Physiology</th>
<th>Shift index (no. of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>30 μM L-NAME (0.5 μL h⁻¹)</td>
<td>28</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td>Cat 2</td>
<td>30 μM L-NAME (0.5 μL h⁻¹)</td>
<td>28</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>Cat 3</td>
<td>22 μM L-NOArg (1 μL h⁻¹)</td>
<td>33</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>Cat 4</td>
<td>22 μM L-NOArg (1 μL h⁻¹)</td>
<td>35</td>
<td>37</td>
<td>42</td>
</tr>
<tr>
<td>Cat 5</td>
<td>2 μM L-NOArg (1 μL h⁻¹)</td>
<td>31</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>Cat 6</td>
<td>30 μM L-NAME (1 μL h⁻¹)</td>
<td>24</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Cat 7</td>
<td>30 μM L-NAME (1 μL h⁻¹)</td>
<td>24</td>
<td>26</td>
<td>31</td>
</tr>
<tr>
<td>Cat 8</td>
<td>22 μM L-NOArg (1 μL h⁻¹)</td>
<td>26</td>
<td>28</td>
<td>33</td>
</tr>
</tbody>
</table>
implanted. We waited 2 days before manipulating visual input in order to allow the infusion drug to achieve a stable intracerebral concentration gradient. Electrophysiological recordings were made after a period ranging from 4 to 9 days of monocular lid suture, which reliably produces a strong shift of responsiveness in favor of the open eye (Klein & Freeman, 1975; Heiter et al. 1986; Hensch et al. 1995).

We were able to detect no consistent differences in the physical appearance of the cortex or cortical vasculature of the two hemispheres. Electrode penetrations were made down the medial bank of the visual cortex in the vicinity of the infusion cannula in both hemispheres. The median number of single units recorded in each penetration was fifteen, typically at 100 μm intervals. All penetrations were made within 2-5 mm along the anteroposterior axis from the cannula to insure that neurons recorded were located in the region affected by the drug infusion. Although the biochemical analysis precluded histological determination of the laminar distribution of cells in this study, we are confident that the population of cells in this study includes at least layers II to IV, as there was a well-defined range of depths in many penetrations in which thalamocortical afferent responses were easily recorded, as is typical of layer IV in area 17. Neurons were characterized for preferred orientation, quality of response, and relative ability of the two eyes to elicit a response (ranked according to the seven point scale of Hubel and Wiesel (see Wiesel, 1982)).

A strong ocular dominance shift towards the open eye was observed in both control and NOS-inhibited hemispheres of all animals studied. Figure 1 compares an ocular dominance histogram from normal kittens of this age with the grouped data from experimental and control

![Figure 1. Ocular dominance histograms show a robust shift in monocularly deprived animals infused with NOS inhibitors or with saline.](image)
hemispheres of deprived animals in this study. The overall ocular dominance distribution for a hemisphere can be quantified as a 'shift index', in which zero represents complete domination by the deprived eye and one represents responses exclusively driven by the open eye. The mean shift index for the confirmed NOS-inhibited visual cortex was 0.89 ± 0.05 (s.e.m., n = 6 animals), versus a shift index of 0.90 ± 0.02 (s.e.m., n = 6 animals) for control hemispheres. Ocular dominance distributions in control and experimental hemispheres of each animal, and of each group of animals pooled by experimental condition, were not found to be significantly different from each other (two-tailed Wilcoxon non-parametric test, $P > 0.05$), except in the case of cat 4 which showed a somewhat greater shift in the experimental hemisphere (0.99) than the control (0.82). Figure 2 illustrates ocular dominance histograms for each of the experimental conditions along with control data.

Figure 2. Ocular dominance histograms for each experimental condition

A, C and E show histograms of ocular dominance for single units recorded in regions treated with 22 mM l-NOSArg ($n = 130$ cells in 3 animals), 2 mM l-NOSArg ($n = 61$ cells in one animal), and 30 mM l-NMMA ($n = 153$ cells in 4 animals), respectively. $B$ ($n = 148$), $D$ ($n = 57$), and $F$ ($n = 92$) are histograms from control hemispheres of the same animals in A, C and E, respectively. There is no significant difference between any of the groups and their controls.
There was also no significant difference in the responsiveness of cortical neurons to visual stimuli between control and experimental hemispheres, with 90% of NOS-inhibited neurons giving strong responses, 9% showing moderate responsiveness, and 1% responding poorly, compared with 85, 13 and 2%, respectively, in control penetrations. Neurons from both hemispheres displayed narrowly tuned orientation selectivity, with only 28% (6/224) of NOS-inhibited neurons and 18% (5/274) of control neurons completely lacking orientation tuning. While acute ischemic lesion application of NOS inhibitor in the visual cortex has been reported to reversibly alter neuronal response properties in a subset of cells (Kara & Friedlander, 1995), it is not known whether these changes are due to local effects on vascular perfusion. We saw no evidence of altered responsiveness or orientation selectivity under conditions of profound, long-term blockade of NOS activity. Indeed, it was impossible on the basis of electrophysiological properties to deduce which hemisphere was infused with the NOS inhibitor.

Nitric oxide synthase activity in visual cortex is greatly reduced or eliminated by chronic infusion of inhibitor

In order to confirm that continuous infusion of L-NMMA or L-NOArg into visual cortex did produce a local inhibition of NOS activity, brain samples were taken for biochemical analysis at the conclusion of electrophysiology. Figure 3A illustrates the effects of 22 mm L-NOArg infusion on cortical NOS activity. In the 3 mm region closest to the cannula, from which all the ocular dominance data were derived, drug infusion decreased levels of NOS activity by an average of 94% below that in the comparable 3 mm segment of control cortex, and by 90% below the mean level for the entire control side. Moreover, in the case of cat 8, NOS activity was below detectable levels in the 2.5 mm region in which electrophysiological data had been collected. Similar results were obtained using 2 mm L-NOArg except that inhibition was slightly less pronounced (90% reduction for the first 3 mm nearest the cannula) and the region of greatest blockade was somewhat smaller (Fig. 3B).

There was considerable intra-animal variation in levels of NOS activity per milligram of total protein between different slices from the control hemisphere, which may perhaps reflect differences in the distribution of cerebral blood vessels. However, the fact that the sections nearest the L-NOArg cannula showed very little variability in NOS activity (Fig. 3A) suggests that L-NOArg was actually above saturation inhibition concentration in the 2.5 mm-long region that had been electrophysiologically characterized. The actual concentration of L-NOArg would be expected to decrease steeply as a function of distance from the cannula, but NOS activity remained almost unchanged with regard to distance until more than 3 mm anterior to the cannula, where it first began to increase with distance.

L-NMMA was considerably less effective at locally inhibiting NOS activity in kitten visual cortex. NOS activity in the first 3 mm of the experimental hemispheres was 25 and

![Figure 3](https://example.com/figure3.png)

**Figure 3. Normalized nitric oxide synthase activity as a function of distance from the infusion cannula.**

Chronic L-NOArg infusion at concentrations of 22 mm (*A*, *n* = 3 animals) and 2 mm (*B*, *n* = 1 animal) produces a region in which NOS activity is profoundly reduced. L-NMMA (30 mm; *C*, *n* = 2 animals) results in a significant, but smaller reduction. The points represent ± SEM (mg protein)"normalized within individual animals to the mean ± SEM (mg protein)" for all tissue sections taken from the control hemisphere (●, inhibitor-treated hemispheres; ○, control hemispheres; error bars are ± SEM). This allows data from different animals to be pooled based on the assumption that the control hemisphere data represent baseline levels of NOS activity. Mean ± SEM (mg protein)"for each control hemisphere are: (A) cat 3, ±0.0.4 + 0.05; cat 4, ±0.0.4 + 0.05; cat 5, ±0.0.4 + 0.05; cat 6, ±0.0.4 + 0.05; cat 7, ±0.0.4 + 0.05. The shaded area indicates the range of distances at which single unit recordings were made.
DISCUSSION

These experiments reveal that normal, robust ocular dominance plasticity does occur in visual cortex in which NOS activity is pharmacologically blocked.

The demonstration of greatly reduced NOS activity in the same tissue from which electrophysiological data were collected ensures that the drugs used in this study accomplished their intended biochemical effect. Previous investigations using the same intracerebral infusion protocols with other drugs, having molecular weights similar to L-NOArg and L-NMMA, have clearly established that a 48 h period prior to eyecup surgery is sufficient for steady-state concentrations of infusion drug to bathe the region of interest in the visual cortex (Beiter et al. 1988). The confirmation that NOS activity continued to be profoundly inhibited after 7 days of continuous infusion of inhibitor makes it highly unlikely that there might have been any window in time during the period of deprivation in which NOS was not inhibited.

While the biochemical assay in this study did not measure NO production directly, it did directly measure the catalytic activity of NOS for the production of the citrulline by-product of NO formation. It is conceivable that the inhibitors infused into cortex were somehow excluded from access to the NOS until the tissue was homogenized for the biochemical assay. This is improbable for two reasons: (1) experiments with NO-sensitive electrodes have directly demonstrated the ability of inhibitors to block NO production when applied extracellularly (Shibuki & Okada, 1991); (2) extracellular NOS inhibitor would have been washed out prior to freezing the tissue samples during the perfusion and sectioning.

The finding that normal ocular dominance plasticity, measured electrophysiologically, occurs in regions of visual cortex where NOS is completely inhibited, or nearly so, rules out any model that requires NO production as a necessary step for this form of cortical plasticity. Nonetheless it is impossible to exclude entirely any role for NO. An alternative explanation for our finding is that NO may be important only for one of several redundant or mutually facilitatory mechanisms that underlie ocular dominance plasticity. For example, homeostatic mechanisms have been shown to compensate for the absence of neuronal NOS in the regulation of cerebral blood flow in mice lacking neuronal NOS gene expression (Erikum et al. 1993).

If redundant mechanisms in ocular dominance plasticity were able to fully compensate for the absence of NOS activity, our assay would fail to detect an effect. On the other hand, if NO were involved in one of several facilitatory mechanisms, ocular dominance plasticity would be predicted to occur more slowly than normal without the contribution of the NO-dependent pathway. In an additional animal that was monocularly deprived for just 2 days, the minimum deprivation known to produce a consistent physiological shift, 22 nm L-NOArg did not impair ocular dominance plasticity (s = ±50 cells, shift index m = 0±88, data not shown) compared with control 2 day-deprived animals.

In addition to the studies that demonstrate a requirement for NO synthesis or release in hippocampal LTP, there have been numerous reports of NO-independent forms of LTP (Griishoff & Laim-Ragus, 1992; Chekirkovich, Klein & Sweets, 1993; Halcy, Malen & Chapman, 1993; Williams, Li, Neya, Errington, Munphy & Bliss, 1995; Cummings et al. 1994). The existence of NO-independent forms of LTP in the CA1 field of the hippocampus, however, does not rule out a contribution of NO in hippocampal plasticity: systemic injection of NOS inhibitors has been reported to impair spatial learning in the Morris water maze (Chapman, Atkins, Allen, Haye & Steinmetz, 1992) and in the radial arm maze (Bohme et al. 1993), two tasks believed to rely on plasticity in the hippocampus. While the specificity of NOS inhibition on hippocampal learning has been contested (Bannerman, Chapman, Klein, Butcher & Morris, 1994), the complete absence of any effect of NOS inhibition on ocular dominance plasticity suggests an even smaller role, if any, for NO in neocortical developmental plasticity. Indeed, no requirement for NO in visual cortical LTP has yet been reported.

LTP induced by electrical stimulation of neural pathways is an artificial condition that may not take place under normal circumstances in vivo. In contrast, ocular dominance plasticity and the examples of spatial learning described above do take place in vivo and can utilize only normal mechanisms. In addition, plasticity of the vestibulo-ocular reflex, believed to reflect cerebellar LTD, has been prevented by subdural application of the NO scavenger haemoglobin to the cerebellum in rabbit and monkey (Nagao & Ito, 1991). Systemic administration of NOS inhibitors also disrupts the development of on-off sublaminar of the lateral geniculate nucleus in the ferret (Cramer & Sur, 1995). Thus there is ample evidence that NO synthesis can have drastic effects in complex, in vivo systems that do depend on NO production.

A final issue that these experiments did not address is the possibility that inhibiting NO synthesis had morphological effects on the thalamocortical terminals that were not revealed by electrophysiological recordings. For example, a failure of the arbor of axon to survive the open eye to expand and of those surviving the deprived eye to shrink in the presence of NOS inhibitors might have gone undetected by electrophysiology if appropriate modifications of synaptic strength had rendered deprived eye inputs ineffective and
strengthened inputs from the open eye. Since the known effects of NO inhibition on neural plasticity demonstrated so far are predominantly physiological, involving the ability of synapses to potentiate or depress transmission, it is unlikely that it would have silent morphological effects in the absence of the physiological ones.

What is the function of the NOS present in neocortical neurons? High levels of NOS are expressed in about 1-2% of cortical neurons distributed diffusely across the cortex. These cells are identical to the NADPH-diaphorase-positive neurons that are highly resistant to loss in certain neurodegenerative diseases and ischemia (Bredt et al., 1991). Both in culture and in experimental in vivo models of ischemia, NO, presumably released by these NADPH-diaphorase-positive neurons, has been shown to mediate glutamate neurotoxicity (Koh & Choi, 1988; Dawson, Dawson, London, Brodt & Snyder, 1991). There is also both anatomical and physiological evidence for a contribution of NO to the metabolic regulation of cortical blood flow (for review see Landreth, 1993). Thus changes in NOS expression in visual cortex seen in response to alterations in the pattern of cortical activity (Aoki et al., 1993) appear more likely to reflect a contribution of NO to general cortical maintenance rather than to plasticity.

Whether NO plays any role in neocortical plasticity will require further study. Nonetheless, the fact that the physiological shift in occular dominance is both as great and as rapid in the absence of NOS activity as in its presence suggests that the role of NO in ocular dominance plasticity is at most a minor one. The basic finding presented above has been replicated recently by a different group using a similar approach to ours (see preceding paper; Reid, Das, Carpathi, Flavin & Sessions, 1996). Our results show that there exists at least one pathway for developmental cortical plasticity that can occur in the absence of cortical NOS activity.


