

CHANGES IN THE VISUAL SYSTEM OF MONOCULARLY SUTURED OR ENUCLEATED CATS DEMONSTRABLE WITH CYTOCHROME OXIDASE HISTOCHEMISTRY

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(Accepted November 23rd, 1978)

SUMMARY

Endogenous cytochrome oxidase activity within the mitochondria of neurons and neuropil was demonstrated histochemically under normal and experimental conditions. Since enzymatic changes were noted with chronic neuronal inactivity in the auditory system (Wong-Riley et al)^{38,39}, the present study sought to examine functionally induced enzymatic changes in the visual system of kittens. Eight kittens were used experimentally: 5 had monocular lid suture for varying periods of time; one had binocular lid suture followed by monocular suture followed by binocular opening; two had monocular enucleation. All initial procedures were performed before eye opening. Materials from other normal kittens and cats were also used as controls. At the end of the experiments, the animals were perfused with aldehyde solutions and frozen sections of the brains were incubated for cytochrome oxidase activity (a detailed protocol was outlined). The results indicated that the deprivation caused by monocular suture produced a decrease in the cytochrome oxidase staining of the binocular segment of the deprived geniculate laminae. Enucleation yielded a greater decrease in the cytochrome oxidase activity in the affected geniculate laminae. However, the staining in the 'normal' lamina extended across the interlaminar border to include a row of surviving large cells in the 'denervated' lamina. The staining of the monocular segment appeared not to be affected by lid suture, but was decreased by enucleation. At the cortical level, lamina IV in area 17 of normal cats was stained darkly as a continuous band. Following lid suture, this pattern was replaced in part by alternating columns of light and dark staining, suggestive of ocular dominance columns. Thus, a decrease in neuronal activity due to reduced visual stimulation or destruction of the primary afferent nerves led to a significant decrease in the level of oxidative enzyme activity one to several synapses away.

INTRODUCTION

The understanding of the dynamic properties of neurons can be approached from morphological, physiological and biochemical means. Neurons can be differentiated from one another according to their sizes, the branching patterns of their dendrites and axons, their synaptic characteristics, their modes of function, or according to the types of neurotransmitters they contain. Another important yet little explored property is the system of endogenous enzymes in neurons. This system is intimately associated with the neurons' metabolic machinery, which is in turn closely related to the levels of neuronal activity.

Among the energy-deriving enzymes, the cytochromes are responsible for electron transport and oxidative phosphorylation, yielding ATP (adenosine triphosphate). Since ATP is needed for vital processes such as protein synthesis, maintenance of the resting membrane potential, and rapid axoplasmic transport within neurons^{23, 24}, it can be reasoned that a more 'active' neuron would engage more vigorously in the above processes and, therefore, would have a more developed cytochrome system. It can also be reasoned that different neuronal groups with varied functional demands may exhibit different levels of cytochrome oxidase activity, and that such levels may change when the degree of maintained neuronal activity changes.

When tested histochemically, the levels of activity of cytochrome oxidase were found to indeed differ from one region of the brain to another³⁶. The reaction products were localized along the outer surface of the inner mitochondrial membrane, in agreement with the original findings of Seligman et al.²⁶ in the heart, liver and kidney. The levels were notably higher in many of the brain stem auditory relay nuclei^{36,38,39}, where the local cerebral blood flow and glucose consumption were reported to be greater than adjacent regions²⁵. With chronic disuse, the staining intensity of this enzyme system decreased with reduced levels of neuronal activity^{38,39}.

The visual system has long been known to suffer transneuronal changes following deafferentation or reduced visual input through lid suture^{3,8,9,33}. Geniculate neurons shrink in size with some dissolution of their Nissl substance. Little is known about the changes that might have occurred in the levels of endogenous enzyme activities other than the brief report by Kupfer¹⁷ describing a decrease in succinic dehydrogenase activity during transneuronal atrophy. A subsequent report by Kupfer and Palmer¹⁹, however, failed to observe enzymatic changes following lid suture in kittens.

The present study was undertaken to examine the effect of unilateral enucleation or lid suture on the level of cytochrome oxidase activity in the dorsal lateral geniculate nuclei and visual cortex of kittens. The purpose was to look for histochemical changes and to correlate these changes with various degrees of decreased afferent input.

A preliminary report of this study has been given elsewhere³⁷.

MATERIALS AND METHODS

Eight kittens were used in this study. Kittens 1, 2, 7 and 8 were from the same litter. Materials from other normal kittens and cats were also used as controls. The experimental procedures are shown in Table I.

TABLE I

Experimental procedures performed postnatally (age in days postnatally)

Kitten no.	(Age)	1st expt.	(Age)	2nd expt.	Age at perfusion
				3rd expt.	
1	(5)	Rt. M.D.	—	148	
2	(5)	Lt. M.D.	—	148	
3	(7)	Rt. M.D.	—	147	
4	(41)	Rt. M.D.	(48) B.O.	65	
5	(41)	Lt. M.D.	(48) B.O.	65	
6	(6)	B.D.	(40) Rt. M.D. (114) B.O.	156	
7	(5)	Rt. M.E.	—	145	
8	(5)	Lt. M.E.	—	54	

Abbreviations: M.D., monocular deprivation (lid suture), B.D., binocular deprivation (lid suture), B.O., binocular lid opening, M.E., monocular enucleation.

A detailed protocol for the demonstration of cytochrome oxidase activity in the central nervous system is outlined below, since the method described earlier^{36,39} has been modified. The previous procedure was applicable for the identification of peroxidases, catalases as well as cytochrome oxidase^{22,36}, although the reaction products in the nervous tissues examined resided mainly within the mitochondria, and the former two enzymes contributed little, if any, to the endogenous reaction with diaminobenzidine. When tissues were processed with both the earlier as well as the modified techniques, the results were virtually identical. The present technique, however, required less DAB, utilized cytochrome C as substrate, and eliminated H₂O₂ from the incubation medium, so that the reaction was more specific for the identification of cytochrome oxidase.

1. The animals were perfused with a mixture of paraformaldehyde (2–4%) and glutaraldehyde (0.5–1%) in 0.1 M phosphate or cacodylate buffer, pH 7.4, and 4% sucrose. A good perfusion was necessary for the clear identification of reaction products at the light and electron microscopic levels.
2. The tissues were left in the fixative for 1 h or longer at 4 °C.
3. The blocks were washed with several changes of the original buffer. Those to be cut with a freezing microtome were subjected to increasing concentrations of sucrose (10%, 20%, 30%) in 0.1 M phosphate buffer for 1–2 days until they sank.
4. Sections were cut with a freezing microtome, vibratome or tissue chopper. Thickness varied from 20–100 µm. Freezing the tissues aided in the penetration of DAB²⁶, but the other two techniques were more suitable for electron microscopic examination.
5. It was important to incubate experimental and control sections under the same conditions at the same time, since a slight variation in the protocol may influence the intensity of staining. Sections were incubated at 37 °C in the dark for 1–2 h. They were checked after 0.5–1 h for light to dark brown reaction products within the tissues.

The incubation medium (modified from Seligman et al. method²⁶) was made up fresh before use: 50 mg diaminobenzidine, 90 ml 0.1 M phosphate buffer, pH 7.4, 15–30 mg cytochrome C, type III, Sigma, and 4 g sucrose. (Catalase (200 µg/ml) had been used to eliminate the presence of any endogenous H₂O₂. However, no detectable difference was noted, and it was not routinely used in the incubation. The concentration of cytochrome C can be varied depending on the types of tissues and the species of animals used. It can be increased if no staining is seen after an hour of incubation.)

6. The incubation was arrested when clear differentiation between highly reactive and non-reactive portions could be discerned. Sections were then rinsed in three changes of 0.1 M phosphate or Michaelis buffer.

7. Sections were mounted, air-dried and cover-slipped. When counterstaining with Nissl was desired, mounted sections were defatted for 1 h in xylene, and stained with cresyl violet as usual.

8. When electron microscopic examination was desired, appropriate portions of incubated tissues were trimmed while they were wet, and postfixed in 1.3% OsO₄ for 1 h. After dehydration and en bloc staining with 1% uranyl acetate in absolute alcohol for 1 h, the tissues were rinsed in propylene oxide and embedded in epon or epon-araldite as usual. At the electron microscopic level, reaction products resided along the outer surface of the inner mitochondrial membranes, including the intracristate space (Fig. 2).

RESULTS

Normal lateral geniculate nucleus

In the lateral geniculate nucleus of normal adult cats, the level of cytochrome oxidase activity was moderately high within laminae A, A1 and the medial inter-laminar nucleus (MIN) (Fig. 1). Reaction products concentrated mainly in the neuropil and in some neuronal perikarya, and different neurons exhibited various levels of oxidative enzymatic activity. The C laminae, in general, were not as conspicuously reactive as the major laminae, and the interlaminar fibrous zones were the least reactive.

Preliminary electron microscopic examination of the LGN suggested that the cytochrome oxidase activity within the retinogeniculate terminals was less than that within other types of terminals such as the 'Fs'³⁵.

Monocular lid suture

Kittens 1, 2 and 3 each had one eye lid sutured from the first week to the fifth month of their postnatal lives. In each case, the geniculate laminae received input from the closed eye showed a decreased level of cytochrome oxidase activity in comparison with the laminae receiving input from the opened eye (Figs. 3 and 4). This was particularly evident in the major laminae, A and A1. A striking feature was that the deprived lamina ipsilateral to the lid suture (lamina A1) always appeared to be more severely affected than the contralateral one (lamina A). In other words, the difference in the levels of enzyme activity between laminae A and A1 was much greater on the

side ipsilateral to the suture than on the contralateral side. The deprived monocular segment in lamina A, however, was still quite reactive (Fig. 4).

Kittens 4 and 5 each had monocular lid suture during the 7th week of their postnatal lives, and they survived another 2.5 weeks. Histochemical findings did not indicate any dramatic changes in the level of cytochrome oxidase activity within the deprived laminae.

Binocular suture — monocular suture — binocular opening

Kitten 6 was binocularly sutured before eye opening until the 40th postnatal day. The left lid was opened at that time, and the right one was opened 74 days later. The animal was then sacrificed after 1.5 months. The levels of cytochrome oxidase activity was low in the laminae that received from the right eye, whereas those laminae that received from the left eye reacted much more intensely (Figs. 5 and 6). In contrast to the purely monocularly sutured kittens, the level of cytochrome oxidase activity within the deprived laminae that received from the right eye was distinctly reduced bilaterally, affecting even the monocular segment of the deprived lamina A. The slight unevenness of staining in the LGN of this animal may be due to differential rate of functional recovery in various portions of the same lamina, or it may be due to other unknown factors. This point is now being further explored.

Monocular enucleation

Kittens 7 and 8 each had one eye removed on the 5th postnatal day and survived 145 and 54 days respectively. In both kittens, the deafferented geniculate laminae exhibited a dramatic decrease in their levels of cytochrome oxidase activity, and the reactive dense band in the normal laminae appeared exceptionally wide (Figs. 7 and 8). When the sections were counterstained with Nissl, the dense band was clearly seen to include not only the normal lamina, but also the adjacent interlaminar fibrous zone, as well as a row of surviving large cells in the deafferented lamina(e) (Figs. 9 and 10). The deafferented monocular segment of lamina A likewise showed a greater decrease of oxidative activity than in the case of lid suture.

Normal striate cortex

The cytochrome oxidase technique revealed an interesting aspect of cortical organization not obvious with conventional Nissl or myelin preparations. A dense, continuous band of high oxidative enzymatic activity was seen within lamina IV of the normal cat striate cortex, with a slight hint of greater reactivity along the upper 1/3 to 1/2 of the lamina. The reaction products resided mainly within the neuropil and within some neuronal perikarya. This densely reactive band stopped short at the boundary zones of area 17 (see Fig. 13), and served as a convenient marker for the striate cortex.

The neuropil within laminae III and VI (particularly the upper half of VI) was moderately reactive, and a few scattered reactive neurons could be seen in lamina III. Lamina V was distinguished by having a random distribution of highly reactive neurons against a rather light background. Preliminary electron microscopic examination revealed that neighboring neurons could indeed differ in their levels of cytoch-

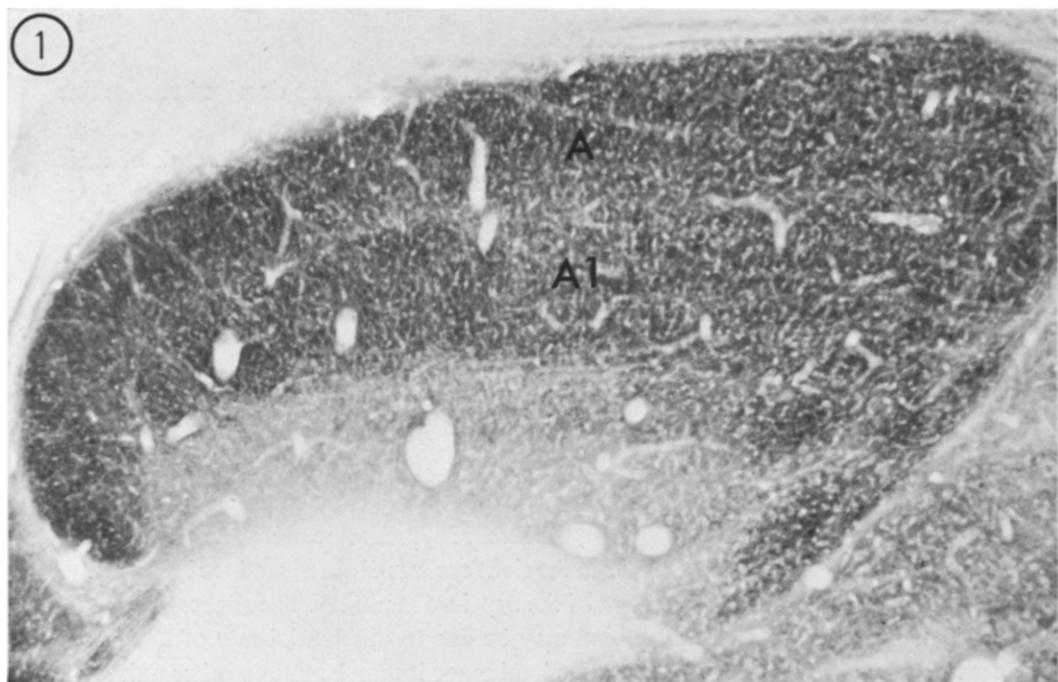
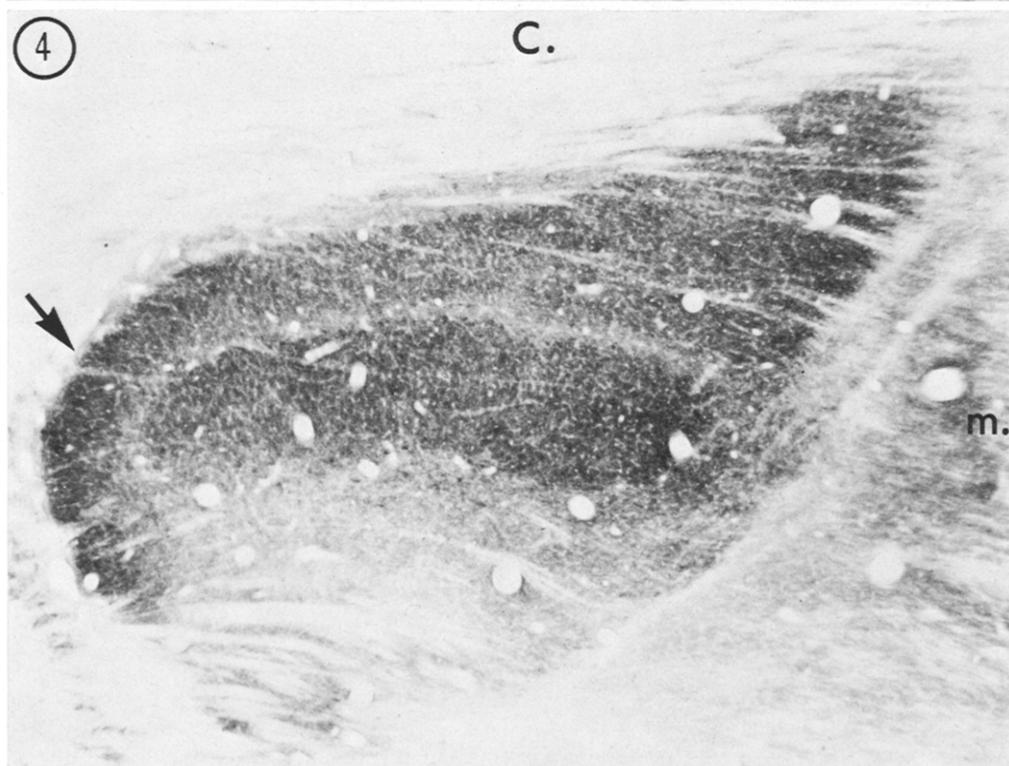
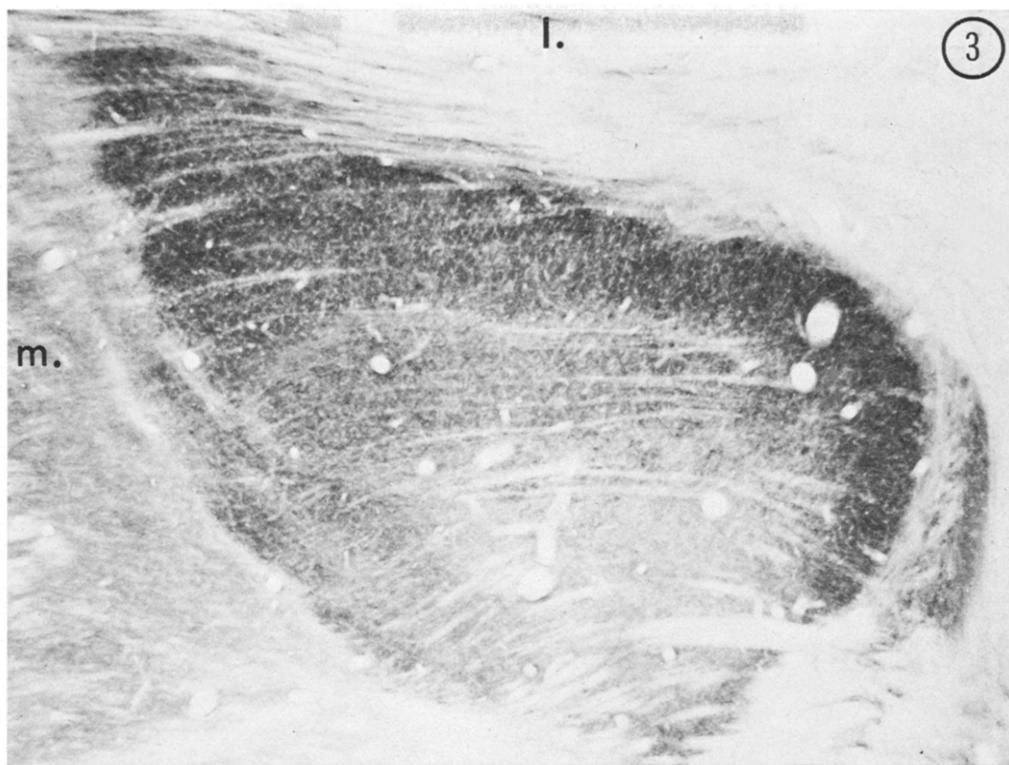
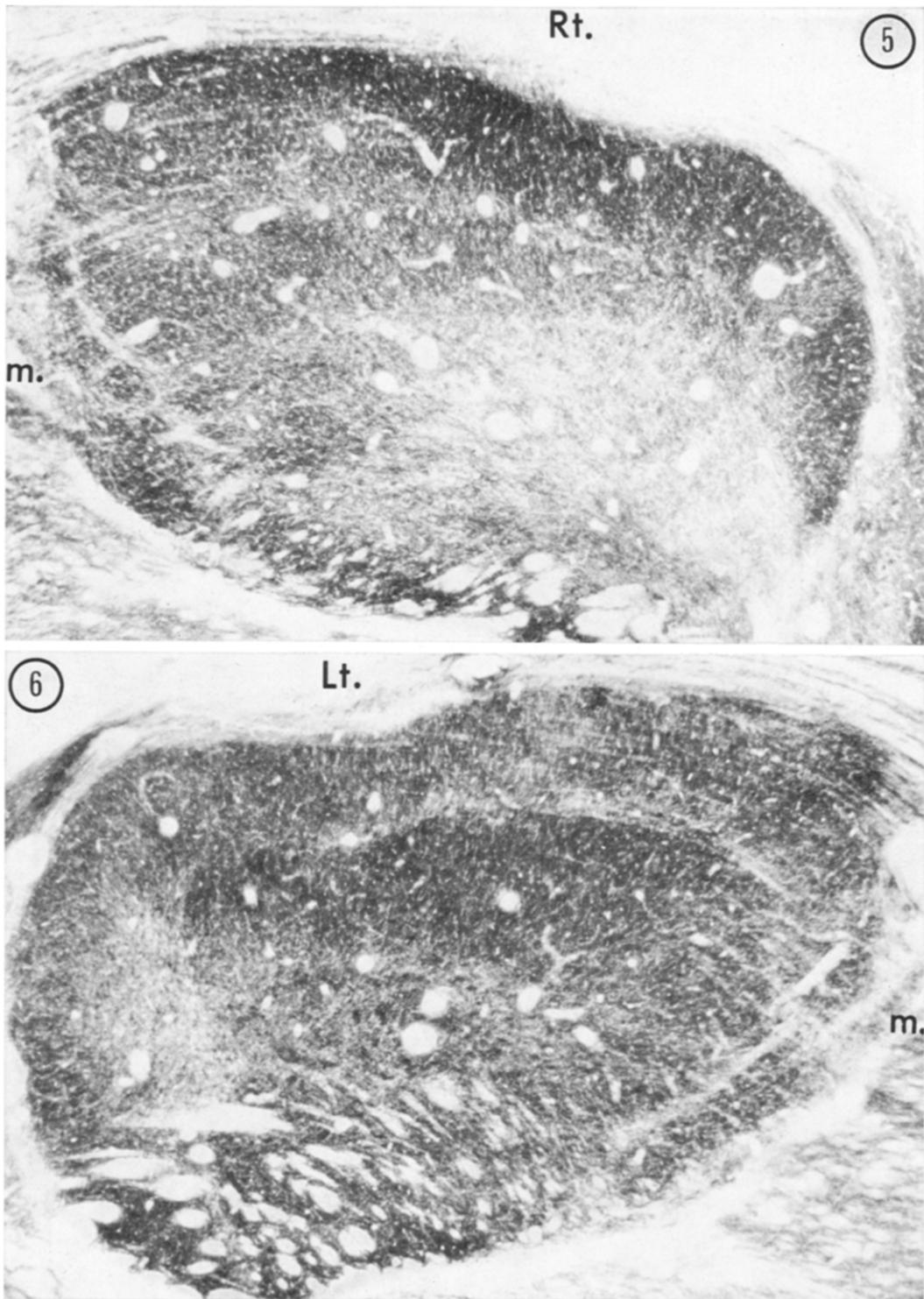


Fig. 1. Endogenous cytochrome oxidase activity in the dorsal lateral geniculate nucleus of a normal cat. Coronal section. $\times 33$.

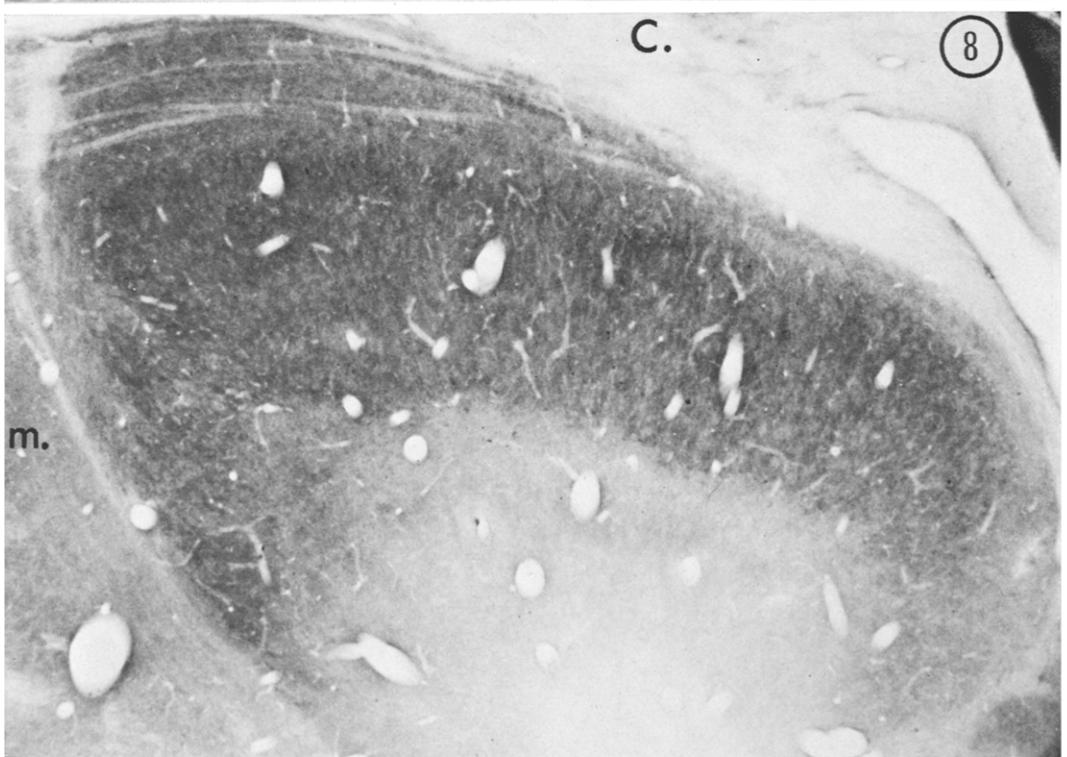
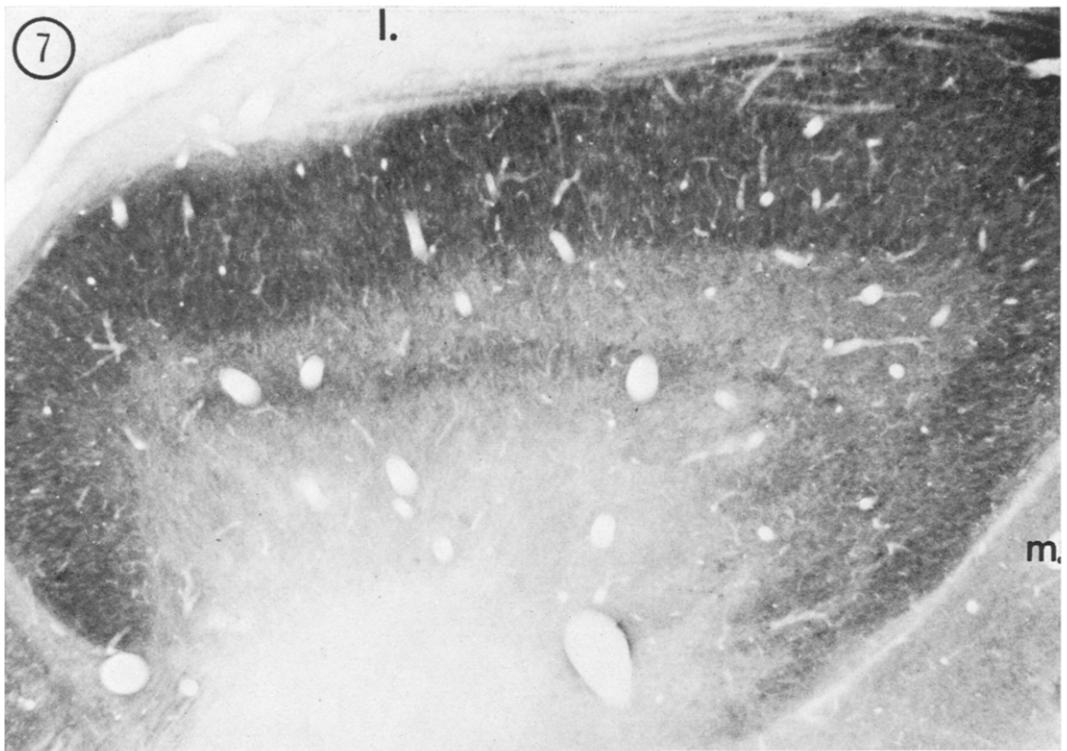
Fig. 2. Electron micrograph of reaction products of cytochrome oxidase activity within the mitochondria of reactive neurons. Normal cat cortex. $\times 36,900$.



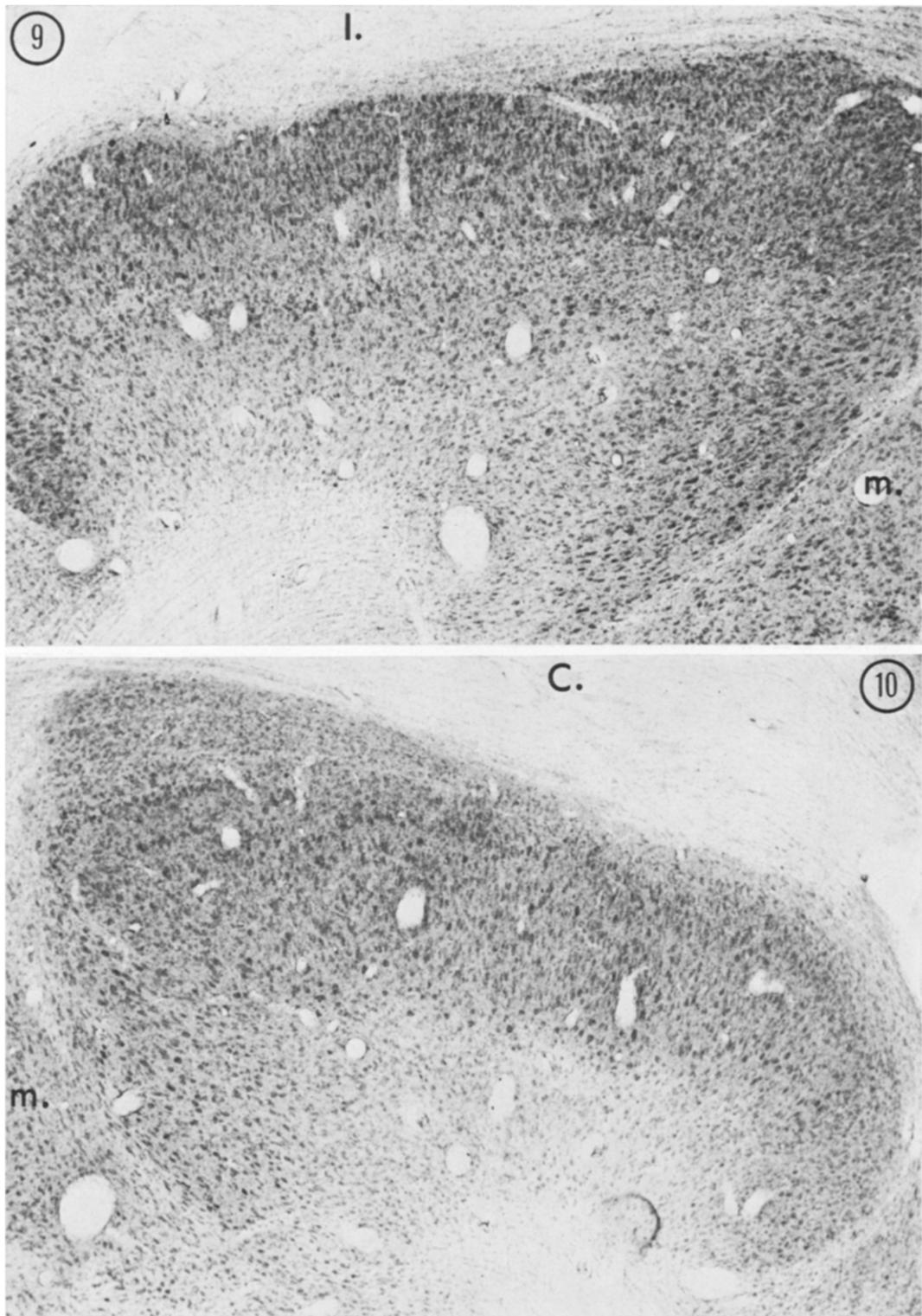
Figs. 3-10. Coronal sections of the dorsal lateral geniculate nuclei of experimental cats. Dorsal is up, and m. = medial; I. = ipsilateral and C. = contralateral to the lid suture or enucleation.
Figs. 3 and 4. Endogenous cytochrome oxidase activity in the LGN of a kitten whose right eye lid was sutured from the first week to the 5th month of its postnatal life. The ipsilateral (I.) lamina A1 showed a dramatic loss in its level of enzymatic activity, whereas the contralateral (C.) lamina A was not affected to the same degree as the ipsi A1. The contralateral monocular segment (arrow), however, still has a high level of enzyme activity. $\times 33$.



Figs. 5 and 6. Effect of monocular suture preceded by binocular suture for the first 40 postnatal days. Note that the laminae which received input from the early-opened eye (left) regained normal levels of cytochrome oxidase activity, whereas those laminae which received input from the late-opened eye (right eye; opened for the entire 5th postnatal month) suffered a dramatic decrease in their level of enzyme activity. The decrease was evident bilaterally, and even involved the monocular segment. Rt. and Lt. = right and left LGN. $\times 40$.



Figs. 7 and 8. Comparison of the two LGN's in a 54-day-old kitten whose left eye was removed during the first postnatal week. Note that the reactive band in each LGN is much wider than the non-reactive band. $\times 33$.



Figs. 9 and 10. Nissl counterstained sections from the same monocularly enucleated kitten as in Figs. 7 and 8, showing that the dense bands each include the 'normal' lamina, the interlaminar fibrous zone, as well as a row of surviving large cells in the 'denervated' lamina which faces the normal lamina. $\times 33$.

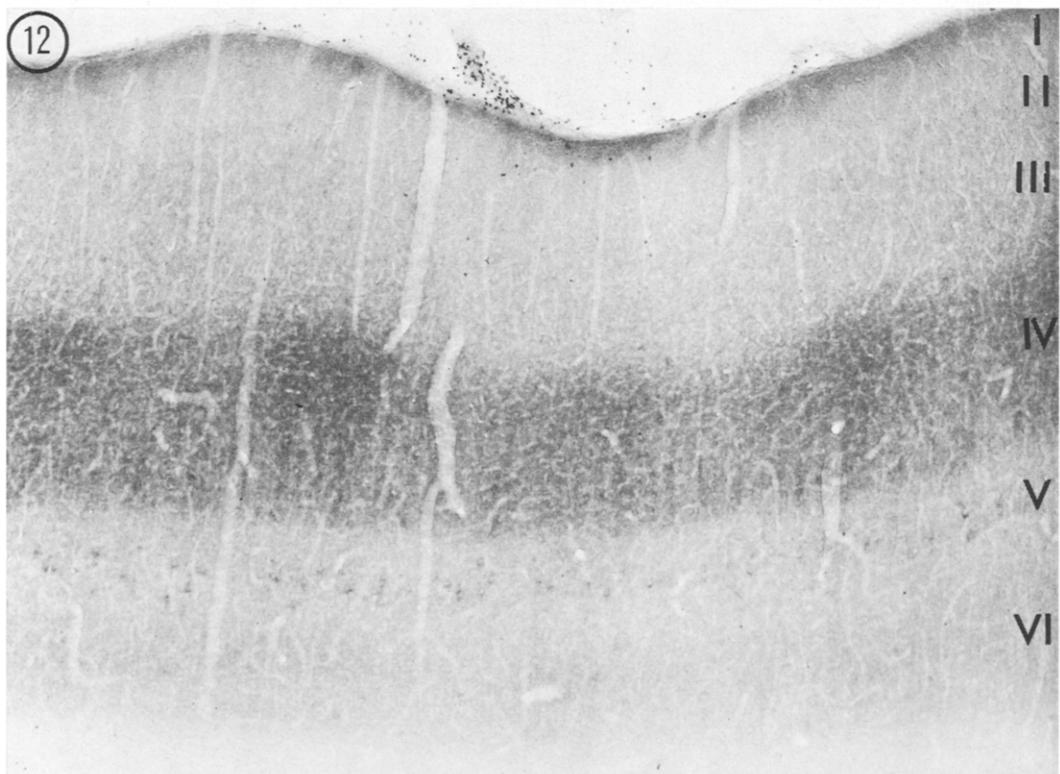
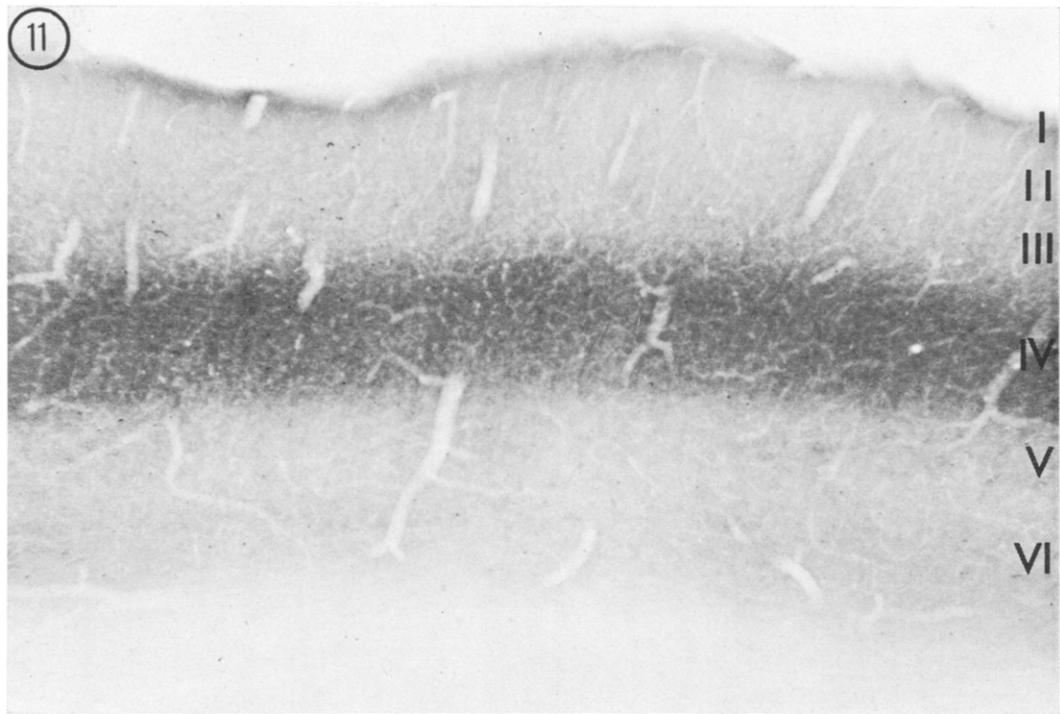


Fig. 11. Endogenous cytochrome oxidase activity in the striate cortex of a normal cat. Lamina IV is seen as a continuous, highly reactive band. Parasagittal section. $\times 54$.

Fig. 12. A portion of the striate cortex ipsilateral to the monocularly deprived eye (deprived for the first 5 months of the kitten's life). Periodic dark and lighter bands are present in lamina IV, indicating alternating areas of high and low cytochrome oxidase activity. Parasagittal section. $\times 34$.

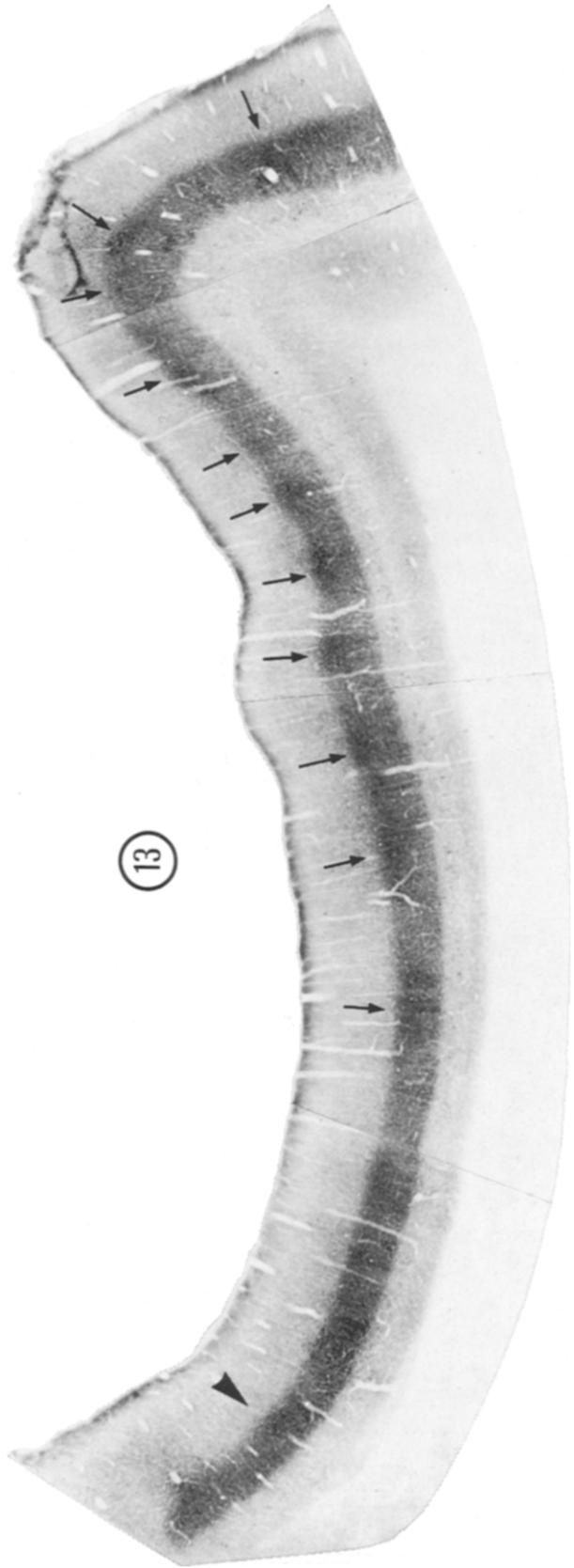


Fig. 13. A montage of micrographs taken from the same ipsilateral striate cortex as in Fig. 12. Note the periodic dark (arrows) and light reactive bands in lamina IV, frequently extending into lamina III. This banding pattern is not evident toward the anterior peripheral border of the striate cortex (arrowhead) and stops abruptly at the very edge of area 17. Parasagittal section taken from the ventromedial portion of the striate cortex. $\times 18$.

rome oxidase reactivity within their respective mitochondria. The outer border of lamina I next to and including the pial surface was rather dark. This may be due partly or mostly to a greater accumulation of DAB along the edges of sections. We cannot as yet rule out the possibility of reactive processes along the outer margin of lamina I.

Cortical effect of monocular deprivation

Monocular deprivation for the first 5 postnatal months produced a different histochemical picture. The continuous dense band within lamina IV was replaced in most parts of area 17 by alternating dark and lightly reactive columns (Figs. 12 and 13). The darkly reactive columns ranged in widths between 300–500 μm , with a center-to-center spacing of approximately 475–760 μm . The reactivity appeared most intense along the upper 1/3 to 1/2 of the lamina, a feature more pronounced in the experimental material than in the normal. The lightly reactive gaps varied in widths between 200–300 μm , although the transition between high and low reactivity was often gradual rather than abrupt. At times, the columns seemed to continue, though to a much less intense degree, into the supragranular and infragranular layers. Scattered neurons within layer V were again reactive. However, their pattern of distribution could not be clearly correlated with that of the columns in lamina IV.

The banding pattern was particularly evident along the medial, ventromedial and ventral portions excluding the monocular segment of the striate cortex. Along the dorsal portion of area 17, the reactive columns appeared to be wider and less regularly spaced. Although the banding pattern could be seen in both hemispheres, it usually was more obvious on the side ipsilateral than contralateral to the monocular deprivation. The monocular segment of the ipsilateral striate cortex remained as an uninterrupted dense band, whereas that of the contralateral cortex appeared less reactive.

The striate cortices of the enucleated kittens were not processed for cytochrome oxidase histochemistry. The cortical effect of this procedure, therefore, awaits to be examined. The normal patterns of cytochrome oxidase reactivity in various non-striate cortical areas will also be the subject of future reports.

DISCUSSION

The present results indicate that the histochemical technique for cytochrome oxidase can be used to demonstrate enzymatic changes in the central nervous system in response to visual deprivation. Previous studies have focussed on the cellular atrophy at the lateral geniculate level^{8,33}, and physiological alterations at the cortical level whereby the number of neurons excitable by the deprived eye was significantly reduced^{1,2,6,33,34}. Behaviorally, the deprived eye was found to be functionally blind in the binocular segment of the visual field^{2,4,5,28,29}. The present study added a new dimension to the above findings by showing that the level of activity of oxidative enzymes, the cytochromes, can be severely affected by visual deprivation. This effect was demonstrated histochemically at both the geniculate and cortical levels.

The important role of the cytochrome system in energy production renders it essential for such active processes as the maintenance of the resting membrane potential by Na⁺-K⁺-ATPase, the synthesis of various cellular components, and fast axoplasmic transport of materials within the neurons^{23,24}. A chronically more active neuron would have greater energy demand and would be expected to have a more active cytochrome system. This is implicated by the recent findings that the spontaneously active brain stem auditory nuclear groups have a higher level of glucose consumption and local cerebral flow than neighboring, less active regions²⁵. These neurons showed a degree of cytochrome oxidase staining that was more intense than surrounding nuclear groups³⁹. Chronic unilateral silencing of the auditory nerve significantly decreased the level of cytochrome oxidase activity within several relay nuclear centers that received their excitatory input mainly from the deafened ear^{38,39}. These changes were detectable from the level of the cochlear nuclei to the inferior colliculi, signifying that the oxidase activity was closely related to the level of neuronal activity modulated by primary afferent input.

The present study demonstrated and reaffirmed the correlation between oxidative activity and the functional state of neurons in another sensory system, the visual system. Monocular deprivation during the critical period (first 3.5 months of the kitten's life)¹⁴ caused not only a decreased percentage of excitatory responses to input from the deprived eye^{33,34}, but the amplitude of the excitatory postsynaptic potentials from the level of the first synapse to the cortical level was also drastically reduced³¹. This reduction in neuronal activity has presumably resulted in reduced energy demand, and therefore a lowered level of oxidative enzyme activity in all of the affected neurons.

Enucleation

Unilateral enucleation during early postnatal life seemed to trigger some unique changes that differed from those of lid suture. First of all, the decrease in cytochrome oxidase activity affected the monocular as well as the binocular segments of the denervated geniculate laminae. Secondly, histochemical changes within the affected laminae were much more severe than in the case of lid suture. Thirdly, the border zone of the denervated lamina that faced a 'normal' lamina retained a level of cytochrome oxidase activity comparable to that of the 'normal' lamina. Thus, the denervated laminae appeared histochemically to be narrower than they were, while the rather wide 'normal' lamina actually encompassed the interlaminar zone plus the border of the deafferented lamina. These findings were consistent with previous morphological studies^{3,9} wherein severe transneuronal atrophy was found in both the monocular and binocular segments of the denervated LGN. They further confirmed and extended Guillory's⁷ original findings that in neonatally enucleated kittens, the border neurons within the denervated lamina did not suffer transneuronal atrophy if they faced a normally innervated lamina. The cytochrome oxidase activity within these border zones also remained high, presumably due to viable innervation of surviving neurons via their translaminar dendrites and/or sprouting axons from the normal lamina.

Preliminary studies of enucleation in the adult indicated that the entire

denervated geniculate lamina suffered a decreased level of cytochrome oxidase activity.

Monocular lid suture

Histochemical changes brought on by monocular deprivation were less severe and qualitatively different from those of enucleation. While the binocular segments of the deprived laminae exhibited a decrease in cytochrome oxidase activity, the monocular segments appeared not to have been affected. This again was consistent with the findings first noted by Guillery and Stelzner¹⁰ in Nissl stained material. In addition, up to the survival period examined in the present study, there was a consistent difference in the degree of cytochrome oxidase reactivity between the ipsilateral and the contralateral sides. That is, the decrease in enzyme activity was more prominent and severe in lamina A1 ipsilateral to deprivation than in the contralateral lamina A. While this phenomenon of bilateral asymmetry has not been widely discussed, a careful search of the literature revealed some interesting and relevant findings. Headon and Powell¹¹ noted that the ipsilateral deprived lamina of the monkey LGN showed greater shrinkage after unilateral lid suture than the contralateral lamina. This was previously reported for enucleated cases^{18,21}. Hoffman and Sireteanu¹³ demonstrated that lamina A1 of the cat LGN suffered greater deprivation effects than lamina A in several respects: first of all, cells in A1 shrank more than those in A, even though the A1 cells were normally larger^{8,12}; secondly, there was a greater depression of input measured physiologically from the deprived eye to the cortex via the ipsilateral lamina A1 than via the contralateral lamina A. This correlated well with their finding that lamina A1 normally contained more Y-type cells than lamina A (58% vs 38%), and that Y-cells appeared to have been more severely affected by visual deprivation than the X-type³⁰. Recovery was also better in the LGN contralateral than ipsilateral to the deprived eye. Finally, there was greater loss in the visual acuity of neurons within the ipsilateral lamina A1 than the contralateral lamina A following lid suture. Apparently the asymmetry was present under normal condition as well, since the visual acuity of normal geniculate neurons was 'significantly higher in lamina A' than in A1. These findings imply that there is normally a contralateral dominance in the cat's visual system and that the contralateral system suffers less as well as recovers more readily from the effects of monocular deprivation. Thus, not only is there binocular competition at the geniculocortical level, but there appears to be some binocular competition at the retinogeniculate level as well.

Binocular deprivation — monocular deprivation — binocular opening

The effect of early binocular deprivation on subsequent monocular lid suture was tested and two consequences were noted: (i) the early-opened eye was able to recover and sustain normal levels of cytochrome oxidase activity within the appropriate geniculate laminae; and (ii) the eye which was opened later (after the known critical period) appeared to suffer 'double jeopardy' — the trauma of visual deprivation plus additional loss in competition. Subsequent lid opening at 3.5 months did not result in significant recovery of the weaker eye, and the decrease in cytochrome oxidase activity

in the corresponding geniculate laminae including the monocular segment was more prominent on both the contralateral and the ipsilateral sides than with monocular deprivation alone. It is possible that within a single lamina, certain portions may recover faster than others, and that at any one point we are capturing the steady state as that time. Whether further recovery occurs after extended period of survival is as yet unknown.

Cortical effect

The remarkably dense band of cytochrome oxidase reactivity in lamina IV of the normal cat striate cortex denotes a high level of metabolic activity within the major site of synaptic interaction between the geniculostriate terminals and the neurons and neuronal processes found within that lamina. This pattern is strikingly similar to that revealed by the [¹⁴C]deoxyglucose technique¹⁶ in the primary visual cortex of the monkey. Lamina IV, then, is marked by a greater rate of glucose consumption, and a significantly higher level of functional activity demanding oxidative metabolism than other layers of the striate cortex. The heightened level of oxidative activity within the upper 1/3 to 1/2 of lamina IV, particularly in reactive columns of monocularly deprived cats, may well denote a region of special synaptic activity. The precise types of axons, dendrites and neurons that contribute to the high reactivity, however, awaits electron microscopic examination.

The moderate degree of reactivity within the neuropil of laminae III and VI probably reflects a level of neuronal activity secondary to that of lamina IV, but is nonetheless greater than those of the remaining layers. The significance of the highly reactive neurons in lamina V remains to be explored. An obvious question will be whether these are the same neurons that project to the superior colliculus.

Monocular lid suture for the first 5 postnatal months brought about a change in the pattern of cytochrome oxidase activity. The dense band within lamina IV of most parts of the striate cortex in both hemispheres was replaced by alternating columns of dark and light reactivity, with the widths of the dark columns being, in general, slightly greater than those of the light columns. The presence of these light columns suggests that some reduction in functional activity occurred there as a result of reduced input from the sutured eye. Since ocular dominance columns have been observed in lamina IV of normal cat striate cortex^{20,27}, it is reasonable to assume that cortical changes subsequent to monocular deprivation may exist in the cat³² similar to those reported in the monkey¹⁵. The pattern of alternating columns of high and low enzymatic activity seen after monocular deprivation suggests that these columns are alternately dominated by input from the non-deprived and the deprived eye. The slightly greater width of the reactive bands suggests that the input from the non-deprived eye is capable of sustaining the activity of border neurons that would otherwise be driven by the deprived eye. This may be the result of axonal sprouting, or of successful competition by the dominant eye in regions of initial binocular innervation. Studies of monocular deprivation in neonatal monkeys¹⁵ favors the second possibility to be the causal factor in sustaining activities of neurons along the border regions. Whether functional recovery or further deterioration of metabolic activity can occur within the lightly reactive bands with prolonged survival remains to be explored.

The cytochrome oxidase system in CNS histochemistry.

The present histochemical procedure can be used at both the light and electron microscopic levels to localize cytochrome oxidase activity within specific regions of the brain, and within neuronal perikarya or neuropil (specific types of axons and dendrites) of such regions. The degree of enzyme activity can be compared between various nuclear groups, and between comparable nuclei under normal and experimental conditions.

The study demonstrates that the cytochrome oxidase reaction can be used to indicate the relative oxidative capacity of neurons, and therefore indirectly of the functional state of these cells. As decreased auditory input induced a significant reduction of cytochrome oxidase activity in several brain stem auditory relay nuclei, so did decreased visual input induce similar enzymatic changes in the visual relay centers of the brain. Various degrees of visual deprivation including deafferentation during the critical period can bring about various levels of decreased oxidative enzymatic activity in affected neurons one to several synapses away. These histochemical changes correlate directly with, and extend, known morphological and physiological alterations subsequent to sensory deprivation.

ACKNOWLEDGEMENTS

It is a pleasure to thank Mrs. Katy Chen and Mr. Dave Akers for their technical assistance. Special thanks to Dr. Ralph Freeman for contributing one of the monocularly deprived cats.

The study is supported in part by NIH Grant NS-12995.

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