0270-6474/85/0507-1925$02.00/0

The Journal of Neuroscience
Vol. 5, No. 7, pp. 1925–1933
July 1985

DSP-4 (*N*-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine) Depletes Noradrenaline in Kitten Visual Cortex Without Altering the Effects of Monocular Deprivation[1]

N. W. DAW,[2] T. O. VIDEEN, D. PARKINSON, AND R. K. RADER

Department of Physiology and Biophysics and McDonnell Center for Study of Higher Brain Function, Washington University School of Medicine, St. Louis, Missouri 63110

Abstract

Kittens were given *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) to deplete cortical noradrenaline (NA) in order to test whether this would affect the results of monocular deprivation. Seven kittens that received DSP-4 systemically had cortical NA depleted by 25 to 98%, and six kittens that received DSP-4 in the lateral ventricle had cortical NA depleted by 72 to 92%. In all of these kittens, suturing shut the eyelids of one eye for 1 to 2 weeks produced a visual cortex in which most neurons responded only or most strongly to the eye that remained open. These results are considered together with previous results from our laboratory on monocular deprivation and NA depletion. There is little difference between the ocular dominance histograms of depleted and undepleted animals and little correlation between the extent of the ocular dominance shift and the extent of NA depletion. We conclude that depletion of cortical NA by itself does not prevent the cortical effects of monocular deprivation and that, where such an effect has been found, it may be due to some other factor.

The kitten visual cortex is mutable for the first few months of life in response to altered visual input. Evidence for this first came from Wiesel and Hubel (1963), who found that closing one eye of a kitten (monocular deprivation) changes the relative responsiveness of visual cortical cells to visual stimulation of the two eyes. Normally, the majority of these cells respond to input from either eye. However, following monocular deprivation, nearly all visual cortical cells can be driven only by the eye that remained open. In other words, monocular deprivation in young kittens shifts the ocular dominance of visual cortical cells from a balanced, binocular distribution, with equal numbers of cells capable of being driven by either eye, to a skewed distribution in which most cells respond only or most strongly to the nondeprived eye (for reviews see Movshon and Van Sluyters, 1981; Sherman and Spear, 1982).

Kasamatsu and Pettigrew (1976, 1979) have proposed that this mutability depends on the presence of noradrenaline (NA) in the cortex. The evidence for this proposal is now controversial. Infusion of 6-hydroxydopamine (6-OHDA), a catecholamine neurotoxin (Jonsson, 1980), directly into the visual cortex consistently reduces or prevents the shift in ocular dominance that usually occurs after monocular deprivation (Kasamatsu et al., 1979; Daw et al., 1983; Paradiso et al., 1983). Interruption of the fibers projecting to the cortex from the locus ceruleus, by electrolytic lesions of the dorsal noradrenergic bundle in the lateral hypothalamus (Daw et al., 1984), by injections of 6-OHDA into the lateral hypothalamus (Daw et al., 1984), or by lesions of the locus ceruleus itself (Adrien et al., 1982), does not prevent the shift in ocular dominance. Also, systemic injections of 6-OHDA into neonatal kittens, while the blood-brain barrier is still permeable, does not prevent the ocular dominance shift (Bear and Daniels, 1983). The original method used by Kasamatsu and Pettigrew (1976)—daily injections of 6-OHDA into the lateral ventricle—has yielded different results in different laboratories. Kasamatsu and Pettigrew (1976, 1979) found that it prevented the ocular dominance shift, whereas Adrien et al. (1982) and Daw et al. (1985) have found that it does not.

Hoping to resolve this controversy, we decided to deplete NA with *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) because this drug has several advantages over 6-OHDA. First, DSP-4 crosses the blood-brain barrier and can therefore be given systemically (Ross, 1976; Jaim-Etcheverry and Zieher, 1980; Jonsson et al., 1981). As a result, we thought that DSP-4 would be less likely to cause the local damage effects that have been found with intracerebral injections of 6-OHDA (Poirier et al., 1972; Butcher et al., 1975). Second, DSP-4 is more selective than 6-OHDA for central NA endings than for peripheral NA endings (Ross, 1976; Jaim-Etcheverry and Zieher, 1980; Jonsson et al., 1981; Dooley et al., 1983). Third, DSP-4 is more selective than 6-OHDA for NA processes compared to dopamine processes (Ross, 1976; Jonsson et al., 1981, 1982). Fourth, whereas previous reports have suggested that DSP-4 may be less selective than 6-OHDA for noradrenergic neurons compared to serotonergic neurons (Ross, 1976; Jonsson et al., 1981, 1982; Dooley et al., 1983), we have found substantial reduction of NA in the kitten visual cortex without much change in serotonin (5-hydroxytryptamine, 5-HT). Finally, DSP-4 reduces NA levels in the cerebral cortex rapidly, and this depletion lasts for several weeks (Ross, 1976; Jaim-Etcheverry and Zieher, 1980; Jonsson et al., 1981; Dooley et al., 1983).

Bear and Daniels (1983) have suggested that the difference between their results after neonatal administration of 6-OHDA and

Received November 14, 1984; Revised January 10, 1985;
Accepted January 14, 1985

[1] This work was supported by National Institutes of Health Research Grant EY00053, Program Project Grant NS 15070, and the McDonnell Center for Study of Higher Brain Function. We thank Elizabeth Coscia for carrying out some of the high pressure liquid chromatography measurements, Jeanette Cohen for the histology, and Janice Wuelling for typing the manuscript. We also thank Tom Robertson, George Seifert, Christine Hahn, Megan Morgan, and Janice Waugh for helping in some of the experiments. Dr. Svante Ross of Astra Läkemedel Research Laboratories kindly arranged to provide us with DSP-4.

[2] To whom correspondence should be addressed, at Department of Physiology and Biophysics, Washington University School of Medicine, 4566 Scott Avenue, St. Louis, MO 63110.

the results of Kasamatsu and Pettigrew (1976, 1979) after intracerebral administration of 6-OHDA could be due to receptor compensation that might occur with long periods of depletion. Since receptor compensation may occur after DSP-4 treatment (Jonsson et al., 1981; Spyraki and Fibiger, 1982; Dooley et al., 1983), we planned to test this suggestion from Bear and Daniels (1983) by comparing animals with short and long periods of depletion using DSP-4.

Materials and Methods

Seventeen kittens were given DSP-4 systemically, 8 for tests of the dose required and duration of NA depletion and 9 for tests of the effect of DSP-4 on monocular deprivation. DSP-4 was dissolved into 0.3 ml of 0.9% NaCl and was usually injected subcutaneously, but occasionally was injected intraperitoneally. Doses varied from 5 mg/kg to 60 mg/kg. Injections were made within 5 min of dissolving the drug. An additional 11 kittens had DSP-4 injected into the lateral ventricle at stereotaxic coordinates A11, L3, U+6. Previous experiments found that this location consistently reached the lateral ventricle (Daw et al., 1985). Kittens were anesthetized with nitrous oxide and halothane and placed in a stereotaxic instrument. A hole was drilled in the skull, and the injection was made with a Hamilton syringe. The DSP-4 was dissolved in sterile 0.9% NaCl, and 5 μl were injected at the rate of 1 μl every 35 sec starting 2 min after mixing and finishing about 4½ min after mixing. Doses varied from 100 μg to 1 mg per kitten (body weight 330 to 620 gm).

Eyelid sutures were done under halothane anesthesia as previously described (Wiesel and Hubel, 1963; Berman and Daw, 1977). Recordings were made in the left cortex in all cases. Intraventricular DSP-4 injections were always made in the left ventricle. Half the animals had the right eye sutured and half had the left eye sutured.

Recording procedures. Procedures followed those previously described (Daw et al., 1984). Anesthesia was induced with 4% halothane in a mixture of 66% nitrous oxide and 34% oxygen. After a tracheotomy and intravenous cannulation, the animal was placed in a stereotaxic instrument. The bone and dura were removed around stereotaxic coordinate AP0 over the lateral gyrus near the representation of the area centralis. After surgery the animal was paralyzed with intravenous pancuronium bromide (Pavulon; Organon Diagnostics, West Orange, NJ), and anesthesia was maintained with approximately 0.5% halothane. Temperature was maintained at 37.5°C with a heating pad controlled by a rectal thermometer.

Recordings were made with lacquer-coated tungsten electrodes (Hubel, 1957). Receptive fields were plotted on a tangent screen, and the animal's retinas were focused on the screen with appropriate contact lenses. Cells were characterized according to ocular dominance (groups 1 to 7 of Hubel and Wiesel, 1962), orientation preference, direction preference, velocity preference, length specificity, type (simple or complex), spontaneous activity, and other characteristics as appropriate. Particular attention was paid to ocular dominance, and at least two experimenters made a judgment on each cell after determining the preferred stimulus for the cell. To avoid sampling bias from staying in the same ocular dominance column, the electrode was angled at 15° to the vertical down the medial bank of the lateral gyrus, and it was moved 300 μm after each cell for the first 1 mm and 150 μm after each cell thereafter (Daw and Wyatt, 1976). At least three penetrations, spaced about 1 mm apart, were made in each animal. About 40 cells were recorded in each animal.

NA analyses. At the end of the recordings the kitten was deeply anesthetized and sacrificed. The skull was removed, and 6 to 14 tissue samples were taken from the lateral, postlateral, and suprasylvian gyri and frozen on a brass block in dry ice. These were stored at −70°C until assayed. Samples were also taken from age-matched normal animals for comparison since the concentration of NA changes with age (Jonsson and Kasamatsu, 1983). Three samples from an experimental animal were compared to three samples from a normal animal to calculate the percentage reduction in NA for the experimental animal.

Catecholamine analyses were performed by ion-paired reverse phase high pressure liquid chromatography (HPLC) with electrochemical detection (Kissinger et al., 1973). Brain samples (10 to 150 mg) were homogenized in acid butanol by sonication after the addition of 5 ng of dihydroxybenzylamine as an internal standard. After centrifugation for 10 min at 12,000 × *g*, the supernatant was added to 200 μl of 0.1 M phosphoric acid and 500 μl of heptane in new tubes. The tubes were mixed for 5 min and centrifuged to separate the layers, and the organic phase was aspirated. The aqueous phase was washed with 200 μl of chloroform, and an aliquot (50 μl) was saved for analysis of 5-HT. Ten milligrams of acid-washed alumina and 1 ml of 0.5 M Tris/10 mM sodium EDTA, pH 8.6, were added to the remainder. The tubes were gently agitated for 15 min and centrifuged to sediment the alumina, and the supernatant was then aspirated. The alumina was washed with 1.5 ml of distilled water, and catechols were eluted with 50 μl of 0.1 M HCl for 30 min, followed by 50 μl of HPLC mobile phase before injecting into the liquid chromatograph.

The high pressure liquid chromatograph consisted of a Waters Associates (Milford, MA) M45 pump, standing column pulse dampener (30 × 1 cm) and a Rheodyne (Cotati, CA) 7125 loop injector (20-μl loop) coupled to a Bioanalytical Systems (Lafayette, IN) electrochemical detector (LC-3A). The 5-μm, 0.46 × 12.5 cm Lichrosorb RP-18 column was eluted with 50 mM potassium phosphate, pH 4.5, containing 0.2 gm/liter of sodium heptanesulfonic acid, 0.1 gm/liter of sodium EDTA, and 8% (v/v) methanol at 35°C with a flow rate of 0.8 ml/min; retention time of norepinephrine was about 6 min. The carbon paste (CP-O) electrode of the detector was maintained at +0.55 V (versus Ag/AgCl). For norepinephrine the lower limit of detection was 40 to 100 pg/sample.

Receptor measurements. β-Adrenergic receptors were measured with ^{3}H-dihydroalprenolol (^{3}H-DHA, 85 Ci/mmol) as ligand. The use of ^{3}H-DHA in cat and kitten visual cortex has already been validated (Wilkinson et al., 1983). Visual cortex was homogenized in 20 vol of 50 mM Tris-HCl, pH 7.2, and centrifuged at 2000 rpm for 10 min to sediment cell debris. The supernatant was centrifuged for 30 min at 12,000 × *g*, and the pellet was then resuspended in buffer and centrifuged again. The final pellet was resuspended in buffer and stored frozen for later use. For the radioligand-binding assay (final volumes, 250 μl), 50 to 100 gm of protein were incubated for 1 hr at room temperature with varying concentrations of ^{3}H-DHA in 50 mM Tris, 150 mM NaCl, pH 8.0. L-Alprenolol (10^{-5} M) was added to some tubes to define nonspecific binding. At the end of the incubation, 2 ml of cold buffer were added, and the mixture was filtered under reduced pressure on Whatman GF/B filters. The filters were washed with two 7-ml aliquots of ice-cold buffer. Tritium bound to the filter was measured by liquid scintillation spectroscopy after addition of 10 ml of Scintiverse E (Fisher Scientific Co., Pittsburgh, PA). Binding parameters (K_D, B_{max}) were calculated from Scatchard analysis (Scatchard, 1949) with at least five concentrations of ^{3}H-DHA (0.5 to 10 nM).

Histology. After taking samples for NA analysis, the anterior part of the left cortex from animals that had DSP-4 injected into the lateral ventricle was submerged in buffered formalin. It was subsequently blocked, frozen sectioned, and stained with cresyl violet for Nissl substance. Serial sections were stained and inspected for location of the needle track and damage around the ventricle.

Results

Systemic administration of DSP-4

Doses required and period of depletion of NA. Since nearly all previous work with DSP-4 has been done on rats, we first needed

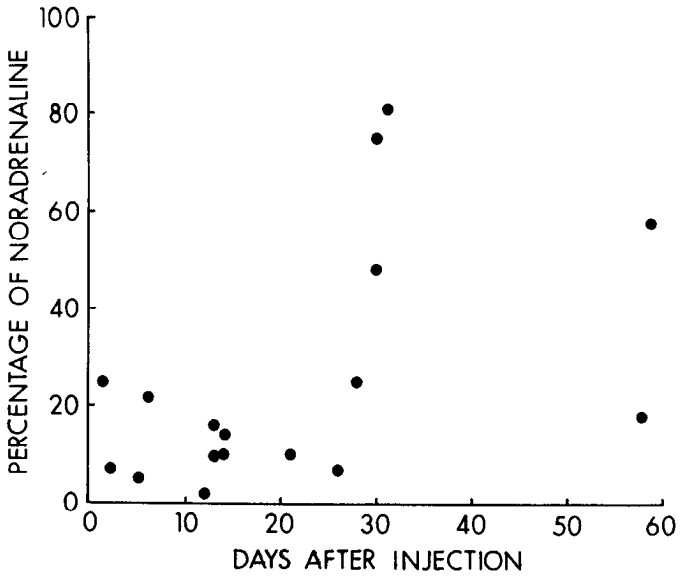

Figure 1. Reduction of NA content of visual cortex after systemic injections of DSP-4. Injections were made at 26 to 34 days of age with one exception—an animal injected at 42 days. The NA content is plotted as a function of the number of days after the injection.

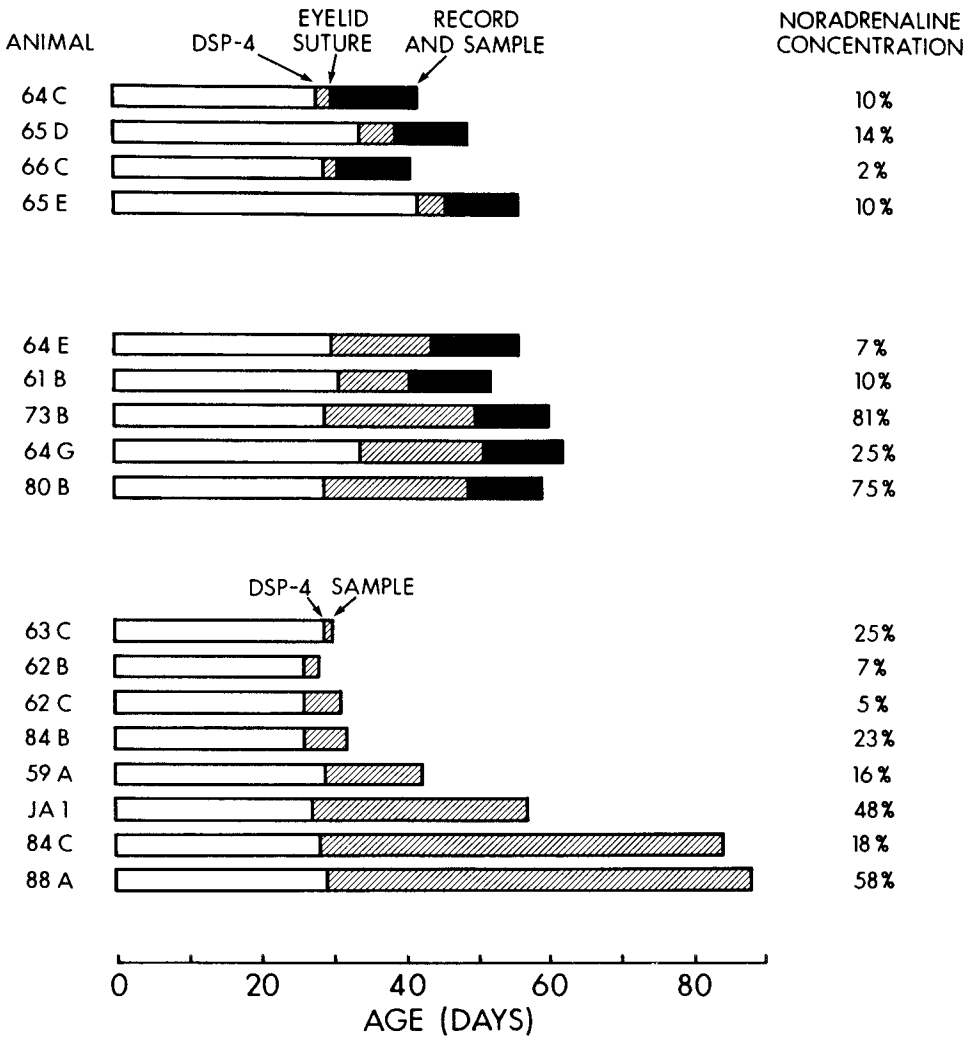

Figure 2. Timing of DSP-4 injections, eyelid suture, electrophysiological recording, and sampling of visual cortex for NA for individual animals together with the measured NA concentration expressed as a percentage of the concentration measured for a normal animal close to the same age.

to establish the dose required for kittens. Preliminary experiments found that a dose of 30 mg/kg reduced NA levels in the visual cortex to less than 10% of normal within 2 days of injection. There was little correlation between dose and extent of NA depletion, with doses ranging from 20 to 45 mg/kg. There was some variability in the amount of NA depletion achieved which did not depend on the dose or the time elapsed between dissolving DSP-4 and injecting it into the animal. Also, there were some signs of recovery of NA levels at 30 days after DSP-4 injection, although one animal showed greater than 80% reduction in NA levels at 58 days after injection (Fig. 1).

Electrophysiology results. The first group of animals (64C, 66C, 65D, and 65E) had their eyelids sutured shortly (2 to 5 days) after the DSP-4 injection. The time between eyelid suture and recording was 10 to 12 days (Fig. 2). In all cases the reduction in NA concentration was substantial (Fig. 2). Two animals (64C and 66C) had the left eyelids sutured and two (65D and 65E) had the right eyelids sutured. The ocular dominance histograms of all animals were shifted toward the open eye by substantial amounts (Fig. 3).

The second group of animals (64E, 61B, 73B, 64G, and 80B) had their eyelids sutured shut at longer times (10 to 21 days) after DSP-4 injection. The length of time between eyelid suture and electrophysiological recording was 10 to 12 days, as in the first group. Three animals (64E, 73B, and 80B) had the left eyelids sutured and two (61B and 64G) had the right eyelids sutured. Again, the ocular dominance histograms of all animals were shifted substantially toward the open eye (Fig. 3). There was no significant difference between the ocular dominance histograms of animals which had eyelid suture shortly after DSP-4 injection and those that had eyelid suture 2 or 3 weeks after DSP-4 injeciton. Since the second group of animals was an average of 10 days older when eye suture began, a slightly greater proportion of binocular cells is expected due to age alone (Hubel and Wiesel, 1970; Olson and Freeman, 1980) and need not involve any receptor or transmitter compensation.

There was a difference between animals that had ipsilateral eyelid suture and those that had contralateral eyelid suture. Combining the results from all animals with ipsilateral suture, 93% (166 of 178) of drivable cells were driven preferentially by the open eye, 75% (133 of 178) could be driven only by the open eye, and 25% (44 of 178) could be driven by either eye. Comparable figures from all animals with contralateral suture were 74% (106 of 143) dominated by the open eye, 47% (67 of 143) driven only by the open eye, and 45% (64 of 143) driven by either eye. These differences are expected from previous results (see summaries in Movshon and van Sluyters, 1981; Sherman and Spear, 1982.

Three animals in the second group (64E, 61B, and 64G) showed substantial reductions in NA concentration (Fig. 2), whereas two others (73B and 80B) did not. A series of control animals (84B, JA1, 84C, and 88A) was used to test whether NA depletion was a function of time after injection of DSP-4. There did seem to be some recovery of NA concentration for periods of 30 days or more between DSP-4 injection and sampling of visual cortex, but the results were quite variable (Fig. 1). In any case, there was little difference between the electrophysiological results from the first and second group of animals and, consequently, no evidence for compensatory effects.

^{3}H-DHA binding was used to assess changes in adrenergic β-receptors in kitten visual cortex after DSP-4 treatment. Five control

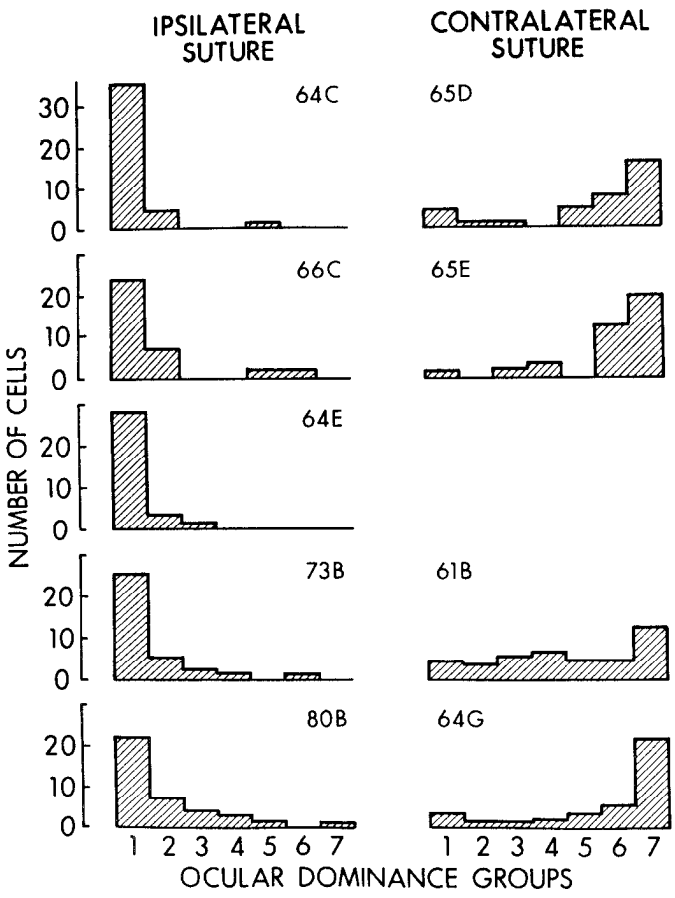

Figure 3. Ocular dominance histograms from nine kittens given DSP-4 systemically followed by eye suture. The left cortex was recorded in all cases.

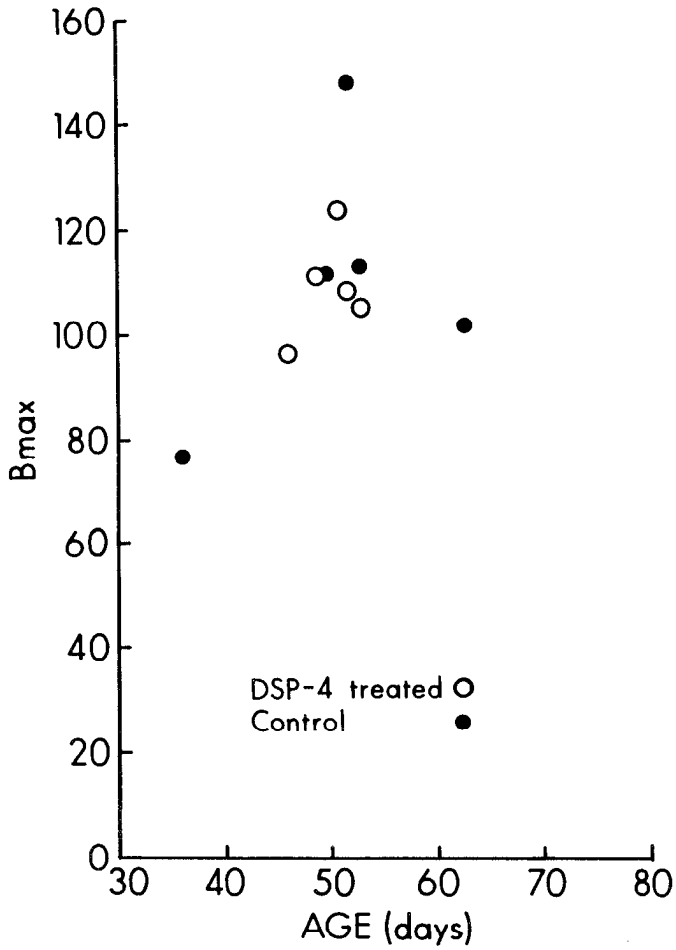

Figure 4. Concentration of β-receptors in the visual cortex as a function of age in control and DSP-4-treated animals. B_{max} was determined by Scatchard analysis of ^{3}H-DHA binding.

kittens (aged 36 to 63 days) were compared with five DSP-4-treated animals (aged 46 to 53 days) in which NA was depleted by greater than 85% (61B, 65D, 90A, 93A, and 93B). The K_D of ^{3}H-DHA was not significantly affected by DSP-4 treatment (control, 2.1 ± 0.7 nM; DSP-4, 2.0 ± 0.6 nM), nor were there any obvious differences in the maximum number of receptors (control B_{max}, 110 ± 12 fmol/mg of protein; DSP-4 B_{max}, 109 ± 5 fmol/mg of protein). There is a tendency for the number of β-receptors to increase with age, but when this is taken into account there is still no obvious trend for increased β-receptors after the DSP-4 treatment (Fig. 4). This was true for the animal that was sampled 21 days after DSP-4 treatment (61B), as well as for the others, which were sampled 14 to 15 days after DSP-4 treatment.

Intraventicular injections of DSP-4

The first series of experiments found that DSP-4 injected systemically could give a 90% reduction in the concentration of NA in the visual cortex without preventing the shift in ocular dominance that usually occurs after monocular deprivation. We wanted to see whether we could achieve a greater reduction in NA concentration by administering DSP-4 directly into the lateral ventricle. Systemic doses of DSP-4 in excess of 50 mg/kg result in high mortality. Furthermore, DSP-4 is believed to act after forming a cyclic azidirinium derivative which does not cross the blood-brain barrier (Zieher and Jaim-Etcheverry, 1980). Consequently, systemic administration does not always give consistent results. We therefore decided to inject a series of animals with a single dose of DSP-4 into the lateral ventricle.

Dose of DSP-4 required, reduction of NA obtained, and behavioral results

Initial results showed that a dose of 500 gm is required for substantial depletion of NA and that increasing the dose to 1 mg does not increase the depletion (Fig. 5). The dose used most regularly was 1 mg, and this reduced the NA content to somewhere between 8% and 28% of normal (Fig. 5). Kittens weighed between 350 and 620 gm; thus, this dose represented a range of 1.6 to 2.8 mg/kg. However, the reduction in NA was not correlated with the dose expressed as milligrams per kilogram of body weight. Two animals that were given low doses (100 gm) of DSP-4 showed NA concentrations greater than normal. The measurements were 125 ng/gm (two samples) for one animal and 101 ng/gm (three samples) for the other, compared to 78 ± 5 ng/gm for seven normal animals of this age. If this turns out to be a consistent finding, it could be a significant observation on the mechanism of action of DSP-4.

Most animals lost weight after the injection of DSP-4. Three of them (93A, 96C, and 98A) showed signs of sham rage over the first 1 to 3 days, followed by placidity. Only one animal had signs of pupillary changes, and there was little indication of circling. We found that the best care for them was to keep them in the laboratory for 24 to 48 hr on a heating blanket and to feed them by hand before returning them to their mothers.

Electrophysiological results

The period between DSP-4 injection and eyelid suture was approximately 1 week, and the period between eyelid suture and electrophysiological recording was always exactly 1 week (Fig. 6).

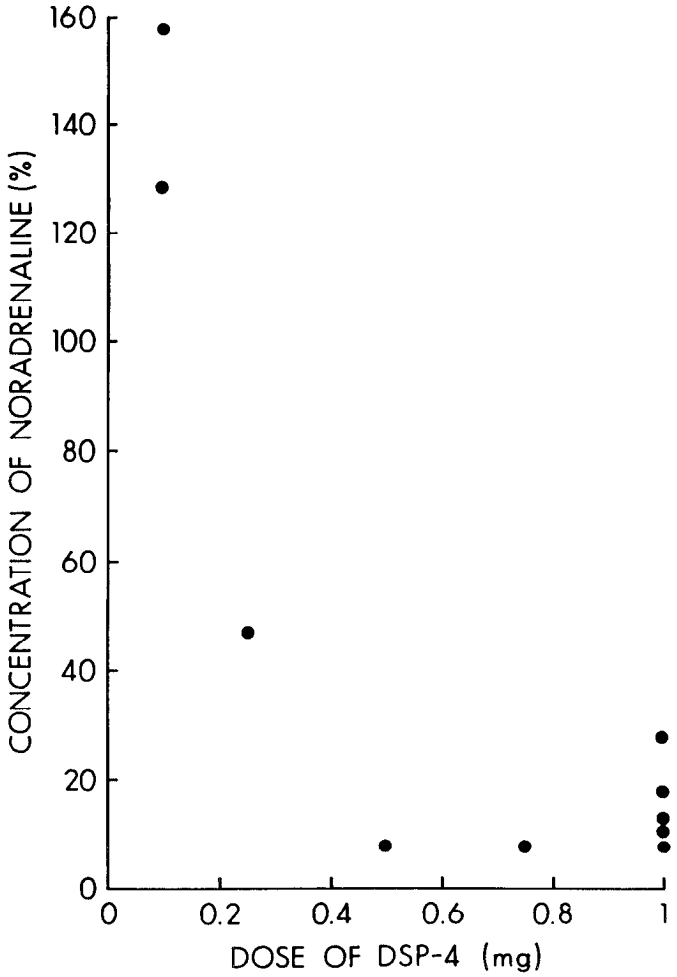

Figure 5. Concentration of NA in the visual cortex plotted as a function of the dose of DSP-4 for intraventricular injections. Kittens were all injected around 5 weeks of age and sampled around 7 weeks of age.

Consequently, animals were sampled for NA concentration about 2 weeks after DSP-4 injection, and the entire period of eyelid suture occurred during the period of maximum NA depletion. We used a period of 7 days of eyelid suture, rather than the 10 to 12 days used with systemic injections of DSP-4 described in the first half of this paper, to accentuate any prevention of ocular dominance shift that might occur.

Again, the ocular dominance histograms recorded from nearly all animals were strongly shifted toward dominance by the open eye (Fig. 7). Kitten 89B can be regarded as a control for animals with contralateral eyelid suture, because this kitten received a dose of 0.1 mg of DSP-4, and there was no reduction in the NA concentration in the visual cortex. There was little difference between the ocular dominance histogram recorded from animal 89B and those recorded from animals 93A, 93B, 96C, and 98A, all of which received higher doses of DSP-4 and had greater than 80% reduction in NA concentration. In kitten 89B, 72% (23 of 32) of the cells were dominated by the open eye, compared to 77% (117 of 151) for the others; 59% (19 of 32) of the cells were driven solely by the open eye, compared to 54% (82 of 151) for the other kittens; and 28% (9 of 32) of the cells were driven by both eyes, compared to 39% (59 of 151) for the other kittens.

Among the animals with ipsilateral eyelid suture, little difference could be seen between kitten 89A, which received a dose of 0.25 mg and had a 53% reduction in NA concentration, and the others, which had higher doses of DSP-4 and lower NA concentrations. In kitten 89A, 73% (24 of 33) of the cells were dominated by the open eye, compared to 95% (63 of 66) for the others; 48% (16 of 33) of the cells were driven solely by the open eye, compared to 79% (52 of 66) for the other kittens; and 42% (14 of 33) of the cells were driven by both eyes, compared to 21% (14 of 66) for the other kittens. If anything, the animals with greater NA depletion showed a larger shift in ocular dominance with fewer binocular cells.

Histology. Nine of the kittens were perfused, and the cortex in the region of the DSP-4 injection was blocked, sectioned, and stained to observe the needle track and any damage. In all cases the needle tip reached the ventricle. There was little damage from the needle to areas that it passed through, and it rarely went beyond the ventricle (in one case it reached the caudate nucleus). The ventricle was usually enlarged, and there was some damage to cells around the ventricle reminiscent of that seen with 6-OHDA injections into the ventricle (Butcher et al., 1975). There was some damage to the white matter leading to the cingulate gyrus in three animals, and some damage to the cingulate gyrus itself in one animal.

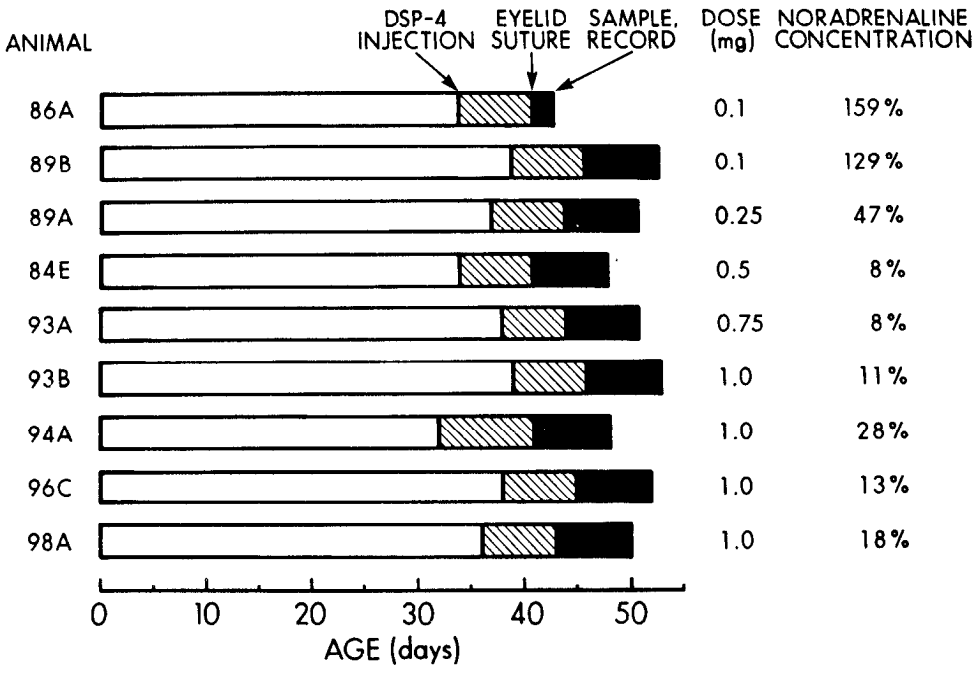

Figure 6. Timing of DSP-4 injection, eyelid suture, recording, and sampling for animals with intraventricular injections along with the measured NA concentration.

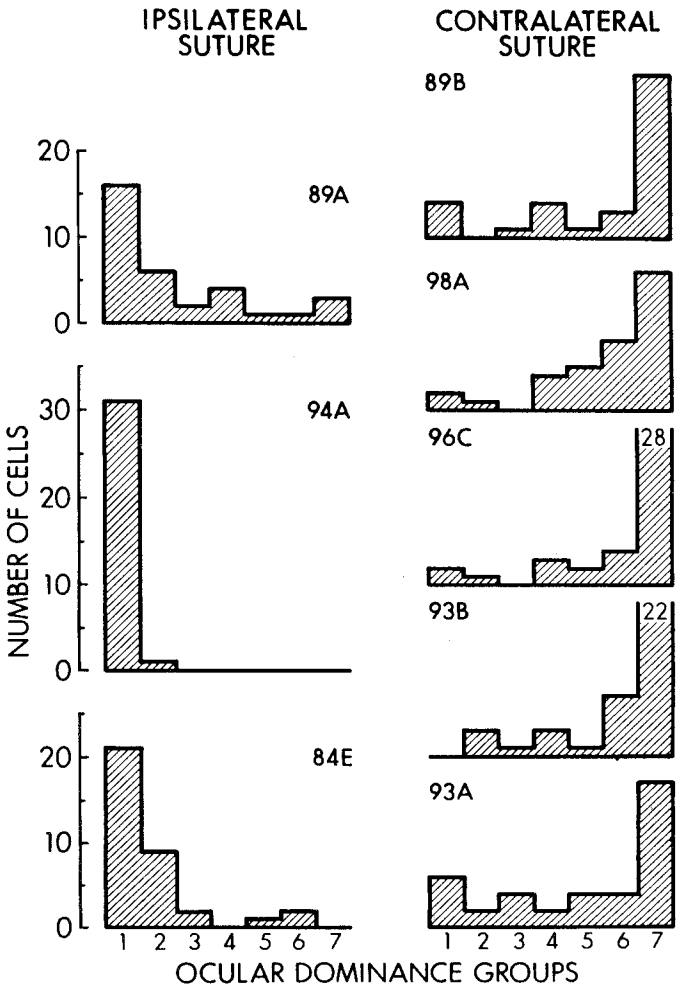

Figure 7. Ocular dominance histograms from eight kittens given DSP-4 intraventricularly, followed by eye suture. Injections were into the left ventricle and recordings were from the left cortex.

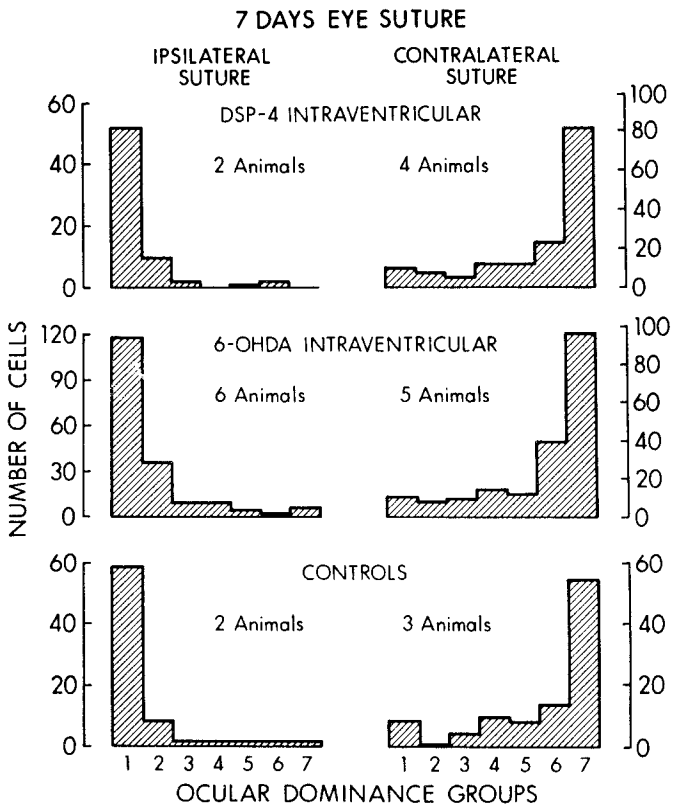

Figure 8. Ocular dominance histograms from kittens with intraventricular injections of DSP-4, animals with intraventricular injections of 6-OHDA, and controls, all monocularly deprived for 7 days. The control group includes two animals with intraventricular injections of ascorbate, one animal with an intraventricular injection of saline, one animal with a low dose of DSP-4 injected intraventricularly and no resulting depletion of NA, and one animal with a unilateral electrolytic lesion of the dorsal noradrenergic bundle where the contralateral side was recorded.

Summary of results and comparison with previous results

We have now accumulated results on more than 33 kittens depleted of NA by various methods, and it seems useful to review the results. With some animals we used exactly 7 days of eyelid suture between about 6 and 7 weeks of age. With others the length of eyelid suture was longer—10 to 14 days. Since the length of eyelid suture can be expected to affect the results, we have divided the results into two sets.

There were two groups of animals for which we used exactly 7 days of eyelid suture—animals with intraventricular injections of DSP-4 and animals with intraventricular injections of 6-OHDA. Ocular dominance histograms from these groups of animals are shown in Figure 8 and compared with histograms from five control animals. There are no detectable differences between the experimental animals and the control animals in terms of percentage of cells dominated by the open eye, percentage of cells driven solely by the open eye, or percentage of cells driven by both eyes.

There were also two groups of animals for which the length of eyelid suture was 10 to 14 days—animals with systemic injections of DSP-4 and animals with lesions of the dorsal noradrenergic bundle. Comparison of the ocular dominance histograms from these two groups and control animals with ipsilateral eyelid suture shows little difference (Fig. 9). Although there appears to be a slight difference between the groups of animals with contralateral eyelid suture, this is within the range of variability that we have found for small groups. The greater number of cells responding to the deprived eye in the group that had systemic DSP-4 injections can be largely attributed to a single animal (61B), which was not exceptional in other respects.

Another question that we considered was whether there might be some correlation between ocular dominance changes and the extent of NA depletion. Two measures of ocular dominance changes were defined by Kasamatsu et al. (1981b) and are calculated by the following formulas:

$$\text{weighted shift} = \frac{n_1 + \frac{5}{6}n_2 + \frac{2}{3}n_3 + \frac{1}{2}n_4 + \frac{1}{3}n_5 + \frac{1}{6}n_6)}{\text{total number of responsive cells}}$$

$$\text{weighted binocularity} = \frac{n_4 + \frac{2}{3}(n_3 + n_5) + \frac{1}{3}(n_2 + n_6)}{\text{total number of responsive cells}}$$

where n_1 is the number of cells in ocular dominance group 1, n_2 is the number in group 2, and so forth. For weighted shift, the formula given is for the case in which the ipsilateral eye is sutured.

Figures 10 and 11 are scatter plots of weighted shift and weighted binocularity versus concentration of NA in the recorded cortex for 46 cortices (44 animals). There is no apparent decrease in weighted shift with decreasing NA (Fig. 10), nor is there any apparent increase in weighted binocularity with decreasing NA (Fig. 11). An indication of the lack of any monotonic relation between NA content and weighted shift or binocularity is evident in the small values of the Spearman rank correlation coefficient: $r_s = 0.12$ for weighted shift versus NA content, and $r_s = -0.20$ for weighted binocularity versus NA content.

Although the points in Figures 10 and 11 represent animals with both contralateral and ipsilateral eye sutures of different durations (7 to 14 days) beginning at different ages (30 to 51 days), it is evident that there is considerable overlap between the values for NA-

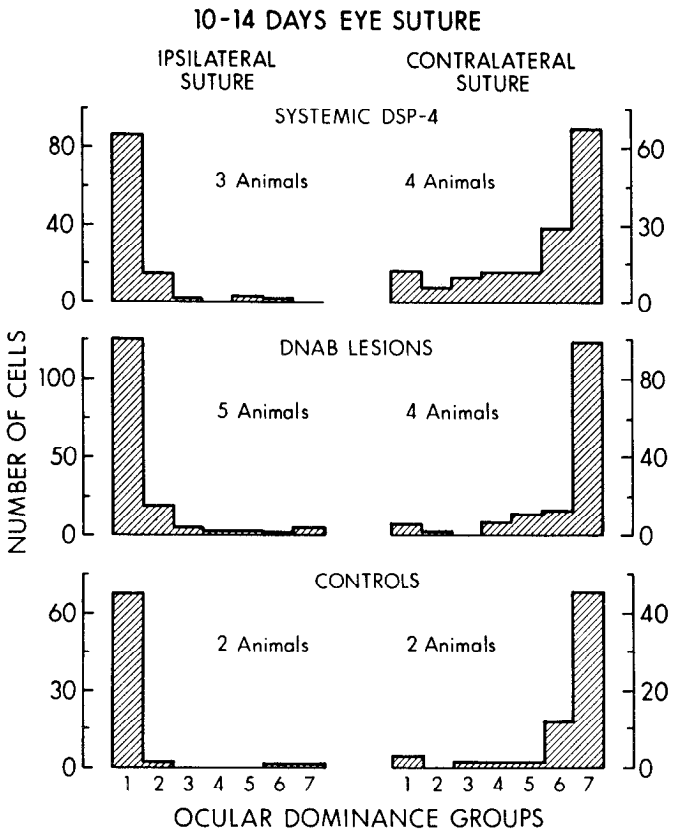

Figure 9. Ocular dominance histograms from kittens with systemic injections of DSP-4, animals with lesions of the dorsal noradrenergic bundle (*DNAB*), and control animals. The control group includes one animal with a unilateral electrolytic lesion of the dorsal noradrenergic bundle where the contralateral side was recorded, one animal where 6-OHDA was intended to be injected into the dorsal noradrenergic bundle but the needle was blocked, one animal with NaCl injected into the ventricle, and one animal with an eyelid suture but no other treatment.

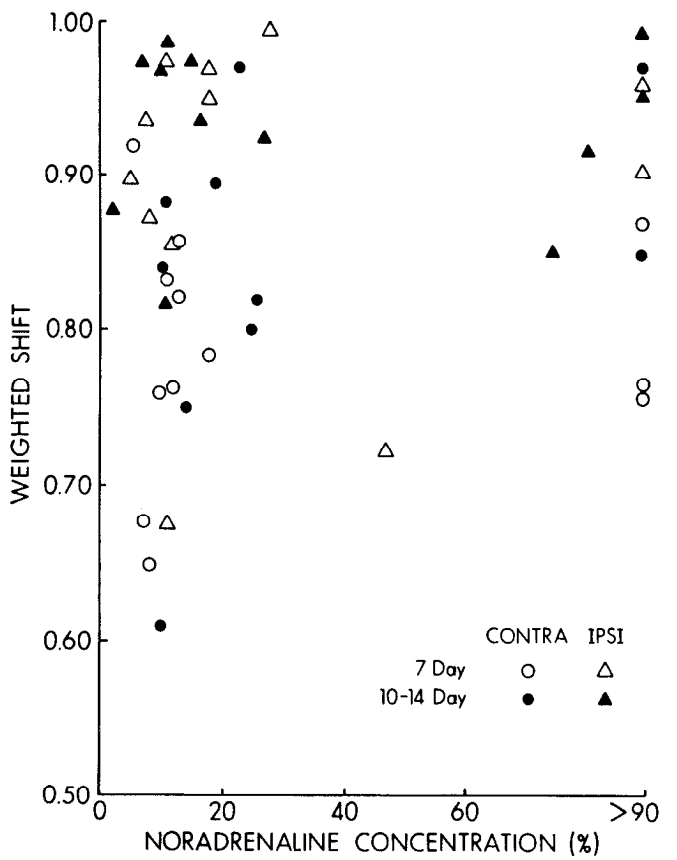

Figure 10. Weighted shift versus NA concentration in the visual cortex for 46 cortices from 44 animals. *Open symbols* designate animals which had exactly 7 days of monocular deprivation; *solid symbols* designate animals which had 10 to 14 days of deprivation. *Circles* designate animals in which the eye contralateral to the recorded cortex was closed; *triangles* designate animals in which the ipsilateral eye was closed. $r_s = +0.12$.

depleted and undepleted animals. Although there were a few NA-depleted animals with a smaller weighted shift and a greater weighted binocularity than in the undepleted animals, this is not statistically significant. Some comparisons of weighted shift using a Mann-Whitney test are: comparing the 9 undepleted cortices (mean age at suture = 40 days, mean duration of suture = 10.4 days) with the 29 cortices that were depleted of NA by at least 80% (mean age at suture = 40 days, mean duration of suture = 8.9 days) yields $z = -0.91$; comparing the same 9 undepleted cortices with the 11 cortices that were depleted of NA by at least 90% (mean age at suture = 39 days, mean duration of suture = 8.5 days) yields $z = -0.87$; comparing the 5 undepleted cortices from animals with contralateral eye sutures (mean age at suture = 41 days, mean duration of suture = 8.6 days) with the 14 cortices from animals with contralateral eye sutures and with at least 80% depletion of NA (mean age at suture = 42 days, mean duration of suture = 8.4 days) yields $z = -0.93$. All values of z are well within the range of values that one would expect by chance in both NA-depleted and undepleted cortices belonged to the same population.

In Figure 12, weighted shift is plotted versus weighed binocularity for each of the 46 recorded cortices. The strong negative correlation between these two measures is readily apparent ($r_s = -0.90$). This indicates that, not surprisingly, these two measures of the effect of monocular deprivation are closely related.

Discussion

These results re-emphasize the point that cortical NA can be depleted by substantial amounts without altering the effects of monocular deprivation. To summarize, NA has now been depleted by neºHatal injections of 6-OHDA (Bear and Daniels, 1983), electrolytic lesions of the dorsal noradrenergic bundle (Daw et al., 1984), 6-OHDA lesions of the dorsal noradrenergic bundle (Daw et al., 1984), 6-OHDA lesions of the locus ceruleus (Adrien et al., 1982), intraventricular injections of 6-OHDA (Adrien et al., 1982; Daw et al., 1985), and systemic injections of DSP-4 and intraventricular injections of DSP-4 as reported in this paper. In all of these cases the depletion of NA was 70 to 90%. The only method of depleting NA that has consistently had an effect on monocular deprivation is injection of 6-OHDA directly into the visual cortex (Kasamatsu et al., 1979; Daw et al., 1983; Paradiso et al., 1983). In some hands, injection of 6-OHDA into the lateral ventricle has reduced the ocular dominance shift by either a large (Kasamatsu and Pettigrew, 1979) or small amount (Allen et al., 1984; Gordon et al., 1984); in other hands, intraventricular 6-OHDA injections have had no significant effect (Adrien et al., 1982; Daw et al., 1985).

A number of suggestions have been made to reconcile these various results. One is that, where NA depletion fails to affect monocular deprivation, there may be some compensation for the NA depletion, such as an increase in the number of β-receptors (Bear and Daniels, 1983). We measured the concentration of β-receptors after DSP-4 injections and found little compensation in animals depleted for either short or long periods of time. We also found the ocular dominance histograms to be shifted substantially in both cases. The apparent lack of β-receptor up-regulation after substantial depletion of cortical NA with DSP-4 was unexpected. Some authors have observed an up-regulation of β-receptors in rat after DSP-4 treatment (Jonsson et al., 1981; Dooley et al., 1983), whereas others have not (Dunwiddie et al., 1983). Any up-regulation

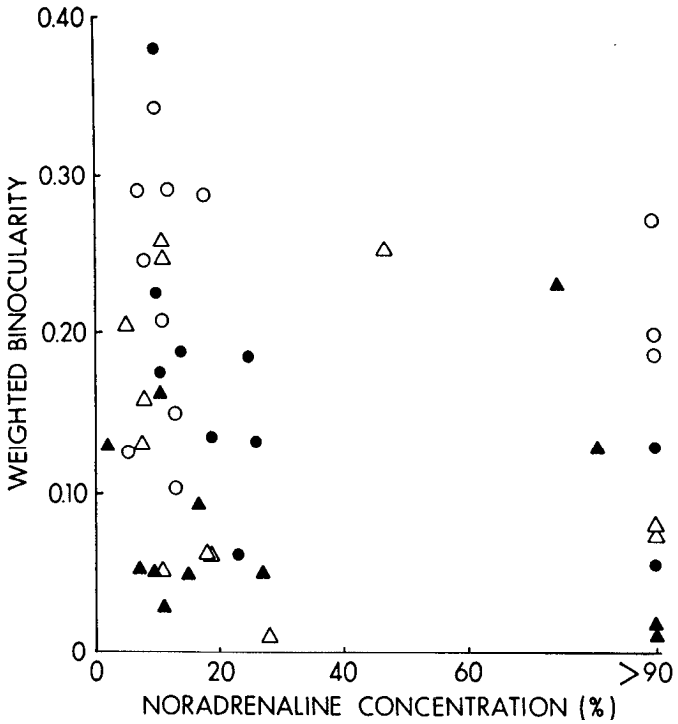

Figure 11. Weighted binocularity versus NA concentration in the visual cortex for 46 cortices from 44 animals. *Symbols* are the same as in Figure 10. $r_s = -0.20$.

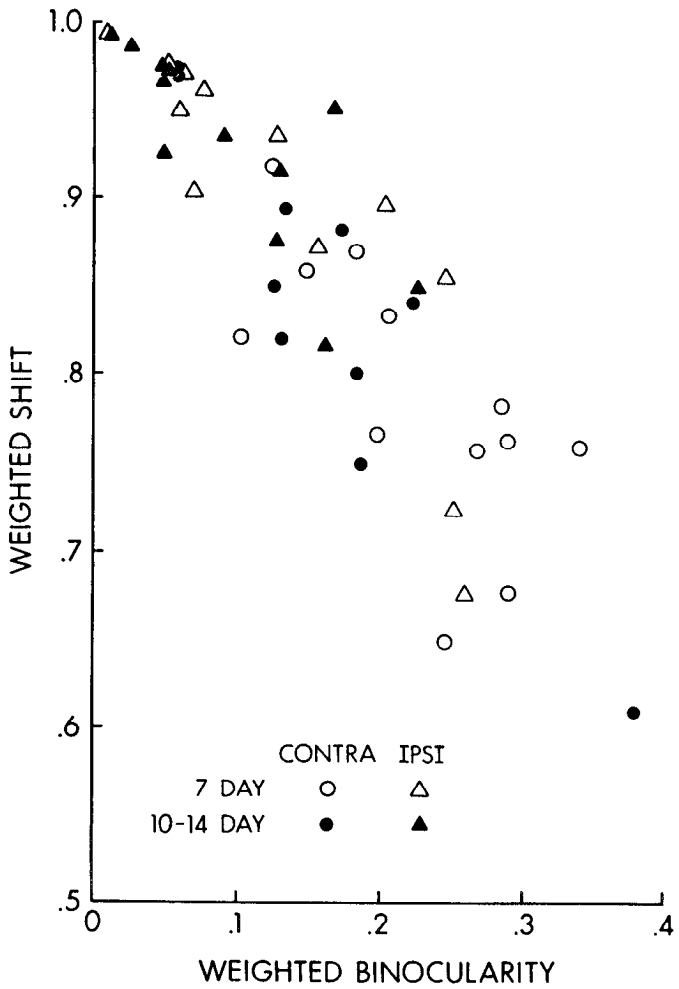

Figure 12. Weighted shift versus weighted binocularity for 46 cortices following monocular deprivation. *Symbols* are the same as in Figure 10. $r_s = -0.90$.

of β-receptors in our animals may have been masked by the significant developmental accumulation of β-receptors in kitten cortex during the period of our experiments (Wilkinson et al., 1983). Moreover, the supersensitivity reaction of maturing cortical neurons may be different from that of adult neurons. Further work is needed to clarify these points. In any case, β-receptor compensation is not an adequate explanation for the lack of an effect of DSP-4 on monocular deprivation. Other types of compensation also seem unlikely as an explanation for our negative findings because we have depleted NA by a variety of methods and have begun eyelid sutures with as short a delay as was done in those experiments in which positive findings have been reported.

Some previous suggestions have already been discarded. One is that the ocular dominance shift fails to occur because of behavioral effects caused by intraventricular injections of 6-OHDA. However, this is unlikely to be a factor in experiments in which 6-OHDA was injected directly into the visual cortex. Also, it is unlikely that the effect of 6-OHDA is due to depletion of dopamine (DA) because either DA is not depleted substantially with injections of 6-OHDA directly into the visual cortex (Paradiso et al., 1983) or else the lateral extent of DA depletion is much narrower than that of NA (Kasamatsu et al., 1981a).

We cannot rule out the idea that NA has to be depleted by close to 100% before there is an effect on monocular deprivation. However, most of the evidence suggests that this is unlikely. Kasamatsu and Pettigrew (1979) reported 50% NA depletions in the original intraventricular administrations of 6-OHDA, although these measurements were done before modern HPLC techniques became available and may be inaccurate. Kasamatsu et al. (1981a) and Paradiso et al. (1983) reported 50 to 90% NA depletion in animals with 6-OHDA injected directly into the visual cortex. However, these values are also hard to interpret because the depletion of NA in these experiments falls off with distance from the tip of the needle inserted into the cortex, because it is hard to get an accurate measurement on the small amounts of tissue involved, and because it is impossible to relate any value obtained to the precise position of the electrode tracks since one cannot do histology and biochemistry on the same tissue. With each new method that we tried we hoped to obtain a larger depletion of NA, but the values have always remained around 70 to 90%. Furthermore, we see no tendency for less pronounced ocular dominance shifts with greater depletion of NA. The possibility, although unlikely, therefore remains open.

At the present time, the hypothesis that seems most likely to reconcile the various results is that experimental methods which affected monocular deprivation involved general damage to the cortex. 6-OHDA has general neurotoxic effects which are not restricted to catecholaminergic neurons (Poirier et al., 1972; Butcher et al., 1975). Injection of 6-OHDA directly into the visual cortex is done by inserting a 26 gauge hypodermic needle, and damage is apparent within a radius of 1.5 mm around the tip of the needle (Kasamatsu et al., 1979; Daw et al., 1983; Paradiso et al., 1983). Electrode penetrations are usually made outside this area, but there could well be damage to the tissue that does not show up with the Nissl stains used to examine the sections. Moreover, unless many electrode penetrations are made, one cannot be certain whether any particular penetration has sampled cells in a region of damaged, NA-depleted, or normal cortex or whether any particular penetration has sampled cells from single or multiple ocular dominance columns. In other experiments Shaw and Cynader (1984) have found that infusion of glutamate directly into the visual cortex prevents the effects of monocular deprivation, whereas we have found that putting penicillin on the surface of the cortex does not (unpublished observations). Both procedures increase the level of activity in the cortex substantially, but the first involves histologically observable

damage to the tissue, due to insertion of a needle into the cortex and possibly due to neurotoxic effects of glutamate (Olney, 1978), whereas the second does not. The "reversal" of the effects of 6-OHDA with local infusions of NA (Pettigrew and Kasamatsu, 1978; Kasamatsu et al., 1979) may have been due to an initial lack of sufficient cortical damage to prevent the ocular dominance shift. In all cases where NA infusions "prevented" the effect of intraventricular 6-OHDA injections, the injections were made in opposite hemispheres. This hypothesis does not explain the suggestion that NA infusions may make adult cortex more plastic (Kasamatsu et al., 1981b), but it may account for results with intracortical injections of 6-OHDA. Whatever hypothesis finally reconciles the various experimental results, it is clear that visual cortical NA can be depleted by a large variety of methods with little or no effect on the course of monocular deprivation. Where the ocular dominance shift has been prevented in monocularly deprived animals, the crucial factor is most likely something other than a reduction in the effectiveness of the noradrenergic system.

References

Adrien, J., P. Buisseret, Y. Frégnac, E. Gary-Bobo, M. Imbert, J. -P. Tassin, and Y. Trotter (1982) Noradrénaline et plasticité du cortex visuel du chaton: Un réexamen. C. R. Acad. Sc. Paris III *295:* 745–750.

Allen, E. E., P. Trombley, J. Soyke, and B. Gordon (1984) Norepinephrine depletion: Relation to visual cortical plasticity. Soc. Neurosci. Abstr. *10:* 1079.

Bear, M. F., and J. D. Daniels (1983) The plastic response to monocular deprivation persists in kitten visual cortex after chronic depletion of norepinephrine. J. Neurosci. *3:* 407–416.

Berman, N., and N. W. Daw (1977) Comparison of the critical periods for monocular and directional deprivation in kittens. J. Physiol. (Lond.) *295:* 249–254.

Butcher, L. C., G. K. Hodge, and J. C. Schaeffer (1975) Degenerative processes after intraventricular injection of 6-hydroxydopamine. In *6-Hydroxydopamine as a Denervation Tool in Catecholamine Research. Chemical Tools in Catecholamine Research*, G. Jonsson, T. Malmfors, and C. Sachs, eds., Vol. 1, pp. 83–90, Elsevier Biomedical Press, New York.

Daw, N. W., and H. J. Wyatt (1976) Kittens reared in a unidirectional environment: Evidence for a critical period. J. Physiol. (Lond.) *257:* 155–170.

Daw, N. W., R. K. Rader, T. W. Robertson, and M. Ariel (1983) Effects of 6-hydroxydopamine on visual deprivation in the kitten striate cortex. J. Neurosci. *3:* 907–914.

Daw, N. W., T. W. Robertson, R. K. Rader, T. O. Videen, and C. J. Coscia (1984) Substantial reduction of cortical norepinephine by lesions of adrenergic pathway does not prevent effects of monocular deprivation. J. Neurosci. *4:* 1354–1360.

Daw, N. W., T. O. Videen, R. K. Rader, T. W. Robertson, and C. J. Coscia (1985) Substantial reduction of noradrenaline in kitten visual cortex by intraventricular injections of 6-hydroxydopamine does not always prevent ocular dominance shifts after monocular deprivation. Exp. Brain Res., in press.

Dooley, D. J., H. Bittiger, K. L. Hauser, S. F. Bischoff, and P. L. Waldmeier (1983) Alteration of central alpha and beta adrenergic receptors in the rat after DSP-4, a selective noradrenergic neurotoxin. Neuroscience *9:* 884–898.

Dunwiddie, T. V., A. L. Mueller, P. C. Bickford, and N. R. Zahniser (1983) Electrophysiological and biochemical response of the destruction of hippocampal noradrenergic afferents by DSP-4. Brain Res. *269:* 311–317.

Gordon, B., J. Moran, P. Trombley, and J. Sorke (1984) 6-OHDA: Protection against behavioral effects of monocular deprivation. Soc. Neurosci. Abstr. *10:* 1079.

Hubel, D. H. (1957) Tungsten microelectrode for recording from single units. Science *125:* 549–550.

Hubel, D. H., and T. N. Wiesel (1962) Binocular interaction and functional architecture in the cat's visual cortex. J. Physiol (Lond.) *160:* 106–154.

Hubel, D. H., and T. N. Wiesel (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J. Physiol. (Lond.) *206:* 419–436.

Jaim-Etcheverry, G., and L. M. Zieher (1980) DSP-4: A novel compound with neurotoxic effects on noradrenergic neurons of adult and developing rats. Brain Res. *188:* 513–523.

Jonsson, G. (1980) Chemical neurotoxins as denervation tools in neurobiology. Annu. Rev. Neurosci. *3:* 169–187.

Jonsson, G., and T. Kasamatsu (1983) Maturation of monoamine neurotransmitters and receptors in cat occipital cortex during postnatal critical period. Exp. Brain Res. *50:* 449–458.

Jonsson, G., H. Hallman, F. Ponzio, and S. B. Ross (1981) DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine)—a useful denervation tool for central and peripheral noradrenaline neurons. Eur. J. Pharmacol. *72:* 173–188.

Jonsson, G., H. Hallman, and E. Sundstrom (1982) Effects of the noradrenaline neurotoxin DSP-4 on the postnatal development of central noradrenaline neurons in the rat. Neuroscience *7:* 2895–2907.

Kasamatsu, T., and J. D. Pettigrew (1976) Depletion of brain catecholamines: Failure of ocular dominance shift after monocular occlusion in kittens. Science *194:* 206–208.

Kasamatsu, T., and J. D. Pettigrew (1979) Preservation of binocularity after monocular deprivation in the striate cortex of kittens treated with 6-OHDA. J. Comp. Neurol. *185:* 139–162.

Kasamatsu, T., J. D. Pettigrew, and M. Ary (1979) Restoration of visual cortical plasticity by local microperfusion of norepinephrine. J. Comp. Neurol. *185:* 163–182.

Kasamatsu, T., T. Itakura, and G. Jonsson (1981a) Intracortical spread of exogenous catecholamines: Effective concentration for modifying cortical plasticity. J. Pharmacol. Exp. Ther. *217:* 841–850.

Kasamatsu, T., J. D. Pettigrew, and M. Ary (1981b) Cortical recovery from effects of monocular deprivation: Acceleration with norepinephrine and suppression with 6-hydroxydopamine. J. Neurophysiol. *45:* 254–266.

Kissinger, P. T., C. Refshauge, F. Dreiling, and R. N. Adams (1973) An electrochemical detector for liquid chromatography with picogram sensitivity. Anal. Lett. *6:* 465–477.

Movshon, J. A., and R. C. van Sluyters (1981) Visual neural development. Annu. Rev. Psychol. *32:* 477–522.

Olney, J. W. (1978) Neurotoxicity of excitatory amino acids. In *Kainic Acid as a Tool in Neurobiology*, E. G. McGeer, J. W. Olney, and P. L. McGeer, eds., pp. 95–121, Raven Press, New York.

Olson, C. R., and R. D. Freeman (1980) Profile of the sensitive period for monocular deprivation in kittens. Exp. Brain Res. *39:* 17–21.

Paradiso, M. A., M. F. Bear, and J. D. Daniels (1983) Effects of intracortical infusion of 6-hydroxydopamine on the response of kitten visual cortex to monocular deprivation. Exp. Brain Res. *51:* 413–422.

Pettigrew, J. D., and T. Kasamatsu (1978) Local perfusion of noradrenaline maintains visual cortical plasticity. Nature *271:* 761–763.

Poirier, L. J., P. Langelier, A. Roberge, R. Boucher, and A. Kitsikis (1972) Non-specific histopathological changes induced by the intracerebral injection of 6-hydroxydopamine (6-OHDA). J. Neurol. Sci. *16:* 410–416.

Ross, S. B. (1976) Long term effects of *N*-2-chloroethyl-*N*-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurons in the rat brain and heart. Br. J. Pharmacol. *58:* 521–527.

Scatchard, G. (1949) The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci. *51:* 660–672.

Shaw, C., and M. Cynader (1984) Disruption of cortical activity prevents ocular dominance changes in monocularly deprived kittens. Nature *308:* 731–734.

Sherman, S. M., and P. D. Spear (1982) Organization of the visual pathways in normal and visually deprived cats. Physiol. Rev. *62:* 738–855.

Spyraki, C., and H. C. Fibiger (1982) Clonidine induced sedation in rats: Evidence for mediation by postsynaptic α_2 adrenoreceptors. J. Neurol Transm. *54:* 153–163.

Wiesel, T. N., and D. H. Hubel (1963) Single cell responses in striate cortex of kittens deprived of vision in one eye. J. Neurophysiol. *26:* 1503–1517.

Wilkinson, M., C. Shaw, I. Khan, and M. Cynader (1983) Ontogenesis of β-adrenergic binding sites in kitten visual cortex and the effects of visual deprivation. Dev. Brain Res. *7:* 349–352.

Zieher, L. M., and G. Jaim-Etcheverry (1980) Neurotoxicity of *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4) on noradrenergic neurons is mimicked by its cyclic azidirinium derivative. Eur. J. Pharmacol. *65:* 249–256.