Two methods of catecholamine depletion in kitten visual cortex yield different effects on plasticity

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As first clearly demonstrated by the experiments of Wiesel and Hubel[1], the developing visual cortex is exquisitely sensitive to sensory deprivation. Temporary closure of one eye of a kitten during a critical period that extends from 3 weeks to 3 months of age results in a dramatic cortical reorganization such that most neurones, originally binocularly driven, are dominated exclusively by the open eye. Recently, attention has been directed to chemical factors which may influence the degree of plasticity during the critical period. The work of Kasamatsu and Pettigrew[2–6] suggests that cortical catecholamines, especially noradrenaline (NA), are essential for the normal plastic response to visual deprivation. In an effort to clarify the role of NA in visual cortical plasticity, we have monocularly deprived kittens whose cortex had been depleted of catecholamines by the neurotoxin 6-hydroxydopamine (6-OHDA)[7–9]. We used two strategies to deplete cortical NA: the first, pioneered by Kasamatsu *et al.*[5], utilized osmotic minipumps to deliver 6-OHDA to visual cortex; the second involved systemic neonatal injections of 6-OHDA, a technique which has proved effective in rodents[10–12]. We found, using high-pressure liquid chromatography (HPLC), that both techniques produced a substantial reduction in the level of cortical NA. However, single unit recording in area 17 revealed that the plastic response to monocular deprivation (MD) was only diminished in the kittens depleted by minipump.

In the first group of kittens ($N = 10$) we followed the 6-OHDA delivery procedures of Kasamatsu *et al.*[5,6]. Animals normally reared to 4–8 weeks of age were fitted with two osmotic minipumps (Alza) connected to cannulae inserted in each hemisphere, 0.5–2.5 mm below the cortical surface, near the areae centrales representations in area 17. One pump contained 4 mM 6-OHDA in vehicle (0.4% ascorbate in saline) and the other contained vehicle alone. Seven of these kittens were used for physiological analysis. In this group of kittens, one hemisphere served as control for the other. Each animal in a second group of kittens ($N = 7$) was given two intraperitoneal injections of 6-OHDA within 48 hours after birth (doses appear in Table 2). Five animals in this group were used for neurophysiological analysis. Littermates received neonatal injections of vehicle and served as a control group.

Minipump-fitted kittens received 7 days of monocular deprivation concurrently with their drug delivery, starting between 4 and 8 weeks of age (Table 1). In the neonatal series, four 6-OHDA treated kittens and four littermate controls received 10 days of monocular deprivation between 4 and 6 weeks of age. In addition, one 6-OHDA treated kitten and its littermate control were deprived for over 1 month (Table 2). The physiological recording procedure[13,14] was identical for all kittens studied. Receptive fields of visually responsive cells in area 17 were plotted and ocular dominance discriminations were made according to the criteria of Hubel and Wiesel[15]. In the minipump kittens, recording sites were 0.5–2 mm anterior to the cannulae sites. Our recording techniques were similar to those of Kasamatsu and Pettigrew except that we recorded from both hemispheres simultaneously (two electrodes mounted in the same microdrive advance) and we recorded blind, without knowledge of the animal's drug history. The codes were broken after single unit recording.

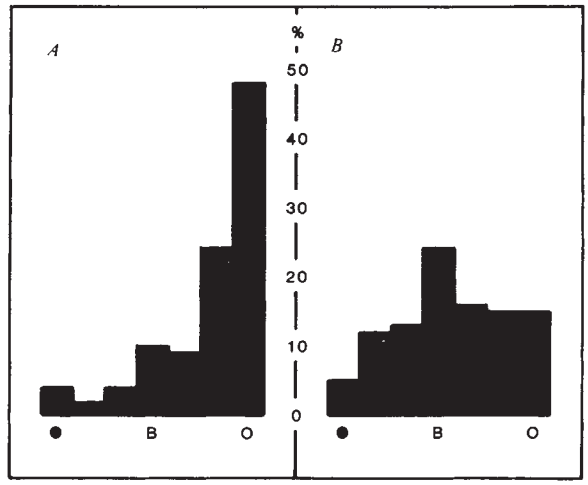

Fig. 1 Composite ocular dominance histograms of six kittens in the minipump 6-OHDA series. 6-OHDA was delivered by osmotic minipump to visual cortex of one hemisphere for 1 week while the animal was monocularly derived. The opposite hemisphere received vehicle solution. *A*, The percentage of cells found in each of the seven ocular dominance groups in the control hemispheres. The open circle is under the monocular open eye group, the filled circle is under the monocular closed eye group, and B labels the strictly binocular group (group 4). As expected, a substantial shift of neurones to the open eye is observed. *B*, The data obtained from the drug-treated hemispheres. In contrast to the controls, most cells in these hemispheres were binocularly activated and the ocular dominance shift was slight.

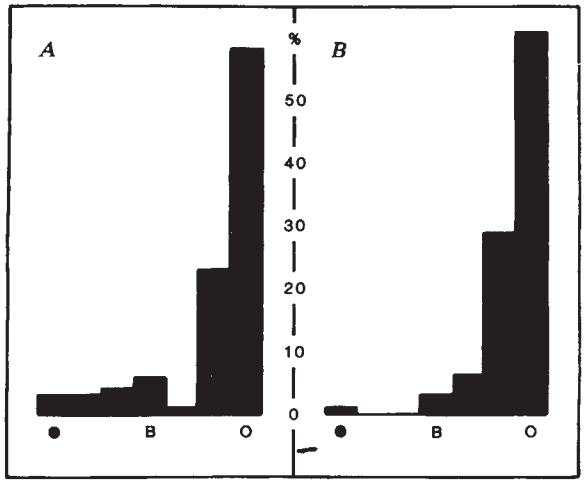

Fig. 2 Ocular dominance histograms from the neonatal 6-OHDA series. In this series, kittens were injected intraperitoneally as neonates with 6-OHDA. Littermates served as vehicle injected controls. *A*, The composite histogram from the control kittens. These animals displayed the expected shift to the open eye. *B*, Data obtained from drug-treated kittens. Unlike the 6-OHDA-treated hemispheres of the minipump series, these kittens displayed a complete ocular dominance shift, virtually indistinguishable from control. All conventions are as in Fig. 1.

Following each recording session, samples of cortical tissue from near the electrode sites were removed and frozen overnight at −30 °C. In the minipump kittens each sample was a 5 mm length of the postlateral gyrus measured posterior to the site of drug infusion (mean wet weight 102 mg). In the systemically treated neonates, the samples were larger (mean wet weight 1.46 g) and included all of cortical areas 17 and 18. Tissue catecholamines were purified by a batch aluminium oxide chromatography technique based on a column procedure described by Anton and Sayre[16]. Dihydroxybenzylamine (DBA) was added as a standard to monitor recovery. Aliquots

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of alumina purified samples were applied to an HPLC column (Bioanalytical Systems) and the separated catechols (approximate retention times: NA, 7 min; DBA, 14 min; dopamine (DA), 24 min) were measured by an electrochemical detector[17].

The ocular dominance results for the six minipump-fitted kittens that were successfully infused with 6-OHDA are shown in Table 1 and Fig. 1. Neurones from the control hemispheres overwhelmingly preferred stimulation through the open eye. However, in the 6-OHDA treated hemispheres we found many more binocular neurones and the ocular dominance shift was greatly attenuated. The biochemical analysis confirmed the effectiveness of the drug treatment: 6-OHDA treated tissue contained an average of only 21% the NA of controls (Table 1 and Fig. 3*A*). We conclude that the minipump delivery of 6-OHDA, introduced by Kasamatsu and Pettigrew, indeed depletes cortical NA and diminishes the plastic response to monocular deprivation.

Figure 2 illustrates pooled ocular dominance results for kittens injected with 6-OHDA at birth compared with vehicle-injected littermates. Data from individual kittens are shown in Table 2. Again, the control kittens displayed large ocular dominance shifts to the open eye. However, the drug-treated animals also showed large ocular dominance shifts, despite having a mean cortical NA content of less than 12% of control (Table 2 and Fig. 3*B*). In fact, one kitten (K119) with 10 days of monocular deprivation displayed a complete ocular dominance shift with less than 1% of control cortical NA (Table 2). We conclude that in the case of animals treated neonatally with 6-OHDA, a large depletion of neocortical NA is not a sufficient condition for reducing plasticity.

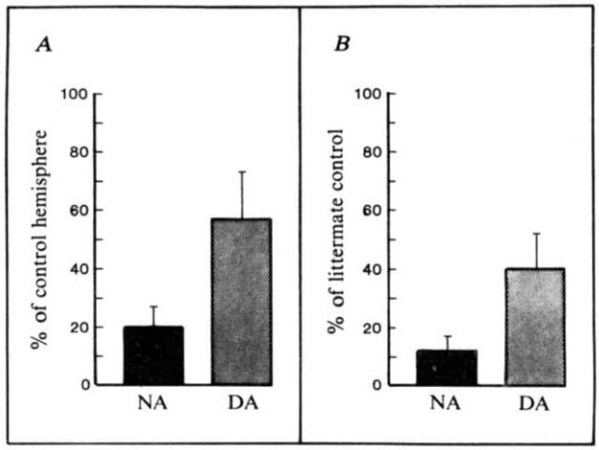

Fig. 3 HPLC confirmation of cortical catecholamine depletion by both the minipump delivery and neonatal injection of 6-OHDA. *A*, NA in the 6-OHDA treated tissue of the minipump series was only 21% of the same animal control hemispheres. DA levels were less affected. *B*, The catecholamine depletion in the 6-OHDA treated kittens of the neonatal injection series, relative to littermate controls. The mean cortical NA level of these animals was less than 12% of control.

Thus although both intracortical and neonatal 6-OHDA delivery techniques effectively destroy the ascending noradrenergic innervation of cortex, only the intracortical procedure results in a loss of plasticity. One possible explanation

Table 1 Minipump 6-OHDA series

Physiology

Kitten	Age at deprivation (days)	Age at recording (days)	Hemisphere	Drug	No. of units	Binocularity
K134	44	51	Left	6-OHDA	13	0.69
			Right	Vehicle	18	0.56
K135	51	58	Left	6-OHDA	20	0.65
			Right	Vehicle	28	0.36
K136	58	65	Left	Vehicle	22	0.32
			Right	6-OHDA	25	0.68
K137	71	79	Left	6-OHDA	28	0.86
			Right	Vehicle	16	0.69
K138	31	38	Left	6-OHDA	27	0.78
			Right	6-OHDA	27	0.70
K140	45	52	Left	Vehicle	30	0.70
			Right	6-OHDA	32	*
K143	40	47	Left	Vehicle	21	0.62
			Right	6-OHDA	13	0.92

Biochemistry†

Control hemispheres

Kitten	K134	K135	K136	K137	K143	K140	$\bar{x}$ ± s.e.m.
Cortical NA (ng per g)	135	406	710	189	1169	303	485 ± 160
Cortical DA (ng per g)	110	163	71	228	325	118	169 ± 38

6-OHDA hemispheres

Kitten	K134	K135	K136	K137	K143	K138L	K138R	$\bar{x}$ ± s.e.m.
Cortical NA (ng per g)	17	207	87	71	261	28	48	103 ± 36
Cortical DA (ng per g)	63	109	66	93	236	35	46	93 ± 26
NA (% of control hemisphere)	13	51	12	36	22	6‡	10‡	21 ± 6

Physiological and biochemical data from kittens which had received minipump infusion of 6-OHDA for 1 week concurrently with monocular deprivation. In all cases the left eye was sutured closed. Also indicated are the number of units isolated from each hemisphere and the binocularity. Binocularity is defined as the number of cells in ocular dominance groups 2–6 divided by the total number of cells recorded. The binocularities of 6-OHDA and control hemispheres were different at the $P < 0.0025$ confidence level. The 6-OHDA treated hemispheres had a mean of 21% of the NA level in the same animal control hemispheres (Fig. 3*A*).

* The right hemisphere in K140 was not successfully depleted of catecholamines because of an obstruction in the pump tubing. This animal is not included in the ocular dominance histogram.

† The variations in catecholamine levels for the minipump-treated animals is possibly a result of the small size of the cortical samples (mean wet weight 102 mg). However, for each animal the 6-OHDA treated hemisphere always had an NA level of 50% or less of the control hemisphere level, a difference that is significant at the $P < 0.05$ confidence level.

‡ Animal K138 received 6-OHDA in both hemispheres and its %NE was calculated relative to the mean NA level for all control hemispheres.

Table 2 Neonatal 6-OHDA series

Physiology

Kitten	6-OHDA dose (mg per kg)	Age at deprivation (days)	Age at recording (days)	Hemisphere	No. of units	Binocularity
K115	2×100	32	42	Left	0	—
				Right	37	0.46*
K116	Control	34	44	Left	12	0.50
				Right	29	0.21
K119	2×200	26	36	Left	8	0.38
				Right	15	0.20
K122	Control	35	45	Left	15	0.73
				Right	30	0.40
K127	2×200	7	54	Left	29	0.38
				Right	18	0.17
K129	Control	7	57	Left	19	0.21
				Right	20	0.30
K130	Control	39	49	Left	24	0.46
				Right	14	0.29
K131	2×200	26	36	Left	40	0.50
				Right	0	—
K132	Control	28	38	Left	18	0.44
				Right	25	0.68
K133	2×200	30	40	Left	6	—†
				Right	6	—†

Biochemistry

Control animals								
Kitten	K114	K116	K120	K122	K129	K130	K132	$\bar{x}$ ± s.e.m.
Cortical NA (ng per g)	138	77	111	192	81	80	58	105 ± 17
Cortical DA (ng per g)	60	30	78	59	21	19	19	41 ± 9
6-OHDA animals								
Kitten	K119	K123	K127	K128	K131	K133	$\bar{x}$ ± s.e.m.	
Cortical NA (ng per g)	<1	<17	<32	<11	4	7	<12 ± 5	
Cortical DA (ng per g)	28	17	16	<2	41	22	<21 ± 5	
NA (% of control mean)	<1	<16	<20	<10	4	7	<12 ± 4	

Physiological and biochemical data from kittens injected as neonates with 6-OHDA and their littermate controls. In all cases, the left eye was sutured closed. Binocularity of 6-OHDA treated and control kittens was similar regardless of the hemisphere studied. The mean NA content of 6-OHDA treated kittens is less than 12% of control (Fig. 3*B*). Conventions are the same as in Table 1.

* Ocular dominance data not included in Fig. 2 because biochemical determination of cortical catecholamines was not made in this kitten.

† Ocular dominance data not included in Fig. 2 because of a corneal lesion on the deprived eye.

is that intracortical infusion of 6-OHDA has a direct effect on plasticity which is independent of the NA depletion. Two lines of evidence argue against this possibility. First, 6-OHDA treatment prevents the ocular dominance shift even when the treatment is stopped 1 day before the monocular deprivation begins[4]. Second, Kasamatsu *et al.*[5] report that plasticity may be restored in 6-OHDA treated tissue by providing exogenous NA. It therefore seems that the conflicting results, from the minipump and neonatal kittens, must stem from differences in the timing and (or) duration of NA depletion produced by the two procedures. In the neonatal series, the cortical NA depletion was initiated during the first postnatal week and lasted for at least 1 month before physiological recording. In the minipump 6-OHDA series, the NA depletion was initiated at ages in excess of 1 month and lasted only for 7 days while the kitten was monocularly deprived. More experiments are required to pinpoint the critical difference. Nonetheless, we believe that the explanation which best fits the available data is, first, that a critical level of NA is normally required for the plastic response to MD and second, that mechanisms exist to compensate for the chronic loss of cortical NA in kittens treated with 6-OHDA at birth.

One compensatory mechanism for chronic denervation is receptor supersensitivity[18]. However, while it is known that cortical beta receptors proliferate in response to locus coeruleus lesions[19], the evidence suggests that this response alone may not be adequate to compensate for a 90% loss of NA[20]. Recently, Harik *et al.*[20] have documented a compensation phenomenon, independent of receptor supersensitivity, that occurs 4 weeks after locus coeruleus lesions in adult rats. The mechanism for this recovery of function remains obscure. Whatever the mechanism, the idea of cortical compensation for chronic depletion of NA offers an explanation that is consistent with available data. This idea predicts that deficits in cortex stemming from early destruction of the noradrenergic innervation would only be transient.

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