NORADRENALINE AND FUNCTIONAL PLASTICITY IN KITTEN VISUAL CORTEX: A RE-EXAMINATION

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SUMMARY

1. A quantitative re-examination was made of the influence of noradrenergic depletion on the epigenesis of kitten visual cortex. Two methods were used to deplete noradrenaline at the cortical level: (1) stereotaxically controlled injection of 6-hydroxydopamine (6-OHDA) in the coeruleus complex, from which the noradrenergic input to visual cortex arises; (2) intraventricular injection of 6-OHDA. The latter chemical lesion also depleted dopamine levels in the brain.

2. Lesion of the noradrenergic or catecholaminergic systems was performed neonatally or at an age of 3-4 weeks in kittens submitted to five different rearing procedures: normal rearing, dark rearing, monocular rearing, monocular exposure following dark rearing and monocular deprivation following normal rearing.

3. Forty-two kittens between 3 and 12 weeks of age were used for this biochemical and electrophysiological study. Noradrenaline and dopamine levels were measured by a radioenzymatic method in the primary visual cortex of twenty-six kittens. A total of 1263 cells were recorded in area 17 of twenty-six kittens. Combined biochemical and electrophysiological data were obtained in ten 6-OHDA-lesioned kittens.

4. Whatever the mode of chemical lesion used, cortical noradrenergic depletion failed to block either maturation or vision-dependent processes which are known to affect orientation selectivity and/or ocular dominance during the critical period. However, in some cases, the amplitude of the epigenetic functional modifications was slightly reduced in 6-OHDA-treated kittens.

5. The cortical effects of monocular deprivation starting from the age of 5 weeks were studied quantitatively both in lesioned and intact kittens. Disappearance of noradrenaline in area 17 did not prevent the loss of binocularity in cortical cells. However, even when monocular occlusion had been maintained for 2 or 3 weeks in 6-OHDA-treated kittens, ocular dominance shifts were limited to a stage equivalent to that observed in the intact kitten after 5-8 days of monocular occlusion.

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6. The amplitude of this partial protective effect was found to be unrelated either to the delay following the chemical lesion, or to the level of noradrenaline remaining in lesioned kitten cortex.

7. Although a putative gating role of noradrenaline cannot be excluded in the development of the intact animal, this report shows that its presence is not required for functional plasticity to occur in kitten area 17.

INTRODUCTION

Noradrenergic ascending projections have for a long time fascinated theoreticians of brain mechanisms (Crow, 1968; Kety, 1970; Jouvet, 1974; Gilbert, 1975) by their rather precise origin and the diffuse pattern of their terminals throughout the neocortical mantle (Nakamura, 1977). Most cell bodies are grouped in a restricted region of the brain stem formed by the locus coeruleus and the subcoeruleus complex (Maeda, Pin, Salvert, Ligier & Jouvet, 1973; Moore & Bloom, 1979). Along its course, one coeruleo-cortical axon forms several thousand monoamine-containing boutons, only a limited fraction of which appear to be junctional to surrounding neuronal elements (Descarrries, 1974; Morrisson, Grzanna, Molliver & Coyle, 1978; Itakura, Kasamatsu & Pettigrew, 1981; review in Foote, Bloom & Aston-Jones, 1983). These characteristics constitute the ideal morphology sought for a global rewarding or gating system, which could act as a modulator of the transmission of more specific information, in particular within the neocortex.

Catecholaminergic endings are already present in 6–8-week-old kittens in regions as distal as the occipital cortex and are mainly confined to the supragranular layers (Itakura et al. 1981). Retrograde horseradish peroxidase labelling techniques confirm that areas 17, 18 and 19 of the adult cat receive afferents from locus coeruleus (Türk, Leventhal & Stone, 1979). This cortical noradrenergic input could selectively affect the signal/noise ratio in the processing of sensory information as described at the lateral geniculate level (Nakai & Takaori, 1974; Rogawski & Aghajanian, 1980). Such a filtering action might be critically dependent on the arousal state of the behaving animal (Livingstone & Hubel, 1981).

A more fundamental role of noradrenaline in cortical function comes from the proposal that the endogenous level of noradrenaline could locally gate epigenetic modifications of visual cortical neurones in response to visual manipulation (review in Kasamatsu, 1983). An impressive series of studies gives strong experimental support for the implication of catecholamines during a critical post-natal period in modulating the functional cortical effects of monocular deprivation (Kasamatsu & Pettigrew, 1976, 1979; Kasamatsu, Pettigrew & Ary, 1979, 1981a; Kasamatsu, Itakura & Jonsson, 1981b). Closure of one eye in 4–6-week-old normally reared kittens, even for a few hours, produces a disruption of binocular integration, with most neurones responding only through the remaining eye as the ultimate result of the deprivation (Hubel & Wiesel, 1970; Movshon & Dürsteler, 1977; Olson & Freeman, 1980). Kasamatsu and collaborators showed that this effect of environmental manipulation could be completely prevented if the kitten was submitted to one of two types of chemical lesions shortly before the start of the visual deprivation
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period: (1) intraventricular injection of 6-hydroxydopamine (6-ODHA) (up to 12 mg in Kasamatsu & Pettigrew, 1979), (2) intracortical perfusion of the same neurotoxin at a smaller dose (several hundred micrograms in Kasamatsu et al. 1979).

These authors suggested that both protocols produced the same protective effect because they both depleted the endogenous cortical catecholamine content to below a certain level. The implication of the intracortical β-adrenoreceptor adenosine cyclic monophosphate (cyclic AMP) system was furthermore suggested by the restoration of cortical sensitivity to monocular deprivation in 6-OHDA pre-treated kittens, following local perfusion of exogenous noradrenaline or cyclic AMP in the cortical tissue itself (Pettigrew & Kasamatsu, 1978; Kasamatsu et al. 1979; Kasamatsu, 1980, 1982).

The starting point of the present study was to know whether this 'gating' effect is found only for monocular deprivation or if it applies to other kinds of sensory manipulation; consequently we explored a possible catecholaminergic control of epigenetic modifications of both ocular dominance and orientation selectivity. The chosen protocols were normal rearing, dark rearing and retarded visual experience during the critical period which are known to affect the orientation selectivity level with quite distinct kinetics (Imbert & Buisseret, 1975; Buisseret & Imbert, 1976; Frégnac, 1979, 1985).

The second aim of this study was to investigate the noradrenergic nature of the observed effects. Intraventricular and intracortical injection of 6-OHDA are known to alter both cortical noradrenergic and dopaminergic contents. Lesion of the coeruleus complex itself, although technically more difficult to achieve, would produce a more specific and complete cortical noradrenergic depletion. In addition the use of limited amounts of 6-OHDA (48 μg) in the coeruleus complex avoids major drawbacks of the protocols initially designed by Kasamatsu & Pettigrew (1979); large doses of the neurotoxin could severely damage cortical homeostasis and impair general behaviour. In most cases we performed bilateral lesions of the coeruleus complex since ascending fibres of the locus coeruleus project to both sides of the brain (Moore & Bloom, 1979).

An additional parameter taken into account in this study is the delay between the start of the chemical lesion and that of the sensory manipulation, or the post-natal age at which the 6-OHDA injection is performed. Coeruleus complex lesion has different consequences for paradoxical sleep according to the age at which it is made (Adrien, 1978, 1981). This in turn could correspond to different levels of spontaneous activity in the geniculocortical pathway; consequently, we compared the effects of these lesions on cortical plasticity when performed neonatally and in kittens older than 3 weeks of age.

Our results show that coeruleus complex lesions appeared ineffective in protecting cortical neurones from the epigenetic effects of visual experience, although they produced significant cortical noradrenergic depletion. Since the selective lesion of the ascending noradrenergic pathway did not block the effects of monocular deprivation, we reproduced some of the original protocols of Kasamatsu & Pettigrew (1976, 1979), using intraventricular injection of 6-OHDA. The apparent contradiction between our data and the original findings of Kasamatsu & Pettigrew, and the very limited effects
produced by lesion of the coeruleus complex, raise some doubt concerning the importance of the integrity of the ascending noradrenergic system during the development of kitten visual cortex.

Preliminary results have been presented (Adrien, Buisseret, Frégnac, Gary-Bobo, Tassin, Trotter & Imbert, 1982a, b; Frégnac, 1982) or reviewed elsewhere (Frégnac & Imbert, 1984).

METHODS

A blind procedure was chosen, dissociating the research teams in charge of the drug treatment (J.A.), of the electrophysiology (P.B., Y.F., E.G.B., M.I., Y.T.) and of the biochemistry (G.B., J.P.T.). Thus, when the electrophysiology was performed on kittens, the previous drug treatment (if any) was unknown at the time of the experiment. In addition two separate teams recorded from one cortex without communication of each other’s results. Samples of cortical tissue were analysed under a code name unknown to the biochemists.

Drug treatment

Coeruleus complex lesions. Bilateral lesions of the coeruleus complex were performed by intracerebral infusion of 6-OHDA (6-hydroxydopamine hydrobromide, Sigma) directly into the lateral pontine tegmentum according to a technique used previously in the rat pup (Lanfumey, Arluison & Adrien, 1981). The younger kittens (1–4 days of age) were anaesthetized with ether and the older kittens (23–33 days) were anaesthetized with pentobarbitone. They were maintained in a stereotaxic apparatus (Kopf) and the needle of a Hamilton syringe was stereotaxically positioned after craniotomy at three different locations on each side of the pontine tegmentum. Eight micrograms of 6-OHDA dissolved in 0.8 μl of saline containing 0.2% of ascorbic acid (pH = 3.5), were slowly infused at each location over 5 min.

The stereotaxic coordinates of the locus coeruleus in both age groups were provided by histological data from the cat colony and the three infusion sites (S1, S2, S3) calculated from the ear bars zero were the following: (1) 1st age group (1–4 days): S1 = (P3.5, L2, V − 4); S2 = (P2.5, L2, V − 3); S3 = (P1.5, L2, V − 2). (2) 2nd age group (23–33 days): S1 = (P3, L2.5, V − 3.5); S2 = (P1.8, L2.7, V − 3); S3 = (P0.6, L2.9, V − 2.5). After completion of the infusions, the scalp was sutured and the kittens were returned to the litter. On the next day they could suckle or eat normally.

Intraventricular injection of 6-OHDA. This procedure was performed according to the original protocols designed by Kasamatsu & Pettigrew (TJ9 and TJ13 in Kasamatsu & Pettigrew, p. 145 and 153, 1979). Under pentobarbitone anaesthesia, seven kittens aged from 26 to 33 days underwent the stereotaxic implantation of a stainless-steel cannula (0.9 mm external diameter, 21 mm long), whose tip was positioned in the lateral ventricle (A11, L2.5, V − 10 from brain surface) to allow subsequent injection of 6-OHDA. The cannula was cemented to the skull and a trocar was inserted to prevent leakage of cerebrospinal fluid. The scalp was then sutured and the kittens were returned to the litter. Two to five days later the 6-OHDA treatment was started: the neurotoxin was infused into the ventricle by means of a Hamilton syringe while the experimenter gently held the kitten in his hands. 6-OHDA was dissolved at a concentration of 16 μg/μl in saline containing 0.2% of ascorbic acid (pH = 3.8), and was infused at doses which were increased daily: 12.5 μl (200 μg) on the first day of treatment, 25 μl on the second day, 50 μl on the third, and 100 μl on the fourth day (16 mg of 6-OHDA). The latter dose was repeated daily for another 4–8 days in order to reach a cumulative dose of 10 mg or above.

The treatment induced severe behavioural side effects, which were scored semi-quantitatively using the following scale: vomiting = 0–2.5, sham rage = 0–5, epileptic seizure = 1–4. The total score for each kitten gave a global estimation of the behavioural side-effects of the neurotoxin.

Rearing conditions and noradrenergic depletion protocols

Forty-four kittens were separated into four experimental groups corresponding to the different drug treatments and the control condition:

- group I: neonatal lesion of the coeruleus complex (seven kittens);
- group II: lesion of the coeruleus complex performed during the critical period (twelve kittens, of which two were used only for biochemical control);
group III: intraventricular injection of 6-OHDA during the critical period (seven kittens); group IV: control group (eighteen intact kittens, of which fourteen were used only for biochemical controls).

The rearing procedure consisted of five types of visual environment: (1) normal rearing (n.r.), (2) dark rearing (d.r.), (3) monocular rearing with eye-suture performed before eye-opening (m.r.), (4) delayed monocular visual experience of 6 h following 6 weeks of dark rearing (m.e.), (5) monocular deprivation with durations ranging from 6 to 25 days following 5 weeks of normal rearing (m.d.). After delayed visual experience (m.e.), the recording session was begun after a return to dark of 12 h (Imbert & Buisseret, 1975). Fig. 1 summarizes the rearing and lesion protocols for the twenty-six kittens in which recordings of visual cortex were carried out.

Fig. 1. Rearing procedures and lesion protocols. For each kitten used for the electrophysiological recordings the timing and duration of visual experience given to each eye are represented by open rectangles. Filled rectangles correspond to dark rearing or periods of unilateral eyelid closure. Lesions of the coeruleus complex are represented by circles (♀: bilateral; ☐: unilateral). The onset (▼) and the duration of the series of daily intraventricular injection of 6-OHDA (dashed line) are indicated below the appropriate rectangles. At the bottom, post-natal age scale is expressed in days. K.P.: Kasamatsu & Pettigrew.

Control electrophysiological recordings in group IV were limited to monocularly reared or deprived kittens, since control data were obtained at the same time by the same experimenters in area 17 of 6-week-old kittens, either normally reared or dark reared (five n.r. kittens and three d.r. kittens in Frégnac, Trotter, Bienenstock, Buisseret, Gary-Bobo & Imbert, 1981) or after delayed monocular exposure of 6 h (five m.e. kittens in Trotter, Frégnac & Buisseret, 1983). For the sake of clarity, since they were used in some statistical tests, relevant data taken from these two latter studies have been included in Table 4.

Recording
Kittens were anaesthetized with an intramuscular injection of Alfatesine (Glaxo: 1-2 ml/kg, i.e. 10-8 mg alfaxone/kg and 3-6 mg alfadolone acetate/kg) and after intravenous cannulation received a continuous perfusion of Alfatesine (0-3 ml/kg . h), and gallamine triiodoethylate (Specia: Flaxedil
Fig. 2. Lesion of the coeruleus complex produced by in situ injection of 6-OHDA. Fluorescence histochemical photomontages of transverse sections from kitten pons around level P4. A, control animal. Fluorescent NA neurones are observed in the locus coeruleus (densely packed cell body zone) and in the subcoeruleus and parabrachialis lateralis nuclei, dispersed in the dorsolateral tegmentum. A low density of fluorescent CA fibres is present over the whole section of the pons (reprinted with permission from Arluison et al. 1980). B, lesioned animal. An 8-week-old kitten, taken from the same breeding colony as the control animal, was injected bilaterally with 6-OHDA in the coeruleus complex area according to protocol II. Fluorescent neurones are no longer observed, except for a single cell body (indicated by a filled arrow) situated in the ventrolateral part of the subcoeruleus nucleus. Calibration bars indicate 1 mm. L.c., locus coeruleus; n.p.b.l., nucleus parabrachialis lateralis; n.t.m., nucleus tractus mesencephalici nervus trigemini; p.c.s., pedunculus cerebellaris superior (brachium conjunctivum); s.c., subcoeruleus nucleus.
Receptive field classification and data tabulation

The electrode was positioned above the skull opening and inclined at various angles relative to the frontal plane thus avoiding sampling in the same slab of ocular dominance (Shatz, Lindström & Wiesel, 1977; Tieman & Tumosa, 1983) or of orientation preference (Singer, 1981). Receptive field analysis was performed at regular intervals (50–100 \( \mu m \)). In monocularly deprived kittens, orientated multi-unit activity was taken into account in a few cases, when no single cell could be isolated over 100 consecutive \( \mu m \). This was done to ensure regular sampling throughout the cortical track. Geniculate fibres were discarded from the analysis.

Three types of cells were classified according to their dynamic and static responses to bars and spots of light: (1) visually unresponsive cells, (2) non-oriented or ‘aspecific’ cells activated equally well by bars and spots of light moving in any direction across the receptive field, (3) orientation-sensitive cells, responding better and more selectively to bars than spots. This latter category includes immature and specific types defined by Imbert & Buissere (1975). The proportion of orientation-sensitive cells was calculated relative to the total number of visual cells (see Tables).

Orientation preference was established using a back projector and directly drawn on the projection screen. In a restricted number of cases (twenty), orientation tuning curves were plotted using an automated exploration of the receptive field to periodically control the good fit between the computed value (Frégnac et al. 1981) and that found with the back projector. Orientation preference distributions were established with a 22.5 deg bin width and Chi-square tests with Yates’ correction were carried out to reject the hypothesis of a uniform polar distribution.

Ocular dominance was assessed on a 5-class scale, ranging from monocular activation through one eye to monocular activation through the other eye via three groups of binocular activation. By convention, class 1 represents monocular activation through the eye contralateral to the recording site in binocularly experienced or deprived kittens (n.r. and d.r.) or monocular activation through the open eye in monocularly experienced kittens (m.r., m.e. and m.d.). From the ocular dominance distributions, two parameters were calculated similarly to those defined by Kasamatsu et al. (1981a). Binocularity (\( B \)) is the proportion of visually responding cells which are driven through both eyes. Weighted binocularity (\( \tilde{B} \)) is given by eqn. (1), assuming that binocular convergence fluctuates linearly between 0 (classes 1 and 5) and 1 (class 3). Capture index from the open eye (\( S \)) is the proportion of all visually responsive cells receiving an excitatory influence from this eye. Weighted shift (\( \tilde{S} \)) is given by eqn. (2) with a weighting factor fluctuating linearly between 0 (closed eye) and 1 (open eye).

\[
\tilde{B} = \frac{(c_3 + (c_2 + c_4)/2)}{c_1 + c_2 + c_3 + c_4 + c_5} \cdot B, \\
\tilde{S} = \frac{(c_1 + \frac{c_2}{2} + \frac{c_4}{2} + \frac{c_4}{2} \cdot c_3)}{c_1 + c_2 + c_3 + c_4 + c_5} \cdot S,
\]

with \( c_i \) being the number of visual cells in the \( i \)th class of the ocular dominance histogram.

Histology

Coeruleus complex lesion. Brain stems in groups I and II were processed according to the Glenner enzymatic technique (Glenner, Burkner & Brown, 1957) in order to visualize the monoamine oxidase activity in the coeruleus complex. Fresh tissue was immediately frozen at \(-20^\circ C\) and sectioned in the frontal plane at 40 \( \mu m \). Alternate sections were processed with the Glenner and the Nissl methods. This histological procedure allowed a precise estimation of the extent of the lesion at the level of the coerulean cell bodies (Lanfumey et al. 1981). However, in order to verify the actual destruction of the noradrenergic neurones in the kitten, fluorescence histochemistry was performed in one lesioned and one control animal (see Biochemistry, last paragraph). A modified Falck–Hillarp method was used, employing a perfusion with glyoxylic acid (Arluison, de la Manche, Adrien, Hamon, Laguzzi & Bourgoin, 1980).

Intraventricular injection of 6-OHDA. Because previous reports described no profound histo-
ological modifications in the locus coeruleus of the cat after such treatment (Bourgoin, Adrien, Laguzzi, Dolphin, Bockaert, Hery & Hamon, 1979; Arluison et al. 1980), the hind brains of kittens in group III were not used for histological analysis. However, careful examination of the forebrain was performed, especially on the side of the cannula: in most cases there was a small enlargement of the ventricle around the tip of the cannula, and necrosis was observed all along its track. This phenomenon was probably due to repeated injections of large doses of 6-OHDA because it did not appear in previous experiments in which, with the same technique of infusion, only small doses of the neurotoxin were used (Arluison et al. 1980).

Biochemistry

Dopamine and noradrenaline levels were measured using a radioenzymatic method initially described by Gauchy, Tassin, Glowinski & Cheramy (1976). Samples taken from the occipital cortex (10-20 mg of fresh tissue) were removed rapidly and immersed in 110 µl of a 0·1 N-perchloric acid, 0·01 N-thioglycollic acid solution to be kept frozen at -20 °C. They were then homogenized by sonication (10 s). After centrifugation (20000 g, 20 min at 4 °C) the catecholamines (CA) contained in the supernatants were isolated by adsorption on alumina microcolumns and eluted with 200 µl of a 0·1 M-acetic acid, 0·005 N-oxalic acid solution. Eluates were then evaporated to dryness and CA were simultaneously transformed into their radioactive methylated derivatives by catechol-O-methyl transferase using 3-H]-S-adenosyl methionine (10·2 Ci/mmol, Amersham) as the methyl donor. Radioactive 3-methoxy-4-hydroxyphenylethylamine and 3-methoxy-4-hydroxyphenyl-ethanolamine were separated by organic extraction and isolated by silica-gel chromatography (Hervé, Blanc, Glowinski & Tassin, 1982). Blank values ranged between 50 and 100 pg, both for noradrenaline (NA) and dopamine (DA), depending on the biochemical series.

In order to reduce intrinsic variability in the estimates of catecholaminergic depletion when comparing CA levels in 6-OHDA-treated and intact kitten cortices, the following precautions were taken. (1) Identical measurement sensitivity and blank values were ensured by comparing experimental assays only with the control ones which had been processed through the same series of biochemical treatments. (2) In addition to the kitten group used for the electrophysiology, we sacrificed thirteen normally reared kittens (included in group IV) under the same anaesthesia conditions coming from four separate litters, at ages varying from 3 to 12 weeks. NA and DA levels were shown to be significantly linearly correlated with age (see Results, first section). Since control and experimental kittens were not always the same age, age correction factors (established from samples taken from kittens of the same litter at 3 and 7 weeks of age) were given by the mean relative increases with age of NA and DA contents. We assumed that these factors would be the same for all the other series of biochemical measurements. According to these conventions, noradrenergic levels in 6-OHDA-treated kitten cortex were expressed in Tables and Fig. 3B as a percentage of control value found in the same biochemical treatment series, after correction for age. Absolute levels of NA and DA are also indicated in the Tables.

Finally, CA levels were measured at 31 days in area 17 of three normally reared kittens of the same litter: one was intact (included in group IV) and the two others had a lesion (either bilateral or unilateral) of the coeruleus complex performed at 23 days of age (included in group II). The delay between the coeruleus complex lesion and the sacrifice date was the same as the shortest interval between the lesion and the onset of monocular occlusion in kittens which were subsequently used for electrophysiology (see Table 2). These biochemical controls were made to assess the level of NA depletion at the start of the monocular deprivation period and compare the relative effects of unilateral and bilateral lesions of the coeruleus complex on area 17 NA content in each hemisphere.

RESULTS

Lesion protocols and noradrenergic depletion

Our control data in n.r. kittens indicated a significant linear dependence of the area 17 CA contents (average value of four samples) on age during the first 10 weeks of post-natal life (NA: \( r = +0.74 \); \( F \) test: \( F = 21.8 \), d.f. = 1,18; DA: \( r = +0.90 \); \( F \) test: \( F = 76 \), d.f. = 1,18). The NA level was 40 ng/g of wet tissue at 6 weeks of age
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Fig. 3. NA levels in normal (A) and lesioned (B) kitten visual cortex. A, dependence of NA and DA levels (ng/g) on age (days) under 8 weeks of age. All kittens belonged to the same litter. Two samples of fresh grey matter were taken from the medial bank of each visual cortex around P0 and P4. Assays taken from a given kitten are represented by identical symbols. A significant increase of NA ($r = +0.77$; $F = 26.6$; d.f. = 1, 18) and DA ($r = +0.94$; $F = 125.4$; d.f. = 1, 18) is found between 3 and 7 weeks of age. The age correction factors are given by the relative slopes of the regression lines which have been plotted both for NA (dashed line) and DA (half-dashed line). B, noradrenergic depletion in kitten visual cortex after injection of 6-OHDA in the right ventricle (▼) or in the coeruleus complex (⊕). The injection onset ranged from 24 up to 35 days. Abscissa: delay from the injection onset expressed in days. Ordinates: NA levels in each sample taken from lesioned animals (whatever the extent of the lesion) given as a percentage of the value in intact control animals of the same age (see Methods, Biochemistry section). The lower levels (25%) obtained for the younger kittens where lesion was complete, i.e. for the shortest delays from the lesion, were found in the range of their associated blank values.

and increased to 80 ng/g 6 weeks later. At a given age, variability between litters appeared larger than between kittens of the same litter and intrinsic variability between the four measurements made in one kitten increased with age. The NA level was systematically 2–3 times higher than the DA level. For kittens less than 8 weeks old, corresponding to the period when most recordings were made, the age correction factor (estimated from animals belonging to the same litter) was found to be around 1 ng/g per day for NA and 0.5 ng/g per day for DA (Fig. 3A).
The injections of microdoses of 6-OHDA at three different locations in the coeruleus complex resulted in the disappearance of monoamine oxidase enzymatic activity revealed by the Glenner reaction. Fig. 2 exemplifies the difference in fluorescence produced by CA neurones in the pons in lesioned and control kittens coming from the same rearing colony. Two observations can be drawn from the comparison at 31 days of age of NA levels in each hemisphere of a bilaterally lesioned, a unilaterally lesioned and an intact kitten from the same normally reared litter. (1) Eight days after the complex coeruleus lesion, the unilateral lesion produced a noradrenergic depletion in the ipsilateral cortex comparable to that observed in each cortex of the bilaterally lesioned kitten. The NA values found were indistinguishable from the blank level (which in this case was as high as 25% of the control value). (2) The NA level in the cortex contralateral to the lesion was comparable to the value found in both cortices of the intact kitten. In summary, a unilateral lesion protocol seems to affect the cortical NA level only on the ipsilateral side; a delay as short as 8 days appears to be sufficient to observe a significant noradrenergic depletion, at least in the visual cortex.

The two modes of lesion of the NA ascending system, 6-OHDA injection in the coeruleus complex or in the right ventricle, were found to be equally effective in producing a cortical NA depletion. The ratio of NA content found in individual samples of visual cortex of lesioned kittens to that found in intact kittens decreased significantly with increasing delay separating the sacrifice from the lesion or the first day of 6-OHDA injection (Fig. 3B). The only experimental point whose level seemed unaffected by the lesion was taken from one cortex of an incommpletely lesioned kitten (m.e.-2). Note that values given in the Tables are the mean calculated from all samples taken in both cortices. This decrease was independent of the mode of lesion chosen to deplete NA. No compensatory noradrenergic reappearance seemed to occur for longer delays (2 or 3 weeks).

The two 6-OHDA treatments also affected the cortical DA content but not in the same way. DA was completely (see kitten K.P.-3 in Fig. 1 and Table 3) or partially depleted in 6-OHDA intraventricularly treated kittens (see Table 3); after coeruleus complex lesion, the DA level showed very large variance around normal values for a delay of 8–10 days, before definitely increasing above normal levels for longer delays (see Table 2).

**Electrophysiological recordings in area 17**

A total of 1263 cells, among which 1136 were visual neurones, were recorded in both hemispheres of twenty-two 6-OHDA-treated kittens and four intact monocularly deprived kittens. 403 cells, among which 348 were visual neurones, recorded at the same time in thirteen intact kittens and which have been published elsewhere (Frégnac et al. 1981; Trotter et al. 1983) constituted an additional control group. Because NA depletion level and coeruleus complex lesion extent might vary between subjects submitted to the same environmental manipulation, individual data are presented in Tables (Tables 1–4) and Figures represent pooled data from kittens submitted to the same lesion and rearing protocols.

**Neonatal lesion of the coeruleus complex.** Both unilateral and bilateral lesions were used. Since, except for one case (n.r.-2), electrophysiological results obtained in
unilaterally lesioned animals were similar for each hemisphere, data were pooled from both cortices. Table 1 and Fig. 4 show clearly that manipulations of visual input during the critical period induce functional changes in lesioned kittens comparable to those observed in control intact kittens (Frégnac et al. 1981; Trotter et al. 1983).

Most cells were orientation sensitive at 6 weeks of age in n.r. lesioned kittens although the proportion of orientated neurones was significantly smaller than found in intact ones (compare n.r.-1 and n.r.-2 in Table 1 and control data from Frégnac et al. 1981 in Table 4). Neurones were mainly binocularly activated. All orientation preferences were equally represented. However, in the unilaterally lesioned n.r.-2 kitten, more orientation-selective cells were found in the contralateral cortex than in the cortex ipsilateral to the lesion, but this difference was not statistically significant. In dark-reared lesioned kittens, most cells were binocularly activated and non-selective to orientation as reported in intact control kittens (Frégnac et al. 1981). In the two lesioned kittens who experienced vision through only one eye (m.r.: m.d.-1 and m.d.-2), most cells were found to be orientation sensitive and strongly dominated by the open eye. The level of orientation selectivity in these 6-OHDA-treated kittens was indistinguishable from that found in control kittens (control data from Trotter et al. 1983 in Table 4).

In conclusion, neonatal lesion of the coeruleus complex does not protect visual cortical neurones from the epigenetic functional changes produced by environmental manipulation during the critical period.

Late lesion of the coeruleus complex. All attempted lesions were bilateral and half of them were complete. Two rearing protocols were used: monocular deprivation (m.d.-4–m.d.-11) and delayed monocular experience of short duration following dark rearing (m.e.). In all cases a clear dominance of the open eye was found, and levels of orientation selectivity were indistinguishable from those observed in control

| Table 1. Neonatal lesion of the coeruleus complex and cortical effects of visual experience |
|---------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Visual experience                          | Normal rearing | Dark rearing   | Monocular rearing | Total         |
| Code name                                  | N.r.-1          | N.r.-2         | D.r.-1          | D.r.-2        | D.r.-3        | M.d.-1         | M.d.-2         |
| No. of cells                               | 36              | 28             | 15              | 30            | 40            | 69             | 97             |
| No. of visual cells                        | 32              | 25             | 10              | 28            | 34            | 69             | 95             |
| Proportion of oriented cells               | 0.55*           | 0.59*          | 0.00            | 0.00          | 0.15         | 0.91           | 0.88           |
| Proportion of binocular cells              | 0.75            | 0.74           | 0.67            | 0.74          | 0.85         | 0.10**         | 0.15**         |
| Extent of the lesion                       | B.              | U.             | U.              | B.            | B.            | B.             | B.             |

Lesion of the coeruleus complex: bilateral (B.), unilateral (U.). Bilateral lesions which were found to be incomplete after histological control (see Methods) are referred as 'partial' (P. on both sides, P_u on one side). Electrophysiological indices which are significantly different from values obtained in control kittens (Table 4) are indicated by one (α = 0.05) or two (α = 0.005) asterisks. NA levels were averaged over four assays in both cortices and expressed as a percentage of the value found in the control kitten used in the same biochemical series, after correction for the age (see Methods). Average absolute values of NA and DA found at 6–7 weeks of age in control kittens were respectively 40 ng/g and 15 ng/g.
Fig. 4. Lesion of the coeruleus complex and cortical effects of visual experience. Ocular dominance histograms on a 5-class scale (1: contralateral; 5: ipsilateral eye; O: open eye; ●: closed eye) are normalized relative to the total number of visual cells. The vertical filled bars indicate the 25% level. Visual cells are classified either non-oriented (open columns) or orientation sensitive (filled columns). Non-visual cells are represented by a shaded column beside each histogram. n: total number of cells. N: number of kittens. Normal histograms represent data from coeruleus complex lesioned kittens (l.c.). Pooled results are given for kittens with a neonatal lesion (group I) followed by normal rearing (n.r., code names: n.r.-1 + n.r.-2), dark rearing (d.r., code names: d.r.-1 + d.r.-2 + d.r.-3), or monocular rearing (m.r., code names: m.d.-1 + m.d.-2). The bottom right histogram refers to group II deprived kittens, monocularly exposed for 6 h after a late lesion of the coeruleus complex (m.e., code names: m.e.-1 + m.e.-2). Inverted histograms represent data from control kittens (C.) submitted to identical rearing procedures (see Table 4).
Table 2. Lesion of the coeruleus complex during the critical period and cortical effects of monocular vision

<table>
<thead>
<tr>
<th>Visual experience</th>
<th>Monocular occlusion following normal rearing</th>
<th>Delayed monocular vision</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code name</td>
<td>M.d.-4   M.d.-5   M.d.-6   M.d.-7   M.d.-8   M.d.-9   M.d.-10   M.d.-11   M. e.-1   M.e.-2   10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delay between lesion and monocular</td>
<td>6         6         7         8         8         14        13         13        7       8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vision (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of monocular vision (days)</td>
<td>7         8         14        17        16        19        16         16        0-25    0-25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cells</td>
<td>36        47        52        58        55        45        43         36        46      50      468</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of visual cells</td>
<td>34        45        51        48        50        41        41         35        37      38      420</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of oriented cells</td>
<td>0.88      0.91      0.90      0.96      0.96      0.83      0.85        0.71      0.60    0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of binocular cells</td>
<td>0.47*     0.42*     0.31      0.14      0.54**    0.24      0.23        0.43*     0.22    0.50*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA (% control)</td>
<td>22        80        —         —         —         03        02         02        75      81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA (ng/g)</td>
<td>9         32        —         —         —         1         1          1         30      33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA (ng/g)</td>
<td>5         38        —         —         —         34        17         28        29      25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extent of the lesion</td>
<td>B.        B.        P_u       P.        B.        P_u       B.          B.        P.      P.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For explanation see Table 1 legend.
kittens (Table 4: group IV for m.d. and data from Trotter et al. 1983 for m.e.). In kittens monocularly exposed for 6 h (m.e.), orientated cells were more monocular (74% in lesioned kittens; 79% in intact kittens) than non-orientated ones (50% in lesioned kittens; 52% in intact kittens). This covariation between the degree of monocularity and that of orientation selectivity is in fact a general feature of the effects of delayed monocular exposure in the intact animal (Cynader, Timney & Mitchell, 1980; Trotter et al. 1983). All orientation preferences were equally represented.

However, several results observed in the 6-OHDA-treated group of monocularly deprived kittens (Table 2: m.d.-4–m.d.-11) depart from our control group (Table 4) and stand in contrast with the well documented effects of monocular vision in intact animals (Blakemore & Pettigrew, 1970; Movshon & Dürsteler, 1977; Van Sluyters, 1978). The proportion of binocular neurones did not decrease significantly with the actual duration of deprivation, although in all cases the eyelid closure began at the same age (5–6 weeks). In addition, comparison of individual histograms between Tables 2 and 4 for similar periods of monocular deprivation indicates that in half of the lesioned kittens the proportion of binocular neurones appeared significantly higher than in intact kittens (see asterisks in Table 2). However, the proportion of binocular neurones was found uncorrelated either with the amplitude of the noradrenergic depletion or the extent of the coeruleus complex lesion. It may be noted that the delay between the lesion and the onset of the monocular deprivation period was not critical since the effects of monocular vision were identical whether its onset was fixed 1 week or 2 weeks after the coeruleus complex lesion. The latter observation fits with the biochemical results described in the second paragraph of Results, first section, which states that a noradrenergic depletion is already achieved at the cortical level 8 days after the lesion.

At this point, a qualitative conclusion can be drawn: neonatal or late lesions of the coeruleus complex do not fully protect visual cortex from the effects of monocular deprivation. However, a higher proportion of binocular neurones was found in some lesioned kittens than in intact animals.

**Intraventricular injection of 6-OHDA.** Two groups of kittens received intraventricular injections of 6-OHDA during the critical period and were later monocularly deprived according to protocols originally designed by Kasamatsu & Pettigrew (1979). When the cumulative doses of 6-OHDA reached a certain level (4–5 mg), behavioural side-effects appeared and generally peaked between the 7th and the 9th day following the injection onset. The most dramatic effects were epileptic seizures, which led to death in two cases. For the remaining kittens electrophysiological results failed to reproduce the complete protection of binocularity observed by Kasamatsu & Pettigrew. Binocularity levels found in our lesioned kittens (group III, Table 3) are below 35% (in contrast to 79% in TJ9 in Kasamatsu & Pettigrew, 1979) and are comparable to those observed in kittens injected only with the vehicle solution (saline and ascorbic acid: 23% in TJ11 and 14% in TJ15D in Kasamatsu & Pettigrew, 1979). These latter kittens were referred to as ‘control’ in Kasamatsu & Pettigrew’s original study. Moreover, in our data, no correlation was found between the proportion of binocular cells and the amplitude of the noradrenergic depletion. One may even note the case of a complete depletion (K.P.-3) where only 9% of neurones remained binocularly activated. No significant correlation was
found either between the degree of binocularity and the amplitude of the behavioural side-effects (see Table 3).

In summary, intraventricular injection of 6-OHDA failed to protect visual cortex from the loss of binocular interaction produced by monocular occlusion and these findings contradict the previous results of Kasamatsu & Pettigrew (Pettigrew & Kasamatsu, 1978; Kasamatsu & Pettigrew, 1979).

**Table 3. Intraventricular injection of 6-OHDA and cortical effects of monocular vision**

<table>
<thead>
<tr>
<th>Original protocol: Kasamatsu &amp; Pettigrew (1979)</th>
<th>TJ13B</th>
<th>TJ9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delay between 1st 6-OHDA injection and monocular vision onset (days)</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Duration of monocular vision (days)</td>
<td>25</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>No. of cells</td>
<td>48</td>
<td>64</td>
<td>34</td>
</tr>
<tr>
<td>No. of visual cells</td>
<td>44</td>
<td>58</td>
<td>33</td>
</tr>
<tr>
<td>Proportion of oriented cells</td>
<td>0.95</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Proportion of binocular cells</td>
<td>0.25</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>NA (% control)</td>
<td>—</td>
<td>—</td>
<td>08</td>
</tr>
<tr>
<td>NA (ng/g)</td>
<td>—</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>DA (ng/g)</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Behavioural side-effects</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

For explanation see Table 1 legend.

**Table 4. Control kittens**

<table>
<thead>
<tr>
<th>Visual experience</th>
<th>Monocular rearing</th>
<th>Monocular occlusion following normal rearing</th>
<th>Total</th>
<th>(+)</th>
<th>(+++)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code name</td>
<td>M.d.-3</td>
<td>M.d.-12 M.d.-13 M.d.-14</td>
<td>4</td>
<td>N.r.</td>
<td>Dr.</td>
</tr>
<tr>
<td>Duration of monocular vision (days)</td>
<td>32</td>
<td>7</td>
<td>17</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>No. of cells</td>
<td>79</td>
<td>67</td>
<td>55</td>
<td>25</td>
<td>226</td>
</tr>
<tr>
<td>No. of visual cells</td>
<td>78</td>
<td>65</td>
<td>51</td>
<td>20</td>
<td>214</td>
</tr>
<tr>
<td>Proportion of oriented cells</td>
<td>0.95</td>
<td>0.95</td>
<td>0.92</td>
<td>0.95</td>
<td>—</td>
</tr>
<tr>
<td>Proportion of binocular cells</td>
<td>0.00</td>
<td>0.27</td>
<td>0.18</td>
<td>0.10</td>
<td>—</td>
</tr>
</tbody>
</table>

(+): from Frégnac et al. (1981).

(+ +): from Trotter et al. (1983).

For explanation see Table 1 legend.

Noradrenergic depletion and the cortical effects of monocular deprivation

From the results presented above it can be concluded that: (1) similar levels of NA depletion at the cortical level could be achieved by coeruleus complex lesion and
intraventricular injection of 6-OHDA; (2) no blockade of plasticity was observed whatever the mode of destruction of the NA system (Fig. 5); (3) the level of binocularity found in coeruleus complex lesioned kittens appeared unrelated to the delay between the lesion and the onset of the monocular occlusion period (Fig. 5).

In order to assess more quantitatively the significance of the differences observed between lesioned and intact kittens, we developed an ‘iso-effect’ analysis. For each kitten two parameters were extracted from the ocular dominance histogram, one
characterizing binocular convergence ($\hat{B}$), and the other the open eye dominance ($\hat{S}$). A standard evolution of these parameters as a function of the occlusion time can be established in the intact kitten, on condition that eyelid closure begins at the same age (in this study, at 5–6 weeks). The decrease in $\hat{B}$ and the increase in $\hat{S}$ shown by the dashed curves in Fig. 6A and B, correspond to the progressive transition from a bell-shaped ocular dominance histogram to the classical L shape (all cells being activated monocularly by the open eye after a few weeks of deprivation). These reference or standard curves are smoothed functions fitting data from group IV and from the literature (Fig. 6). It may be noted from Fig. 6A and B that most experimental points correspond to lower $\hat{B}$ and higher $\hat{S}$ values than would normally be expected.
For each experimental point (lesion of the coeruleus complex or intraventricular injection of 6-OHDA) one additional parameter was extracted to define its distance from the standard curves: the iso-duration time is the theoretical duration of monocular occlusion which, in intact kittens, would correspond to the same electrophysiological index (iso-$\hat{B}$ or iso-$\hat{S}$) as that observed in the lesioned kittens. The calculation of iso-effect duration (iso-$t$) is given in the legend of Fig. 6. If no effect on cortical sensitivity to monocular vision were introduced by the lesion, all experimental points in graphs C and D of Fig. 6 would lie centred on the main bisecting line: on average, iso-time should equal the real duration of monocular deprivation. Apart from one kitten (K.P.-3, Table 3), which was more monocularly activated by the open eye than expected in an intact kitten, all points for both indices $\hat{B}$ and $\hat{S}$ lie on the same side of the iso-effect bisecting line (Fig. 6C and D). Iso-effect durations also appear to be non-significantly correlated with the actual duration of deprivation. One can note that lesion protected more cortical cells from capture by the remaining eye than from breakdown in binocularity. The average imposed time of monocular deprivation was 14.2 days in lesioned kittens; this corresponds to an iso-$\hat{B}$ time of 8.6 days and to an iso-$\hat{S}$ time of 5.2 days of occlusion in intact kittens. This analysis indicates that the effects of monocular deprivation appear to be limited in lesioned animals to the level of those observed in 5–8 day monocularly deprived intact kittens, although the actual deprivation period might be extended over more than 3 weeks. In this sense only can we describe a protective effect induced by NA system lesion on the visual cortex sensitivity to environmental manipulation.

DISCUSSION

This report shows that noradrenergic projections are not essential in the expression of epigenetic modifications of visual cortical receptive fields. In kittens submitted neonatally or at 3 weeks of age to lesion of the coeruleus complex, cortical NA depletion failed to block functional plasticity of cortical neurones. Similar negative results were observed when area 17 depletion was achieved by intraventricular injection of massive doses of 6-OHDA. These findings strongly moderate the importance attributed to NA during cortical development by Kasamatsu & Pettigrew (1976, 1979): these authors proposed that the disappearance of NA at the cortical level would freeze the susceptibility of visual cortical integration to incoming retinal signals during the critical period. The only agreement which remains qualitative is on the limitation of the deleterious cortical effects of monocular deprivation following noradrenergic depletion. Whatever its putative importance in the development of the intact kitten cortex, the role of NA in relation to visual cortical plasticity should no longer appear as ‘unique’ and, as proposed in recent reviews (Sillito, 1983; Frégnac & Imbert, 1984), its gating action must stand open to re-examination.

Cortical biochemical effects produced by 6-OHDA injections

Assessment of the absolute level of CA depletion is somewhat obscured by the difficulty of measuring CA levels in area 17 of kittens which have undergone several hours of anaesthesia and paralysis. We report here intrinsic low levels of NA (40–80 ng/g) in 6–10-week-old intact kittens. DA stands at about half the NA level during development. These values are in agreement with recent reports (Jonsson &
Kasamatsu, 1983; Daw, Robertson, Rader, Videen & Coscia, 1984) using techniques of comparable sensitivity (high pressure liquid chromatography with electrochemical detection). It may be noted from other studies that low levels corresponded to a refinement in sensitivity: for the same team of experimenters, absolute NA levels in n.r. kitten cortex tended to decrease with more recent work (compare Kasamatsu & Pettigrew, 1979; Kasamatsu et al. 1981 b and Jonsson & Kasamatsu, 1983 or compare Paradiso, Bear & Daniels, 1983 and Bear, Paradiso, Schwartz, Nelson, Carnes & Daniels, 1983).

The second observation from our control data is that, in spite of large intrinsic variability between litters, a strong dependency of CA levels on age was observed. A linear increase with age was also found by Jonsson & Kasamatsu (1983) up to 9 weeks of age but these findings contradict data from Daniels and colleagues (Paradiso et al. 1983; Bear & Daniels, 1983; Bear et al. 1983) in whose study intrinsic variance seemed to mask age dependency. In addition the average age-dependent slope is higher in our data (1 ng/g per day) than in Jonsson & Kasamatsu’s study (0·3 ng/g per day).

We have attempted two different types of chemical lesion of the noradrenergic system:

1) injection of more than 10 mg of 6-OHDA in the lateral ventricle produced cortical noradrenergic depletion levels (70–90 %) comparable to those obtained by other authors (Kasamatsu & Pettigrew, 1976, 1979; Daw, Videen, Rader, Robertson & Coscia, 1985; Allen, Trombley, Soyke & Gordon, 1984).

2) Stereotaxic injection of 48 μg 6-OHDA restricted to the coeruleus complex produced cortical NA depletion levels as severe as 98 % which were on average, of the same order as those reported after lesion of the ascending noradrenergic forebrain bundle (Daw et al. 1984). However, injection of the neurotoxin at the cell body level ensured a better chance of destroying the majority of noradrenergic neurones since monoaminergic neurones are known to survive severe axotomy. NA levels were indistinguishable from blank values as early as 8 days after the lesion. Similar findings have been obtained in the rat pup (Lanfumey et al. 1981). Our observation of a correlated increase of DA 3 weeks after coeruleus complex lesion is in agreement with biochemical studies in the rat which suggested that selective NA depletion produced by destruction of the ascending noradrenergic bundle or lesion of the coeruleus complex is later followed by a compensatory DA collateral sprouting (Tassin, Lavielle, Hervé, Blanc, Thierry, Alvarez, Berger & Glowinski, 1979; Harik, 1984).

Cortical electrophysiological effects of visual experience following noradrenergic depletion

Orientation selectivity changes

Our results do not confirm the suggestion that early CA depletion might freeze development of orientation selectivity (Pettigrew, 1978). Uncontrolled factors other than the lesion itself (for instance weight loss) might explain the intermediate level of orientation-sensitive neurones in n.r. kittens after neonatal lesion of the coeruleus complex, since most cells were found to be orientation selective in the m.r. lesioned kitten cortex (see m.d.-1 and m.d.-2 in Table 1).
**Ocular dominance shifts**

*Ocular dominance shift following intraventricular injection of 6-OHDA.* We replicated two original protocols designed by Kasamatsu & Pettigrew (1979), but we were unable to reproduce a complete protection of binocularity following 1 to 2 weeks of monocular deprivation in spite of severe NA depletion. Similar negative findings have been very recently reported by Daw et al. (1985) and Allen et al. (1984), which raise further doubt concerning the efficiency of intraventricular injection of 6-OHDA in producing significant blockade of cortical plasticity.

*Ocular dominance shift following late lesion of the NA ascending projection system.* Injection of 6-OHDA limited to the coeruleus complex area and performed 1 or 2 weeks before closure of one eye did not prevent the loss of binocularity of cortical cells. This conclusion appears in agreement with that reported in studies of the effects of lesion of the ascending NA forebrain bundle (Daw et al. 1984) or of systemic injection of drugs (DSP-4(N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) which silence locus coeruleus activity (Daw, Rader, Robertson & Videen, 1983b). However, the ocular dominance shifts we observed following monocular deprivation appeared to be significantly less pronounced in some coeruleus complex lesioned kittens than in intact ones, which indicates the existence of a very limited protective effect.

*Ocular dominance shifts following neonatal lesion of the NA system.* Visual cortical plasticity survives neonatal destruction of the coeruleus complex although cortical NA depletion found at 6 weeks of age is comparable to that produced by later lesions performed during the cortical period. These results are compatible with a recent study of the cortical effects produced by systemic injection of 6-OHDA before blood–brain barrier formation (Bear et al. 1983; Bear & Daniels, 1983). From both reports, it can be concluded that no complete protection of ocular dominance shift was observed whatever the mode of NA lesion attempted neonatally.

**Re-examination of the ‘NA hypothesis’**

Apart from earlier findings of Kasamatsu & Pettigrew (1976, 1979) concerning 6-OHDA intraventricular injections, most studies producing lesions or disfunction of the NA or CA ascending pathways have failed to describe a protection of loss of binocularity of cortical neurones in response to monocular deprivation (Adrien et al. 1982a, b and this study; Bear & Daniels, 1983; Bear et al. 1983; Daw et al. 1983b, 1984, 1985; Allen et al. 1984).

The observed effects are quite different if 6-OHDA is directly perfused in the cortical tissue. In this situation, blockade of cortical plasticity appears to apply to ocular dominance (Pettigrew & Kasamatsu, 1978; Daw, Rader, Robertson & Ariel, 1983a; Paradiso et al. 1983), to orientation selectivity (Daniels, Ellis, Bianco, Garrett, Nelson & Schwartz, 1981) and to direction selectivity (Daw et al. 1983a).

Several factors have been evoked to explain this experimental controversy such as the timing between the neurotoxin injection and the onset of environmental manipulation (Bear et al. 1983; Bear & Daniels, 1983) or the relative importance of NA depletion produced by each protocol (Kasamatsu, Itakura, Jonsson, Heggelund, Pettigrew, Nakai, Watabe, Kuppermann & Ary, 1985).
Influence of the timing of the 6-OHDA injections

Our results raise doubts as to the importance of the age at which the lesion is performed or of the delay between the lesion and the environmental manipulation. The hypothesis of chronic versus acute differences in the protective virtues of CA depletion was in fact introduced by Bear and colleagues when comparing results obtained in neonatally intraperitoneally injected kittens with those obtained following intracortical 6-OHDA injection (Bear et al. 1983; Bear & Daniels, 1983). In view of the data presented here, the importance of these differences might be ignored if one restricts the comparison between systemic and intraventricular or coeruleus complex injection paradigms.

Influence of the level of NA depletion

The second important claim by proponents of the 'NA hypothesis' is the existence of a correlation between the amplitude of the plasticity blockade and the depletion of NA in visual cortex (Kasamatsu & Pettigrew, 1979; Paradiso et al. 1983). The original observation was that for comparable monocular deprivation periods the proportion of binocular cells was roughly proportional to the quantity of intraventricularly injected 6-OHDA. By extension, although NA levels were not systematically measured, Kasamatsu & Pettigrew proposed that this correlation reflected a control of the cortical effects of visual experience monotonously dependent on the endogenous NA level (Kasamatsu & Pettigrew, 1979). In contrast our own results show no significant correlation between the level of binocularity or the open eye dominance index and the relative noradrenergic depletion in lesioned kittens. An additional negative argument comes from comparison of electrophysiological results from both cortices of kittens in which the coeruleus complex lesions were unilateral or incomplete: although NA levels might differ in each cortex, in all but one case (n.r.-2) electrophysiological properties appeared indistinguishable. We conclude that after coeruleus complex lesion or intraventricular 6-OHDA injection no significant correlation is found between the protection in ocular dominance shift and the NA depletion level in visual cortex.

The only remaining convincing argument in favour of a noradrenergic gating of cortical plasticity is the demonstration of the restoration of plasticity by NA intracortical injection following previous 6-OHDA intracortical treatment (Kasamatsu et al. 1981a) and the preliminary report of blockade of cortical plasticity following injection of propranolol (Kasamatsu, 1983, p. 61). However, blockade of plasticity following 6-OHDA intracortical injection is not a proof in itself: indeed intracortical injections of glutamate (Shaw & Cynader, 1984) or of scopolamine (W. Singer, personal communication) as well as electrical stimulations of the visual cortex (Shaw & Cynader, 1984) are all procedures producing qualitatively similar blockades in the expression of cortical plasticity.

Three hypotheses may be put forward.

(1) A conservative view is to propose that an early sign of breakdown in cortical homeostasis in response to in situ exogenous perturbation is a loss of plasticity: this would occur before a more profound disorganization of cortical connexions, affecting in particular the 'static' receptive field properties (Mountcastle, 1979), takes place;
such impairment of the sole 'dynamic' functioning of the cortical network and of its ability to modify neuronal connexions should be observed mainly in the case of intracortical injection. Non-specific side-effects of this lesion technique should indeed be investigated carefully: data from Bear et al. (1983) suggest that the injection of the vehicle solution itself might produce protective effects. In addition, as pointed out by Daw et al. (1984, 1985) no control study has been made on the non-specific effects of 6-OHDA injection following administration of a NA (and 6-OHDA) uptake blocker.

(2) A more speculative proposal holds as follows: on one hand, all procedures which increase the temporal correlation between the afferent geniculate message and the cortical response would improve the transmission efficiency in the activated synapses (Hebb, 1949): the presence of NA in the cortical tissue might suppress spontaneous activity, facilitate visual response and thus increase signal/noise ratio. However, ionophoretic experimental evidence concerning a selective enhancement of visual information transmission by exogenous NA remains contradictory (Kasamatsu & Heggelund, 1982; Videen, Daw & Rader, 1984). On the other hand, all procedures which decrease correlation between pre- and post-synaptic activities would damp the epigenetic influence of incoming signals on cortical function. This could be the case when post-synaptic activity is driven to an abnormally low level (following anaesthesia, paralysis or intracortical scopolamine injection) or an abnormally high level (following intracortical glutamate or 6-OHDA injection or electrical intracortical stimulations). In this respect it would be crucial to record the chronic effects of 6-OHDA injection on cortical single unit activity.

(3) A more simple hypothesis might be proposed from the observation of a fundamental distinction among procedures used to lesion the NA terminals: in most protocols (systemic, intraventricular or coeruleus complex 6-OHDA lesions) the morphological integrity of the NA neurones is affected throughout the brain; in one case (intracortical 6-OHDA injection) the NA lesion is confined to a remote terminal region (occipital cortex). We propose that all lesion procedures which affect the integrity of coeruleus complex NA neurones put into play the modulatory action of compensatory systems thus masking in lesioned kittens the otherwise predominant gating role of NA during cortical development. In this respect it cannot be excluded that injection of 6-OHDA localized in the visual cortex might be the only situation in which a very limited NA terminal deafferentation does not trigger lesion-induced compensatory phenomena.

The authors are indebted to Michel Arluison for the fluorescence histochemical controls. They thank Michèle Gautier for assistance with the Figures, Pierre Godement for helpful comments and Kirsty Grant for correcting the English. The chemical lesions were performed at Unité Inserm U3 (1), the electrophysiological recordings at the Laboratoire de Neurophysiologie du Collège de France (3) and the biochemistry at Unité Inserm U114 (2). This research was supported by grants from CNRS and DGRST.

REFERENCES


NORADRENALINE AND VISUAL CORTICAL PLASTICITY


NORADRENALINE AND VISUAL CORTICAL PLASTICITY


extraoculaire dans les mécanismes de plasticité fonctionnelle du cortex visuel primaire du chaton. 
