STUDIES ON THE ROLE OF AFFERENT ACTIVITY IN THE VISUAL PATHWAYS OF THE CAT

I. MECHANISMS INVOLVED IN THE CONTROL OF GENICULATE CELL SIZE

II. CONTROL OF THE CRITICAL PERIOD AFTER DARK-REARING

Thesis by

Baruch Davidson Kuppermann

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1983

(Submitted May 18, 1983)

to dyg

ACKNOWLEDGEMENTS

Life at Caltech, while not always exciting, has never been dull. I acknowledge Jack Pettigrew for conveying a sense of exhilaration about science in addition to providing me with months (years?) of drama during his period of hemispheric oscillations. David Van Essen ruled me with a gentle hand, allowing me almost enough independence to get into serious trouble before rescuing me in the nick of time. For this combination of freedom and supervision, and for his perceptive comments I am grateful. I thank my committee members for supporting my work with a minimum of interference and an appropriate amount of criticism. Bill Lease made (everyone's) life much easier by being so good at whatever it is he does. Connie Katz saved my thesis from the dreariness of dot-matrix; her generous help and concentrated effort are greatly appreciated.

I have enjoyed my late night conversations with Jeremy Brockes about the way things are, and my morning visits with Derek Fender about the way things were. Andreas Burkhalter, Ruth Ann Eatock, Dan Felleman, Larry Fritz/Katz, Herman Gordon, Rich Lewis, and Kunio Nakai provided a healthy climate of scientific communion in addition to endless hours of entertaining chatter. Greg Lemke has been a continuous source of amazement, as well as a good friend. Edwardo Herrera, Jeff Ray, Roger Zack, and Rick Cole have been instrumental in preserving a sane perspective about excessive work, and Audrey Maynard has been a long distance solace. I thank my parents for teaching me the joys of scientific inquiry, and my family for their love and support through the years.

I am occasionally overwhelmed at my good fortune in meeting and befriending, let alone marrying, a person like Dorothy. I acknowledge my gratitude to her for a continued sense of humor, a new-found awe of life, and a belief in the infrequent magic that the cosmos is wont to visit upon the innocently curious.

I acknowledge with thanks the financial support of the Evelyn Sharp fellowship, NIH training grant GM07737, and the Pew Charitable Trusts. Costs in the preparation of this thesis were defrayed by the Jean Weigle Memorial Fund.

ABSTRACT

Two studies were conducted examining the role of afferent activity on synaptic connectivity in the cat's visual pathways. The first study investigated the effects of retinal inactivity on cell size in the lateral geniculate nucleus (LGN). A complete retinal blockade was produced in one eye of 7-week-old kittens by intravitreal injections of tetrodotoxin (TTX). Within one week, cell shrinkage of ∿20% in laminae with input from the inactive eye was observed in the binocular and monocular segments of the LGN. Cell growth of 10% was observed in active laminae only in the binocular segment. Adult cats subjected to one week of monocular TTX treatment and kittens placed in the dark during the treatment period underwent uniform changes in cell size of ∿20% throughout the binocular and monocular segments of the LGN. The cell size changes in the monocular segment of the kittens left in the light, and throughout the LGN of the kittens placed in the dark and of the adult cats were not indicative of a competitive response to differential activity from the two eyes. The results suggest a strong control of LGN cell size by afferent (retinal) activity.

In the second study, cats were reared in the dark from birth until adulthood, then subjected to various visual exposure paradigms to test their residual cortical plasticity. Animals allowed two weeks of binocular visual exposure after dark-rearing, then monocularly lid-sutured underwent changes in cortical ocular dominance in response to the monocular deprivation. Animals which were monocularly deprived immediately upon removal from the dark and 25 days later reverse-sutured for 4-6 months (closure of initially open eye, opening of previously sutured eye) underwent shifts in ocular dominance towards the experienced eye both after the initial monocular deprivation and again in response to reverse-suturing. The LGN of dark-reared/monocularly deprived animals did not exhibit any morphological abnormalities.

Depletion of cortical norepinephrine reduced but did not abolish the sensitivity of the dark-reared cortex to monocular deprivation. Visual exposure for four weeks after birth, followed by extended dark-rearing did not halt the subsequent prolongation of cortical plasticity in the dark.

Table of Contents

GENERAL INTRODUCTION

During normal development of the mammalian nervous system, neural circuitry undergoes substantial modification. Early in embryonic life, a phase of overproduction of cells throughout the nervous system leads to large amounts of cell death (Hamburger and Levi-Montalcini, 1949; Harris-Flanagan, 1969). The result is an appropriate number of cells between afferent source and target (Cowan, 1978). Later in prenatal development, an overproduction of synapses occurs between the appropriately matched target and source populations of cells. This period of synapse formation results in an initial mapping of source afferents onto the target cell population, which subsequently undergoes topographic refinement (Gaze et al., 1974; Rakic, 1977; Dennis and Harris, 1979). The multiple axonal innervation of target cells is still seen at birth in the peripheral, parasympathetic, and central components of the nervous system. After several weeks of postnatal development, however, the number of axons innervating each target cell is markedly reduced, as is the terminal field of each axon (Brown et al., 1976; Crepel et al., 1976; Lichtman and Purves, 1980). While these plastic processes of synapse formation and elimination have been studied most extensively in the neuromuscular junction and the autonomic ganglia of various mammals, they appear to be common to the development of the specificity of connections throughout the nervous system. The lesser amount of clear evidence on synapse elimination in the central nervous system is due primarily to the difficulty of visualizing all of the axonal inputs for each cell from a large sample of cells. One central system amenable to direct examination for evidence of synaptic elimination is the cerebellum, due to its relatively simple circuitry. The primary output neuron of the cerebellum, the Purkinje cell, is initially innervated by several climbing fibers, but by several weeks postnatally, only one climbing fiber input remains (Crepel et al., 1976). In the visual cortex, a neural system of complex circuitry, the available non-

quantitative techniques (Hubel and Wiesel, 1972; Wiesel et al., 1974; LeVay et al., 1975) indicate a process of central synaptic modification that is analogous to that seen more peripherally.

Despite its complexity, the cat (and primate) visual system is an excellent model for the study of the development and subsequent modification of central connections. Early in embryogenesis, inputs from the two eyes to the lateral geniculate nucleus (LGN) in the thalamus are diffuse, then undergo prenatal segregation into discrete laminae subserved by one eye or the other (Rakic, 1977; Shatz, 1983). The projections of these segregated thalamic neurons to layer IV of the visual cortex are still diffuse, however, so each target cell at this stage in development receives input from both eyes (LeVay et al., 1978, 1980). Near the time of birth, the geniculo-cortical inputs begin to segregate so that discrete groups, or columns, of target cells are innervated by inputs from only one eye. The process of segregation does not go to completion; some cells at the boundaries of the columns retain inputs from both eyes (Shatz and Stryker, 1978; LeVay et al., 1978). The mature state of segregation is reached several weeks postnatally (LeVay, 1978, 1980). While it is not clear that there is an absolute decrease in the number of axons projecting to a single target cell in layer IV of the cortex after segregation, a modification of synaptic connectivity is evident.

The control of the processes of synaptic formation, elimination, and modification is not yet fully understood. A prominent hypothesis is that the presynaptic terminals are competing for some component, perhaps a trophic factor or simply a limited number of synaptic sites, supplied by the target cell (Jansen et al., 1978; Purves and Lichtman, 1978). The presynaptic determinant for successful competition is also unclear, though axons with smaller terminal fields appear to have an advantage in keeping more of their postsynaptic sites (Brown et al., 1976). This advantage may be due to the presumably lesser demands made by a small terminal

field on the cellular apparatus. A second hypothesis is that the level of activity of an afferent is responsible for its successful innervation of the target cells. Such a role for afferent activity in synaptic modification has been demonstrated in the neuromuscular junction. Neuronal blockade of input to the immature muscle reduces the rate of synaptic elimination and afferent (and efferent) stimulation increases its rate (O'Brien et al., 1978; Thompson et al., 1979). Additionally, non-degenerative blockade of afferent activity to the mature muscle results in sprouting of terminals, while direct electrical stimulation of the muscle inhibits sprouting (Brown et al., 1977; Brown and Ironton, 1977). The importance of neuronal activity is not universal, however, since other systems have indicated an apparent disregard for levels of afferent activity in the resultant pattern of connectivity. For example, neuronal blockade does not result in terminal sprouting of heart ganglion cells of the frog (Roper and Ko, 1978). Nor does halting afferent activity in the axolotl affect the development of retino-tectal specificity, though in the goldfish this specificity is disrupted by impulse blockade (Harris, 1980; Meyer, 1983). Regardless of the generality of its role, it is clear that afferent activity has profound influences on connectivity in the mammalian central visual pathways.

If one eye of a kitten is sutured closed for several days during a critical period early in postnatal life, a relative imbalance of activity from the two eyes is produced. Most cells in the primary visual cortex then become driven exclusively by the nondeprived eye (Wiesel and Hubel, 1963b). Such differential activity has been proposed to enhance the competition between thalamic afferents from the two eyes for the presumably limited number of postsynaptic sites in the cortex (Wiesel and Hubel, 1965; Guillery and Stelzner, 1970). This electrophysiological evidence can only prove that changes in synaptic efficacy are produced by differential afferent activity. However, anatomical assessment has demonstrated that structural changes in connectivity occur during this process (Hubel et al., 1977; Shatz and Stryker, 1978).

In addition to a role for differential afferent activity in specifying synaptic connectivity, absolute levels of activity have also been suggested as influencing the neuronal circuitry of the visual cortex (Cynader and Mitchell, 1980). The process of segregation of thalamic inputs to layer IV of the visual cortex is reduced if the animal is reared in the dark, and is halted if retinal activity is completely blocked in both eyes by injection of tetrodotoxin (TTX), a specific inhibitor of electrical conduction (Stryker, 1981; Swindale, 1981).

This thesis investigates the roles of differential and absolute afferent activity on two different visual structures: the LGN and the primary visual cortex. Both the methods of altering the retinal (afferent) activity and assessing the central effects were different in each case, and thereby constitute two independent studies. In Part I, blockade of all retinal activity in one eye of kittens by injections of TTX allowed a comparison of the relative roles of differential and absolute afferent activity on cell size in the LGN. This work is based on prior demonstrations that extended periods of monocular lid-suture of young kittens results in a shrinkage of cells in LGN laminae with input from the deprived eye, and an expansion of cells with input from the experienced eye (Wiesel and Hubel, 1963a; Hickey et al., 1977). The elegant experiments of Guillery and associates showed that changes in LGN cell size occur only in the presence of differential activity from the two eyes, and only in the binocular segment of the LGN, where cells are capable of some binocular interaction (Guillery and Stelzner, 1970; Guillery, 1972; Sherman et al., 1974). The significance of changes in cell size under this binocular competition hypothesis is that cell body area in the LGN is related to the size of its axonal field in layer IV of the visual cortex. Changes in the terminal field would therefore be reflected in corresponding changes in LGN cell size. There is, in fact, indirect evidence to suggest such a correspondence. Successful afferent competition in layer IV during monocular lid-suture has been shown to lead to increases in the width of ocular dominance columns

subserved by the experienced eye (Hubel et al., 1977; Shatz and Stryker, 1978). Significantly, a linear relationship exists between the width of an ocular dominance column and the mean cell size of the corresponding LGN lamina (LeVay et al., 1980). Though one ocular dominance column contains a large number of geniculo-cortical terminal fields, it is likely that change in columnar width involves corresponding changes in terminal fields from individual axons which would be reflected in cell body shrinkage or expansion. In spite of the evidence supporting differential activity from the two eyes in the control of LGN cell size, changes in LGN cell size have been described that cannot be accounted for by the binocular competition hypothesis. For example, if both eyes are sutured shut, so that a relative balance of afferent activity from the two eyes is maintained, long periods of deprivation result in modest amounts of cell shrinkage (Hickey et al., 1977). This suggests that the loss of afferent activity could affect LGN cell size. However, lid suture is not an effective means of completely silencing retinal activity, since retinal ganglion cells fire tonically, even in the dark (Kuffler et al., 1957; Rodieck, 1967). The present study investigated the importance of the role of afferent activity on LGN cell size by completely blocking retinal activity in one eye and examining the varying response throughout the LGN. The results suggest that loss of afferent activity can produce net changes in cell size as great as those seen in response to differential activity from the two eyes.

In Part II, an examination of the role of visual inactivity on the maintenance of cortical synaptic modifiability is described. One of the many striking finds of the studies by Hubel and Wiesel on the susceptibility of the visual cortex of the cat to differential activity from the two eyes was the demonstration that the sensitivity is restricted to a critical period early in life (Hubel and Wiesel, 1970). More recently, Cynader and Mitchell (1980) demonstrated that the critical period for cortical synaptic plasticity can be prolonged by rearing the animal in the dark. The anatomical demonstration of reduced segregation of ocular dominance columns in

dark-reared animals (Swindale, 1981) suggests that the prolonged plasticity may be related to the degree of layer IV segregation. However, plasticity in normal kittens persists after the mature level of segregation is reached (Hubel and Wiesel, 1970; LeVay et al., 1978). It may be that two partially overlapping stages of plasticity could be present during the normal critical period: an early plasticity resulting in structural synaptic modification which gradually declines as segregation proceeds; and a later phase of functional plasticity, where synaptic efficacy can be altered. By subjecting dark-reared adult cats to various visual exposure paradigms and comparing the results with those of young kittens, an attempt was made to evaluate the nature and extent of the cortical plasticity preserved in the dark. The results from this thesis contribute to the mounting body of evidence demonstrating the role of afferent activity in the development and maintenance of synaptic efficacy and connectivity in the central visual pathways.

REFERENCES

Brown, M. C., Goodwin, G. M. and Ironton, R. Prevention of motor nerve sprouting in botulinum toxin poisoned mouse soleus muscles by direct stimulation of the muscle. J. Physiol. Lond. **267**:42-43P, 1977.

Brown, M. C., Jansen, J. K. S. and Van Essen, D. Polyneuronal innervation of skeletal muscle in new-born rats and its elimination during maturation. J. Physiol. Lond. **261**:387-422, 1976.

Brown, M. C. and Ironton, R. Motor neuron sprouting induced by prolonged tetrodotoxin block of nerve action potentials. Nature **265**:459-461, 1977.

Cowan, W. M. Aspects of neural development. Int. Rev. Physiol. **17**:149-191, 1978.

Crepel, J., Mariani, J. and Delhaye-Bouchard, N. Evidence for a multiple innervation of Purkinje cells by climbing fibers in the immature rat cerebellum. J. Neurobiol. **7**:567-578, 1976.

Cynader, M. and Mitchell, D. E. Prolonged sensitivity to monocular deprivation in dark-reared cats. J. Neurophysiol. **43**:1026-1040, 1980.

Dennis, M. J. and Harris, A. J. Elimination of inappropriate nerve-muscle connections during development of rat embryos. Prog. Brain Res. **49**:359-364, 1979.

Gaze,R. M., Keating, M. J. and Chung, S. H. The evolution of the retinotectal map during development in Xenopus. Proc. R. Soc. Lond. **B185**:301-330, 1974.

Guillery, R. W. Binocular competition in the control of geniculate cell growth. J. Comp. Neurol. **144**:117-127, 1972.

Guillery, R. W. and Stelzner, D. J. The differential effects of unilateral lid closure upon the monocular and binocular segments of the dorsal lateral geniculate nucleus in the cat. J. Comp. Neurol. **139**:413-422, 1970.

Hamburger, V. and Levi-Montalcini, R. Proliferation, differentiation, and degeneration in the spinal ganglia of the chick embryo under normal and

experimental conditions. J. Exp. Zool. **111**:457-502, 1949.

Harris, W. A. The effect of eliminating impulse activity on the development of the retino-tectal projection in salamanders. J. Comp. Neurol. **194**:303-317, 1980.

Harris-Flanagan, A. E. Differentiation and degeneration in the motor horn of foetal mouse. J. Morphol. **129**:281-306, 1969.

Hickey, T. L., Spear, P. D. and Kratz, K. E. Quantitative studies of cell size in the cat's dorsal lateral geniculate nucleus following visual deprivation. J. Comp. Neurol. **172**:265-282, 1977.

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

Hubel, D. H. and Wiesel, T. N. Laminar and columnar distribution of geniculo-cortical fibers in the macaque monkey. J. Comp. Neurol. **146**:421-259, 1972.

Hubel, D. H., Wiesel, T. N. and LeVay, S. Plasticity of ocular dominance columns in monkey striate cortex. Phil. Trans. R. Soc. Lond. **B278**:377-409, 1977.

Jansen, J. K. S., Thompson, W. and Kuffler, D. P. The formation and maintenance of synaptic connections as illustrated by studies of the neuromuscular junction. Prog. Brain Res. **48**:3-18, 1978.

Kuffler, S. W., FitzHugh, R. and Barlow, H. B. Maintained activity in the cat's retina in light and darkness. J. Gen. Physiol. **40**:683-702, 1957.

LeVay, S., Hubel, D. H. and Wiesel, T. N. The pattern of ocular dominance columns in macaque visual cortex revealed by a reduced silver stain. J. Comp. Neurol. **159**:559-576, 1975.

LeVay, S., Stryker, M. P. and Shatz, C. J. Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study. J. Comp. Neurol. **179**:223-244, 1978.

LeVay, S., Wiesel, T. N. and Hubel, D. H. The development of ocular dominance columns in normal and visually deprived monkeys. J. Comp. Neurol. **191**:1-51, 1980.

Lichtman, J. W. and Purves, D. The elimination of redundant preganglionic innervation to hamster sympathetic ganglion cells in early post-natal life. J. Physiol. Lond. **301**:213-228, 1980.

Meyer, R. L. Tetrodotoxin inhibits the formation of refined retinotopography in goldfish. Dev. Brain Res. **6**:293-298, 1983.

O'Brien, R. A., Östberg, A. J. C. and Vrbová, G. Observations on the elimination of polyneuronal innervation in developing mammalian skeletal muscle. J. Physiol. Lond. **282**:571-582, 1978.

Purves, D. and Lichtman, J. W. Formation and maintenance of synaptic connections in autonomic ganglia. Phys. Rev. **58**:821-862, 1978.

Rakic, P. Prenatal development of the visual system in the rhesus monkey. Phil. Trans. R. Soc. Lond. **278**:245-260, 1977.

Rodieck, R. W. Maintained activity of cat retinal ganglion cells. J. Neurophysiol. **30**:1043-1071, 1967.

Roper, S. and Ko, C.-P. Impulse blockade in frog cardiac ganglion does not resemble partial denervation in changing synaptic organization. Science **202**:66-68, 1978.

Shatz, C. J. The prenatal development of the cat's retinogeniculate pathway. J. Neurosci. **3**:482-499, 1983.

Shatz, C. J. and Stryker, M. P. Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J. Physiol. Lond. **281**:267-283, 1978.

Sherman, S. M., Guillery, R. W., Kaas, J. H. and Sanderson, K. J. Behavioral, electrophysiological, and morphological studies of binocular competition in the development of the geniculo-cortical pathways of cats. J. Comp. Neurol. **158**:1-18, 1974.

Stryker, M. P. Late segregation of geniculate afferents to the cat's visual cortex after recovery from binocular impulse blockade. Soc. Neurosci. Abstracts **7**:842, 1981.

Swindale, N. V. Absence of ocular dominance patches in dark reared cats. Nature **290**:332-333, 1981.

Thompson, W., Kuffler, D. P. and Jansen, J. K. S. The effect of prolonged, reversible block of nerve impulses on the elimination of polyneuronal innervation of newborn rat skeletal muscle fibers. Neuroscience **4**:271-281, 1979.

Wiesel, T. N. and Hubel, D. H. Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. J. Neurophysiol. **26**:978-993, 1963a.

Wiesel, T. N. and Hubel, D. H. Single cell responses in striate cortex of kittens deprived of vision in one eye. J. Neurophysiol. **26**:1003-1017, 1963b.

Wiesel, T. N. and Hubel, D. H. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J. Neurophysiol. **28**:1029-1040, 1965.

Wiesel, T. N., Hubel, D. H. and Lam, D. N. K. Autoradiographic demonstration of ocular-dominance columns in the monkey striate cortex by means of transneuronal transport. Brain Res. **79**:273-279, 1974.

PART I

Mechanisms Involved in the Control of Geniculate Cell Size in the Cat

INTRODUCTION

When a kitten or infant monkey is subjected to unequal visual inputs through the two eyes, such as that induced by monocular lid suture, striking morphological and physiological abnormalities have been shown to develop in the central visual pathways. In the visual cortex, major changes occur in the organization of the ocular dominance system, which develops postnatally and normally provides for the precise integration of inputs from the two eyes (LeVay et al., 1978; LeVay et al., 1980). After monocular deprivation within a critical period early in life (Hubel and Wiesel, 1970; Blakemore and Van Sluyters, 1974; LeVay et al., 1980), the inputs from the deprived eye throughout the visual cortex are greatly reduced in effectiveness when assessed physiologically (Wiesel and Hubel, 1963b, 1965a; Baker et al., 1974), and the afferent terminal fields to the layer IV of the cortex are shrunken when assessed anatomically (Hubel et al., 1977; Shatz and Stryker, 1977; LeVay et al., 1980). The analysis of the mechanisms of cortical ocular dominance column formation and subsequent modification has proven to be an excellent means for studying competitive interactions of synaptic terminals projecting to a common substrate.

In the lateral geniculate nucleus (LGN), the primary visual thalamic nucleus, segregation of inputs into exclusively ipsilateral and contralateral laminae develops prenatally (Anker, 1977; Rakic, 1977; Shatz, 1983), and minimal binocular interactions exist between retino-geniculate afferents (Bishop et al., 1959; however, see Singer, 1981). Thus, one might not expect extensive plasticity to occur. Indeed, no major redistribution of retinal afferents is seen between LGN laminae after extended periods of monocular deprivation, and even enucleation of one eye does not elicit large-scale translaminar growth of afferents from the normal eye (Guillery, 1972b; Hickey, 1975). What has been obvious, though, is the marked effect on LGN cell size when one eye is closed from birth: cells in LGN laminae which receive input from the sutured eye shrink in size, and cells in corresponding laminae with input from the

experienced eye increase their size (Wiesel and Hubel, 1963a; Guillery and Stelzner, 1970; Hickey et al., 1977; Hoffmann and Holländer, 1978; Kalil, 1980). This morphological response by the LGN has proven to be a valuable system for the study of the role of competition and other factors in the control of neuronal cell size, analogous to the use of cortical changes to study synaptic terminal competition.

A role for competition induced by differential activity from the two eyes in the control of LGN cell size has been implicated by the following evidence: 1) If both eyes of a kitten are sutured shut so that a relative balance of activity from the two eyes is maintained, a much smaller effect on cell size occurs in comparison to that seen after monocular deprivation (Guillery, 1973; Hickey et al., 1977). 2) The effects of monocular deprivation on cell size are not uniform throughout the LGN. In the binocular segment, where cells in adjacent laminae receive input from the same point in visual space but from different eyes, and project to the same portion of the visual cortex, strong changes in cell size are seen in response to monocular eye closure. The monocular segment of the LGN, the region with input from the monocular periphery and having no adjacent lamina, is much less affected by the deprivation (Guillery and Stelzner, 1970; Hickey et al., 1977). 3) If a focal retinal lesion is placed in the normal eye of a monocularly deprived cat, an artificial monocular segment is created, where a small region of the binocular segment with input from the sutured eye has no corresponding region in the adjacent lamina. Cells in this critical segment are not affected by the monocular deprivation, similar to what is seen in the monocular segment (Guillery, 1972a; Sherman et al., 1974).

Binocular competition, therefore, appears to be responsible for much of the change in cell size seen in the LGN after monocular deprivation. However, factors other than competition appear to influence geniculate cell size, since slight reductions in cell size are seen both throughout the LGN after long-term binocular suture (Hickey et al., 1977), and in the monocular segment after extended monocular

suture (Hickey et al., 1977; Kalil, 1980). Binocular competition cannot be invoked in either case, since a binocular balance is being maintained when both eyes are shut, and a basis for binocular interaction does not exist in the monocular segment, since it is completely segregated from inputs belonging to the other eye. These results suggest that loss of afferent activity may also influence geniculate cell size.

No experimental paradigm involving lid suture is ideal for examining the influence of afferent activity on LGN cell size, since the decrease in activity produced by lid suture is incomplete. While the closed eyelid of a kitten attenuates incident light by about 3 log units (Wiesel and Hubel, 1963a; Loop and Sherman, 1977), it is possible, for example, to stimulate cortical units through a shut eyelid (Spear et al., 1978). More importantly, retinal ganglion cells display a high tonic rate of firing of 45 $\pm$ 17 spikes/second in X cells and 24 $\pm$ 15 spikes/second in Y cells (Stone and Fukuda, 1974); this activity persists even in the dark (Kuffler et al., 1957; Rodieck, 1967; Barlow and Levick, 1969).

The question of whether afferent activity has a significant role in controlling LGN cell size and, if so, its influence in comparison to binocular competition was investigated in this study. By injecting one eye of 7-week-old kittens with tetrodotoxin (TTX), a potent sodium channel blocker, it was possible to produce a complete impulse blockade in that eye. The imbalance of activity between the two eyes produced by monocular TTX injections was greater, therefore, than that seen after monocular suture, as was the absolute decrement of activity in the treated eye. In animals subjected to monocular TTX treatment for one week, dramatic changes in cell size were observed in both the binocular and monocular regions of the LGN, suggesting a role for total afferent activity in the control of LGN cell size. These changes in cell size were of sufficient magnitude to permit the assessment of the relative contribution of differential and absolute afferent activity in the control of geniculate cell size.

METHODS

The data used in this series of experiments were obtained from 35 kittens and 4 adult cats. All of the animals were born in the laboratory breeding colony of a partially-inbred line of Tabbies. The kittens used were housed with their mothers until sacrificed. The kittens received a normal visual environment for the first seven weeks of life; no experimental manipulation was performed before that time. Two of the adults were sacrificed at 6 months of age, and two at one year of age.

Intravitreal Tetrodotoxin Injections

The primary experimental manipulation used in this series of experiments was the injection of tetrodotoxin (TTX), a potent sodium channel blocker, into the vitreal chamber of one eye of an animal. The TTX powder (Sigma) was dissolved in sterile water, at a concentration of 10^{-3} M TTX. The TTX is packaged in a mixture with citrate buffer, so the resultant concentration of citrate buffer in the dilute TTX solution was 8×10^{-3} M. The TTX injections were performed under conditions of halothane anesthesia in 66% N_2O/33% O_2. As the sclera is not visible in the cat, an incision of roughly 5 mm was made laterally from the extreme lateral margin of the eye. The surface of the sclera was exposed by cutting through connective tissue and muscle, and the fine membrane covering the eye was removed in a region around the point of injection. All injections were made superior and adjacent to the long ciliary vein that runs along the medial axis of the eye. Injections were made from a 50 µl Hamilton syringe to which a drawn pipette was affixed with epoxy. The diameter of the pipette tip was kept between 50-70 µ. A hole in sclera was made with a loose pipette of similar dimensions. The pipette tip was generally inserted 2 mm deep into the vitreous humor, and angled towards the back of the eye. An antibiotic powder (Neosporin) was applied to the wound before resuturing.

The injection of TTX into the vitreous humor of a cat's eye has been shown to result in a complete silencing of retinal ganglion cell firing (Archer et al., 1982).

Within an hour after an intravitreal injection of 30 µl of 10^{-3} M TTX, the pupillary reflex in the TTX-treated eye in response to light shone in either the treated eye or the normal eye was halted. The recovery of the pupillary response was biphasic. Roughly two days after the TTX injection, direct light stimulation into the non-treated eye was found to elicit a pupillary constriction in the TTX-treated eye. Visual stimulation directly into the TTX-treated eye at that time evoked no pupillary response. Three to four days after TTX treatment, a complete pupillary reflex in response to direct visual stimulation of the treated eye could be observed. The recovery of the pupillary reflex has been shown elsewhere (Archer et al., 1982) to precede the recovery of physiological activity in electrophysiological recordings in both the retina and in the optic tract. The pupillary reflex was closely monitored in all animals and the animals either received a second intravitreal TTX injection or were sacrificed before recovery of the second stage of the pupillary reflex.

All animals subjected to intravitreal tetrodotoxin received two injections, one on the first day and the second, about three days later depending on the observed recovery of the pupillary reflex. All but four animals so treated were then sacrificed at the end of the week-long period of TTX-induced deprivation. The four remaining animals underwent a recovery period of three weeks before sacrifice.

Monocular Lid Suture

Five animals were monocularly sutured for comparison to the effects of monocular TTX injections. For suturing, the lid margins were trimmed and sutured together. Ophthalmic vetromycin was applied to the cornea and lid margins. The kittens were checked every day for openings along the sutured lid margins. No openings were observed in any of the animals during the week-long period of monocular suture.

Histology

After administration of a lethal dose of nembutal, the animals whose LGN were to be celloidin-embedded for cell area measurements were perfused through the heart with warm 0.9% saline followed by 10% formol-saline. Animals whose LGN were to be processed for cytochrome oxidase were perfused through the heart with warm 0.1 M phosphate buffer at pH 7.4, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. For all animals, a single block of tissue containing the entire extent of both lateral geniculate nuclei was cut. For cell area measurements, the blocks were embedded with celloidin using the rapid infiltration techniques described by Humason (1972), whereby the infiltration was done in a span of 24 hours at 56°C. Coronal sections, 40 µm in thickness, were cut from these blocks, mounted on slides treated with Gatenby solution, and stained with cresyl violet for 8 minutes at 23°C.

The blocks to be treated for cytochrome oxidase were placed in a 30% sucrose/ 0.1 M phosphate buffer solution, and 40 µm sections were cut on a freezing microtome once the tissue blocks had sunk in the sucrose solution. The sections were incubated at 37°C in the dark in a solution containing diaminobenzidine, cytochrome c type III (Sigma), sucrose, and phosphate buffer (Wong-Riley, 1979) with the addition of 20 µg/ml catalase (Seligman et al., 1968) for 3-5 hours (until the LGN was well stained), then washed in five minutes three times in 0.25 M phosphate buffer.

Cell Measurements

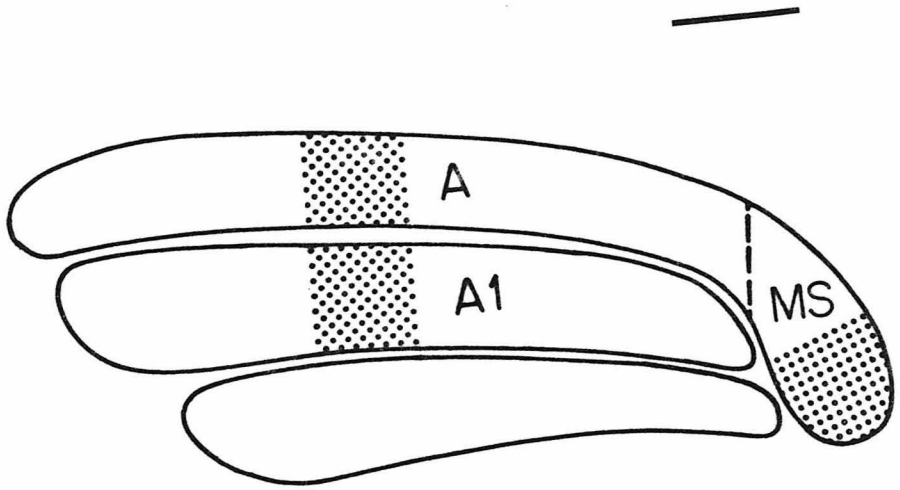
The cross-sectional areas of 420 LGN neurons were measured from each animal. In order to minimize the possibility that prior knowledge of the animal's experimental condition might bias the drawing of cell outlines, each animal's identification number was replaced by a code unknown to the researcher, until quantification of the cell outlines was completed. The measurements were made from cells in the mediolateral extent of the binocular segment of laminae A and A1 and from the monocular

segment of lamina A (the region that extends beyond lamina A1) at an anteroposterior plane corresponding to Sanderson's (1971) coronal 5, representing the central 10° of the visual hemifield (see Figure 1). Seventy cell areas from each of the three regions described above (binocular A, A1, and monocular A) were measured from both lateral geniculate nuclei. Cells were drawn at a total magnification of 900X using a camera lucida and Leitz microscope. Only cell bodies whose nucleoli were visible were scored. Cell area outlines were drawn in an orderly fashion from the dorsal edge of the lamina descending perpendicularly to the ventral border, and back again. These outlines were then processed on a Tektronix 4956 digitizing tablet coupled to a Tektronix 4052 terminal.

Statistical Analysis

Comparison of all the cell sizes between corresponding laminae within the same animal were made using Student's t-test for independent variables, since the number of cells measured per lamina was large enough to meet the conditions of that test. For comparisons of corresponding laminae between animals the Mann-Whitney U test, a nonparametric assay, was utilized. For each animal the mean and standard deviation of the cells measured in each lamina were determined. Each mean was used as a single observation in the nonparametric analysis for significant difference between animals. The means $\pm$ standard error for all the laminae are presented in the two tables. The (grand) mean $\pm$ standard error of the individual mean cell sizes from corresponding laminae of all the animals in a single experimental condition is depicted in the graphs.

Figure 1. A schematic drawing of a coronal section through the middle of the lateral geniculate nucleus (LGN) of a kitten. Medial is to the left, dorsal is up. Lamina A receives input exclusively from the contralateral eye and lamina A1, from the ipsilateral eye. Where they lie adjacent, corresponding regions of each lamina receive input from the same area of visual space and project to the same point in the visual cortex. The lateral extent of lamina A is referred to as the monocular segment, since it receives input only from the monocular visual periphery. The stippled zones indicate the sites of cell measurement. The dotted line represents the border between the binocular and monocular segments of lamina A. The scale bar corresponds to 500 μm.


A
A1
MS

RESULTS

Normal Animals — The Controls

In order to determine whether the changes in LGN cell size seen in the TTX-treated animals were due to shrinkage or hypertrophy or both, a comparison with normal animals was necessary. The control animals were divided into 3 groups, 4 animals per group. Since there is no evidence that geniculate neurons in normal animals are larger in one hemisphere than in the other, cell areas were measured from only one LGN per animal, except for animals 536 and 545 (see 1 below).

1) To test for the occurrence of hypertrophy in the active laminae of the TTX-treated kittens, the appropriate comparison is with normal 8-week-old kittens, the age at which the TTX-treated animals were sacrificed. The four 8-week-old normals used as controls in this study were divided into two groups: two animals (536 and 545) received two monocular injections of citrate buffer, the vehicle solution for TTX, as a control for the injections themselves, and the other two animals (518 and 519) were not subjected to any treatment. Those two animals given citrate injections had larger cell areas overall, but comparison between corresponding laminae indicated no significant difference in cell area due to the injection itself. The mean cell areas for the 4 animals are shown in Table 1; data from only one hemisphere (chosen at random) of the citrate-treated animals are shown.

2) To address the question of whether cell shrinkage or simply cessation of growth occurred in the inactive laminae of the TTX-treated kittens, a control group of 7-week-old normal kittens (the age at the beginning of treatment) was needed. The mean cell areas are shown in Table 1.

3) Since one group of experimental animals was placed in the dark during the week of monocular TTX treatment, a control group was reared similarly, with the exception of the TTX treatment: 7 weeks in a normally lit environment followed by one week in the dark. The mean cell areas are listed in Table 1.

TABLE 1

Mean ± S.E. (μm^2) of laminar cell sizes of all control animals

Animal	Treatment	Side of measurement	A	A1	MS
518	8 wk normal	R	202 ± 6	194 ± 6	184 ± 6
519	8 wk normal	L	216 ± 6	247 ± 8	197 ± 5
536	8 wk citrate	L	250 ± 7	281 ± 9	218 ± 6
545	8 wk citrate	R	254 ± 6	291 ± 7	230 ± 7
552	7 wk normal	R	213 ± 6	240 ± 6	192 ± 5
556	7 wk normal	R	258 ± 5	256 ± 8	236 ± 5
573	7 wk normal	L	208 ± 6	221 ± 7	174 ± 5
580	7 wk normal	L	233 ± 6	282 ± 8	228 ± 5
633	7 wk normal/1 wk dark	L	220 ± 6	255 ± 8	221 ± 5
641	7 wk normal/1 wk dark	R	232 ± 5	262 ± 7	238 ± 6
647	7 wk normal/1 wk dark	L	240 ± 7	301 ± 9	226 ± 5
648	7 wk normal/1 wk dark	R	227 ± 7	270 ± 7	209 ± 5
	Grand mean ± S.E.		229 ± 5	258 ± 9	213 ± 6

Each value is the mean ± standard error of 70 cell areas from the indicated lamina of a single animal given in μm^2. A and A1 refer to laminae A and A1, respectively; MS refers to the monocular segment of lamina A. Rearing conditions are given in weeks (wk). Normal refers to rearing in a normally lit environment. Citrate refers to normally lit rearing and two intravitreal injections of 8×10^{-3} M citrate buffer, the vehicle solution used in the TTX injections, during the eighth week. Dark refers to placement in a completely dark room for the indicated duration. The side of measurement refers to the hemisphere from which cell outlines were drawn. The grand mean ± standard error for each laminae region was calculated by using each mean as a single observation.

A comparison of the mean cell sizes of corresponding laminae between these control groups showed no significant differences ($U = 7\text{-}8$, $p > 0.4$). The lack of difference between the 7-week-old and 8-week-old normals was not surprising since other studies had indicated that LGN neurons stop growing by about 7 weeks of age (Kalil, 1978a; Hickey, 1980). Similarly, the lack of difference between 8-week-old normals and 7-week normal/1-week dark-reared animal was unremarkable, even though Kalil (1978b) has demonstrated a modest slowing of cell growth in animals dark-reared from birth. Since there was no significant difference between these three control subsets, they were grouped together when statistical comparisons were made between normal animals and an experimental group.

The variability of cell areas in these normal animals is of some interest. For every group of experimental and control animals, a large amount of variability in the average cell size was seen. This could be due to several factors, primarily differential shrinkage during preparation of the tissue blocks, and a real difference in cell size between animals. This has been observed in all quantitative studies of geniculate cell area (Kalil, 1978; Hickey, 1980; Kalil, 1980) and does not adversely affect the results presented here, though the amount of statistical analysis possible is reduced.

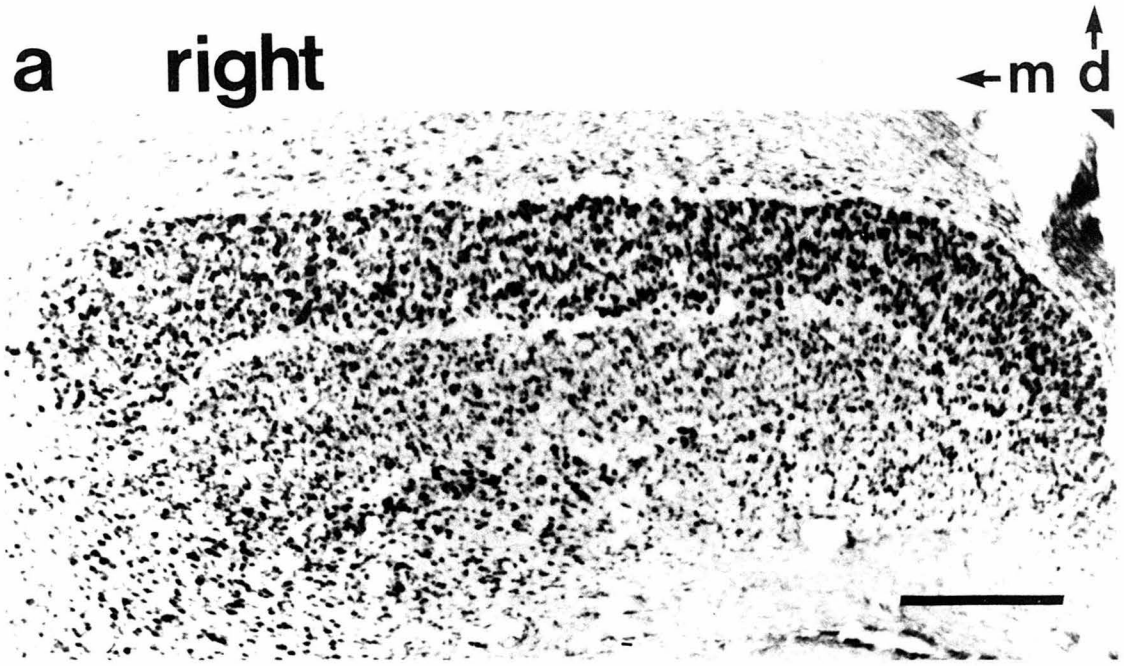
The Effect of Monocular TTX in a Lighted Environment on LGN Cell Size

Four kittens were raised in a normally lighted environment until seven weeks of age. They were then subjected to a one-week unilateral blockade of both visually-evoked and tonic retinal ganglion cell activity induced by monocular TTX injections. One week of monocular impulse blockade resulted in obvious morphological abnormalities in the LGN, so much so that quantitative analysis was not necessary for their detection. Cells in laminae that received input from the TTX-treated eye showed reduced intensity of Nissl stain relative to those with input from the normal

eye, as can be seen in Figure 2. The right LGN has a very heavily stained lamina A and a more lightly stained lamina A1. Lamina A1 received inputs from the ipsilateral (right) eye, leading one to guess (correctly) that the right eye was subjected to TTX treatment. The inverse pattern is observed in the left LGN. There is a constant intensity of stain in both left and right laminae A from the binocular region (medial) to the monocular segment (lateral). This appearance is qualitatively different from animals subjected to long-term monocular suture, where the deprived monocular segment is more lightly stained than the binocular segment (see Guillery and Stelzner, 1970, plate 1). The appropriate comparison of cell sizes is not between lamina A and A1 of the same LGN but between corresponding laminae from each hemisphere. A representative view of this is seen in Figure 3a, with laminae A and A1 of the left LGN on the left, and laminae A and A1 of the right LGN on the right. Differences in cell size can be seen between cells in the inactive laminae (receiving input from the eye given TTX injection) versus the active laminae. Figure 3b compares the relative sizes of the right (nontreated) and left (TTX-treated) monocular segments of the same animal. Even at this low power view, differences in cell size can be discerned.

The cell areas were quantified and the mean cell areas $\pm$ standard error are presented in Table 2. The change in cell size was calculated by subtracting the mean areas of corresponding inactive and active laminae and dividing by the mean cell area of the active lamina. One week of monocular blockade of both visually evoked and spontaneous retinal activity resulted in an average change in mean cell size between inactive and active corresponding laminae of 28% in lamina A (range 23-33%) and 31% in lamina A1 (range 23-37%). This result is similar in magnitude to that seen in animals that were lid sutured from birth for many months (Hickey et al., 1977), but were elicted here in one week. The fact that the percent change in lamina A1 is larger than in lamina A (31% versus 28%) is consistent with the findings in monocularly sutured animals (Hickey et al., 1977; Kalil, 1980) but it is not

Figure 2. Blocking all retinal activity in one eye of a seven-week-old kitten for one week produces striking morphological abnormalities in the LGN. a) Coronal section through the right LGN. Dorsal is up, medial to the left. The right eye of this animal was silenced with intravitreal injections of tetrodotoxin (TTX). Lamina A1, which receives input from the ipsilateral (TTX-treated) eye is thinner and more lightly stained than lamina A, which receives input from the contralateral (active) eye. b) Coronal section through the left LGN. Dorsal is up, medial to the right. An inverse pattern of staining is seen, with lamina A now less heavily stained and thinner than lamina A1, which in this hemisphere receives input from the untreated eye. Note that the staining of lamina A is constant throughout its extent. In animals subjected to long-term monocular lid suture from birth, the monocular segment is much less affected by the deprivation than the binocular segment, so a differential in staining intensity is observed. The scale bar corresponds to 500 μm.


a right
m
d


b left
d
m

Figure 3. The appropriate comparison of the effects of monocular inactivation is between corresponding laminae from the two hemispheres. a) A magnified view of the left and right binocular segments of the coronal sections in Figure 2. Cells in laminae with input from the inactive retina (lamina A at left, lamina A1 at right) are noticeably smaller and more lightly stained than in their corresponding laminae with input from the untreated eye (lamina A at right, lamina A1 at left). The scale bar represents 200 μm. b) A magnified view of the left and right monocular segments of lamina A. The cells in the left (inactive) monocular segment are smaller and more lightly stained than in the right MS. This effect is not seen in kittens monocularly lid sutured from birth for many months. Scale bar indicates 200 μm.

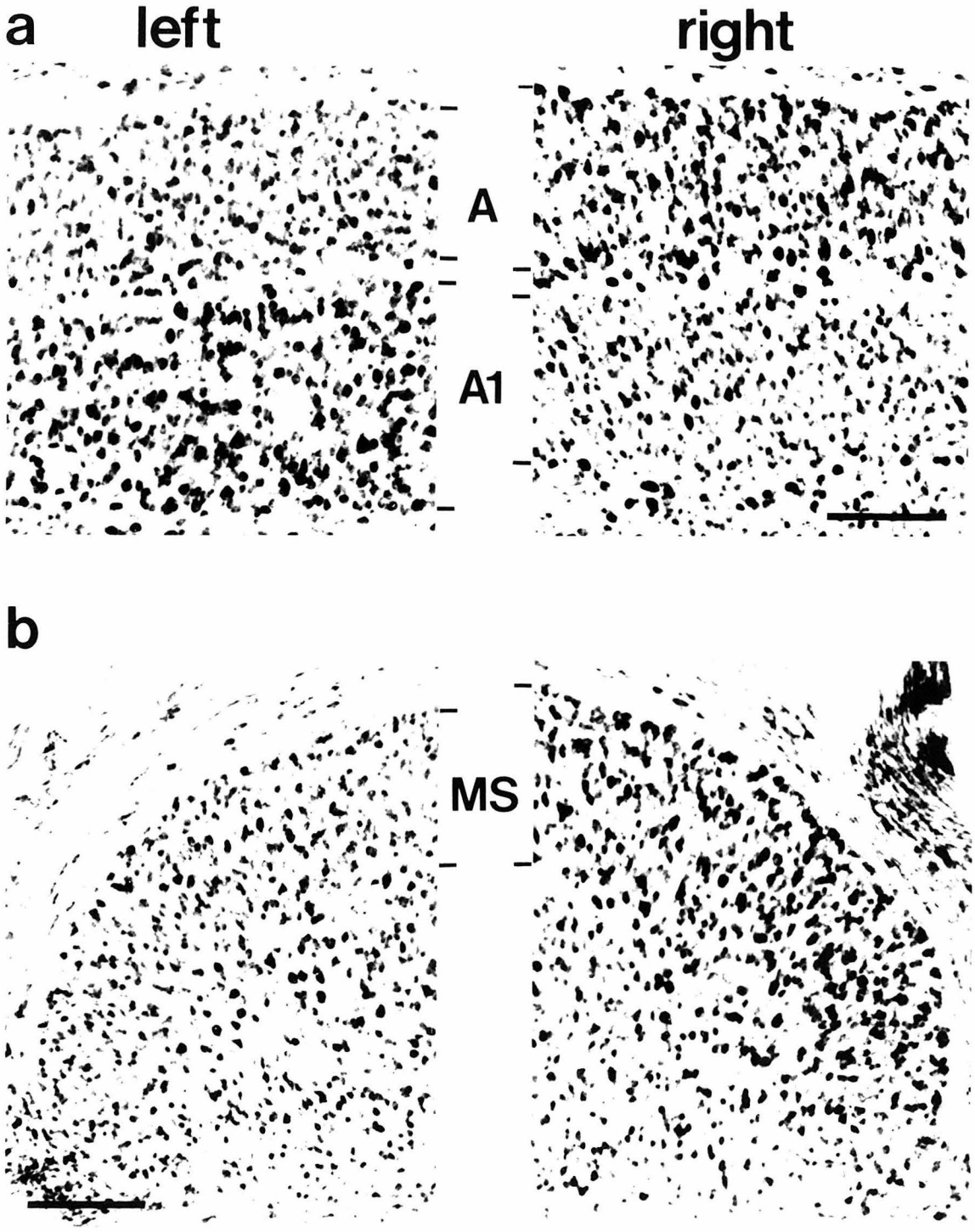

a
left
right
A
A1
b
MS

TABLE 2

Mean ± S.E. (μm^2) of laminar cell sizes of all experimental animals

		A			A1			MS		
Animal	Treatment	Inactive	Active	% Δ	Inactive	Active	% Δ	Inactive	Active	% Δ
TT1	TTX-light	186 ± 5	278 ± 8	33%	201 ± 6	284 ± 9	29%	189 ± 5	228 ± 7	17%
TT3		180 ± 5	249 ± 8	27%	171 ± 5	272 ± 7	37%	170 ± 5	218 ± 6	22%
492		190 ± 5	246 ± 8	23%	219 ± 7	285 ± 7	23%	181 ± 4	226 ± 5	20%
530		196 ± 5	270 ± 10	28%	196 ± 5	298 ± 9	35%	179 ± 4	230 ± 6	22%
Grand mean ± S.E.		188 ± 3	251 ± 8	28%	197 ± 10	285 ± 5	31%	180 ± 4	225 ± 3	20%
327	TTX-dark	181 ± 4	215 ± 5	16%	206 ± 4	244 ± 7	16%	167 ± 5	206 ± 5	19%
331		188 ± 5	236 ± 6	20%	192 ± 5	243 ± 7	21%	169 ± 5	203 ± 5	17%
481		187 ± 5	215 ± 6	13%	215 ± 6	250 ± 7	14%	168 ± 4	202 ± 5	17%
578		200 ± 6	228 ± 6	12%	205 ± 6	277 ± 7	26%	184 ± 4	210 ± 4	13%
Grand mean ± S.E.		189 ± 4	223 ± 5	15%	204 ± 5	253 ± 8	19%	172 ± 4	205 ± 2	16%
TT5	Recovery	216 ± 6	245 ± 10	12%	246 ± 8	249 ± 8	1%[2]	216 ± 6	232 ± 6	7%[1]
560		216 ± 7	248 ± 7	13%	265 ± 8	270 ± 9	2%[2]	209 ± 5	226 ± 7	8%[1]
592		210 ± 6	231 ± 6	9%[1]	257 ± 8	254 ± 8	1%[2]	204 ± 6	212 ± 7	4%[2]
593		217 ± 5	234 ± 7	7%[1]	257 ± 6	244 ± 8	-5%[2]	196 ± 4	216 ± 5	9%[1]
Grand mean ± S.E.		215 ± 3	240 ± 8	10%	255 ± 7	254 ± 11	0%	206 ± 8	221 ± 9	7%

TABLE 2 (continued)

546	Lid suture	222 ± 6	260 ± 9	15%	228 ± 5	267 ± 9	15%	196 ± 3	204 ± 5	4%[2]
557		197 ± 4	204 ± 6	3%[2]	195 ± 5	223 ± 7	13%	174 ± 6	185 ± 5	6%[2]
572		198 ± 6	219 ± 6	10%[1]	227 ± 6	245 ± 7	7%[1]	211 ± 4	204 ± 5	-3%[2]
579		265 ± 7	276 ± 9	4%[2]	257 ± 8	282 ± 11	9%[1]	217 ± 4	235 ± 7	8%[1]
Grand mean ± S.E.		220 ± 16	240 ± 17	8%	227 ± 13	254 ± 13	11%	200 ± 10	207 ± 10	3%
502	Adults	218 ± 6	263 ± 8	17%	233 ± 7	290 ± 8	20%	193 ± 6	247 ± 9	22%
549		307 ± 9	375 ± 14	18%	329 ± 9	405 ± 12	19%	280 ± 8	337 ± 8	17%
574		220 ± 7	276 ± 9	20%	282 ± 8	336 ± 12	16%	235 ± 5	281 ± 7	17%
628		282 ± 6	323 ± 9	13%	271 ± 9	339 ± 7	20%	242 ± 8	277 ± 7	13%
Grand mean ± S.E.		256 ± 22	309 ± 25	17%	279 ± 20	343 ± 24	19%	237 ± 18	285 ± 19	17%

Each value is the mean ± standard error of 70 cell areas from the indicated lamina of a single animal, given in μm^2. Inactive refers to laminae with input from the TTX-treated eye; active refers to untreated laminae. The %Δ was determined by dividing the difference in mean cell area between corresponding active and inactive laminae of single animal by the mean cell area of the active lamina. Absence of a superscript above the %Δ indicates a statistically significant difference at $p < 0.001$. Superscript 1 refers to differences significant at $p < 0.05$. Superscript 2 refers to differences that were not significant at $p < 0.1$. Treatment code is as follows: TTX-light refers to one week of monocular TTX-induced inactivation beginning at age 7 weeks while the animal was left in a normally lit environment. TTX-dark animals were treated similarly with the exception that during the week of TTX treatment the animal was placed in a completely dark room. Recovery animals were subjected to one week of monocular TTX treatment beginning at age 7 weeks and then allowed three weeks of recovery from the TTX treatment. Lid suture animals were normally reared for 7 weeks and then monocularly lid sutured for one week. Adults were normally reared and then subjected to one week of monocular TTX treatment.

statistically significant. The monocular segment of lamina A exhibited a smaller but significant difference in cell size between the inactive and active segments of 20% (range 17-22%). The change in cell size in the monocular segment of TTX-treated animals differs markedly from the results seen after long-term monocular lid suture. Six months of monocular suture are required for a significant difference between the deprived and nondeprived monocular segments to be observed, and even then the magnitude is smaller than seen here (Guillery and Stelzner, 1970; Hickey et al., 1977; however, see Kalil, 1980).

Is the change in cell size in the binocular regions of the geniculate produced by a hypertrophy of cell area in the nondeprived laminae or a shrinkage of cells in the deprived laminae? Figure 4 compares cell areas between the control animals and between active and inactive laminae of the animals given one week of monocular retinal blockade. The statistical analysis for growth in cell size between the active laminae of the TTX-treated ($n = 4$) and normal ($n = 12$) animals showed a significant increase in cell size over normal in the active laminae A ($U = 6$, $p < 0.025$), and in the active laminae A1 ($U = 9$, $p < 0.05$). In the active monocular segment of lamina A, a slightly greater cell size was observed relative to normal controls. This increase, however, was not statistically significant ($U = 15$, $p > 0.1$). These results indicate an actual increase of cell size in the active binocular regions of the LGN, but little or no increase of cell size in the monocular segment.

Analysis for decrease in cell size in the inactive laminae showed that cells in all the inactive regions of the LGN, both binocular and monocular, were significantly smaller than normal (lamina A: $U = 0$, $p < 0.001$; lamina A1: $U = 3$, $p < 0.01$; monocular segment: $U = 4$, $p < 0.01$). The laminar cell area distributions of a TTX-treated kitten are shown in Figure 5. The distributions from corresponding active and inactive laminae are suggestive of an effect on all cell sizes, with both large and small cell populations responding to the TTX treatment.

Figure 4. Changes in geniculate cell size after one week of monocular TTX-induced inactivation. Mean cell areas ± standard error from laminae with input from the active eye are indicated by open circles (O) and those from laminae with silenced input are indicated by filled circles (●). The shaded regions represent the grand mean cell areas ± standard error of the 12 control kittens shown in Table 1. Cells in inactive laminae A, A1, and the monocular segment (MS) were all smaller than normal, while cells in the active binocular segments (A and A1) were larger than normal. The active MS showed no significant increase in cell size. The decrease in cell size in the monocular segment is probably due to the loss of afferent activity, since no binocular interactions exist in that region.

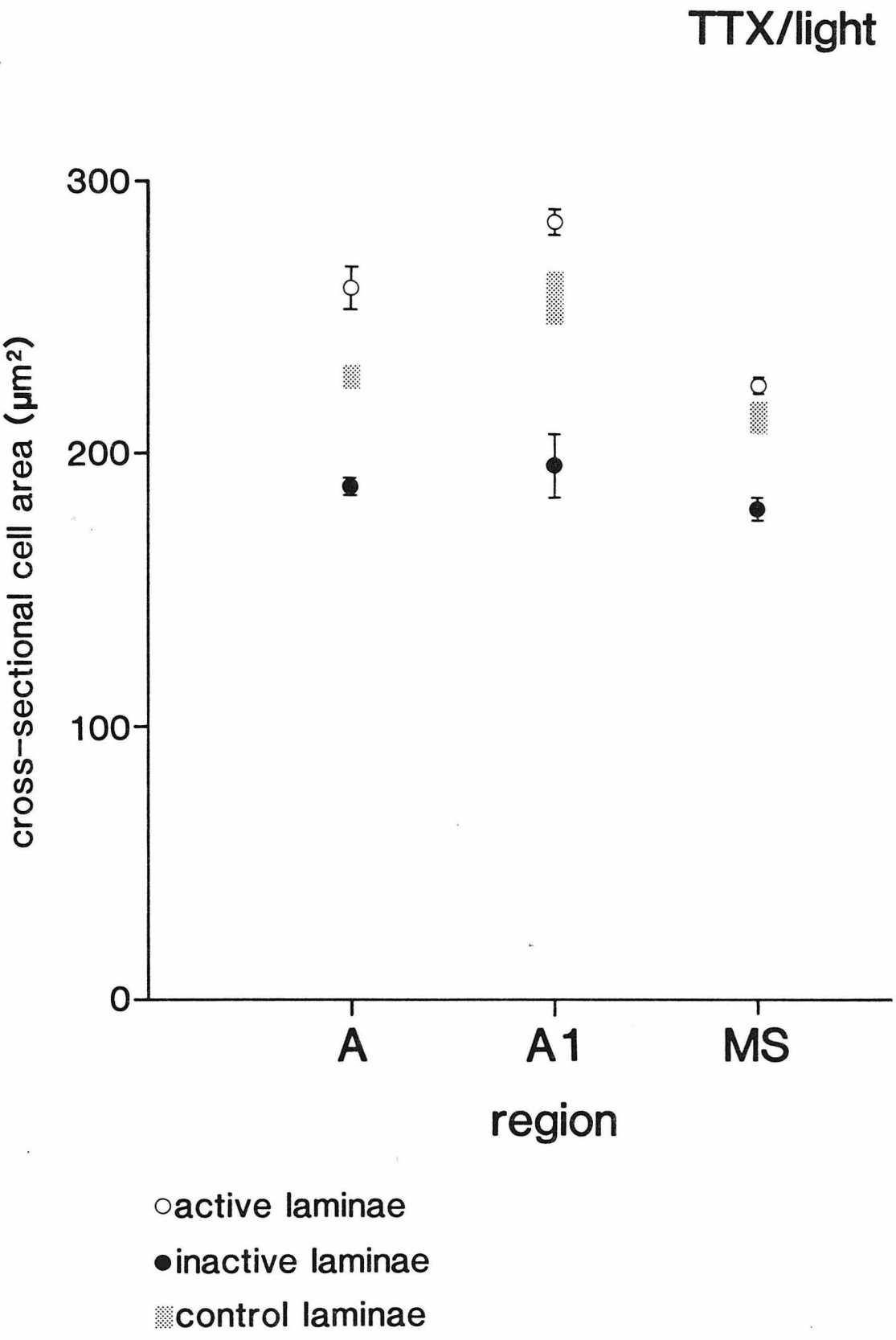
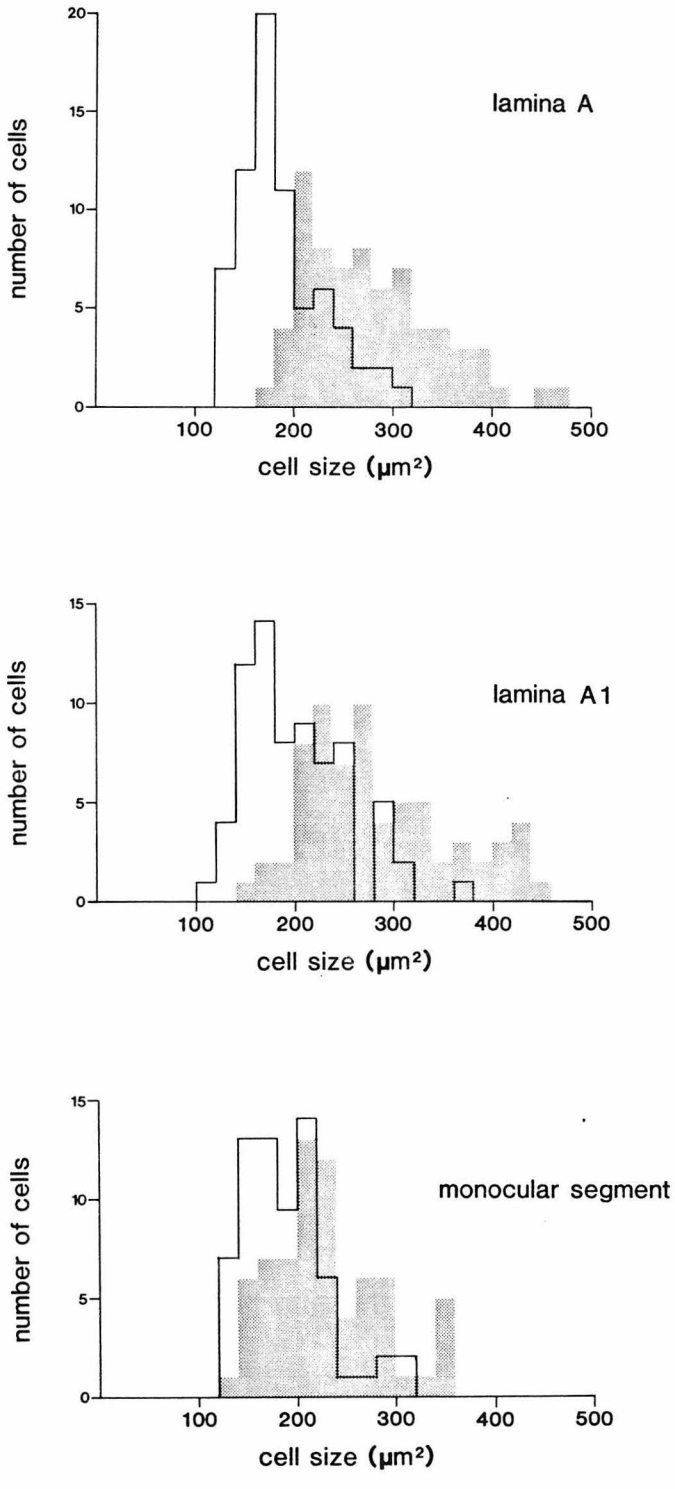

TTX/light
300
200
100
0
cross-sectional cell area (μm²)
A
A1
MS
region
○active laminae
●inactive laminae
▒control laminae

Figure 5. Comparison of the cell size distributions between active and inactive laminae of a kitten (TT1) subjected to monocular TTX treatment for one week. Each histogram is composed of 70 cells. In lamina A (a), lamina A1 (b), and the monocular segment (c), the effect of TTX treatment is seen on both large and small cell sizes; no obvious subpopulation of cells appears to be exclusively responsible for the observed response to the TTX treatment.

LGN CELL SIZE DISTRIBUTION AFTER 1 WEEK MONOCULAR TTX



In summary, 7-week-old kittens subjected to one week of TTX-induced retinal blockade exhibited cell shrinkage and expansion in inactive and active laminae, respectively, in the binocular segment of the LGN. In the monocular segment, only shrinkage of deprived cells was observed, with no significant change in size observed in cells receiving input from the untreated, active eye. The reduction of cell size in the monocular segment, where no binocular interaction exists, suggests that the total loss of afferent activity was responsible for this change.

TTX Blockade While in the Dark

In the previous experiment, the monocular TTX injection produced a severe imbalance of retinal input: both visually evoked and tonic retinal firing in one eye versus no electrical activity in the other eye. In this experiment, four normal 7-week-old kittens were given monocular TTX treatment but placed in a dark room for the week-long period of deprivation. (Since the effective duration of one TTX injection was roughly three days, the animals were removed from the dark for a second TTX injection midway through the week of deprivation. Their eyes were firmly taped over and they were returned to the dark within 15 minutes.) The resulting imbalance of ocular activity was due to tonic activity in one eye versus no activity in the other.

Qualitatively obvious morphological changes in the LGN were observed in these animals. Figure 6 shows the right and left geniculates of an animal that was placed in the dark for the week-long period of TTX treatment.

Quantified results of cell area calculations from each animal are presented in Table 2. Cells in the binocular segment of the inactive laminae A were 15% smaller (range 12-20%) than cells in the active A laminae (Figure 7). Similarly, inactive A1 cells were 19% smaller (range 14-26%) than active A1 cells. In the monocular segment of lamina A, the mean area of cells with input from the inactive eye was

Figure 6. The effects of one week of monocular TTX inactivation while in the dark. a) A coronal section through the right LGN. Medial is to the left. Lamina A, with input from the untreated eye, is more heavily stained than lamina A1. b) Coronal section through the left LGN, where the inverse pattern of staining is seen. Medial is to the right. Scale bar represents 500 μm.

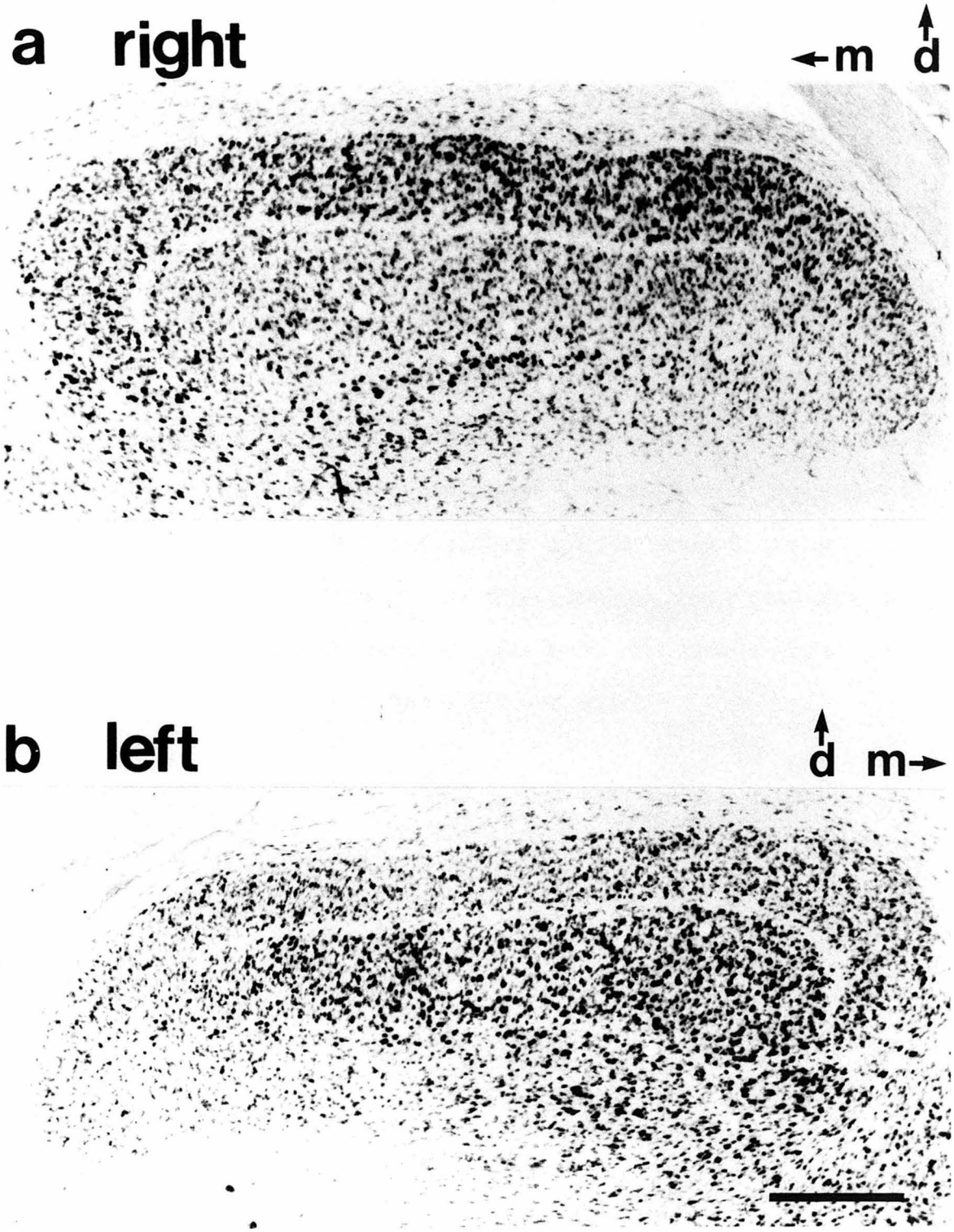
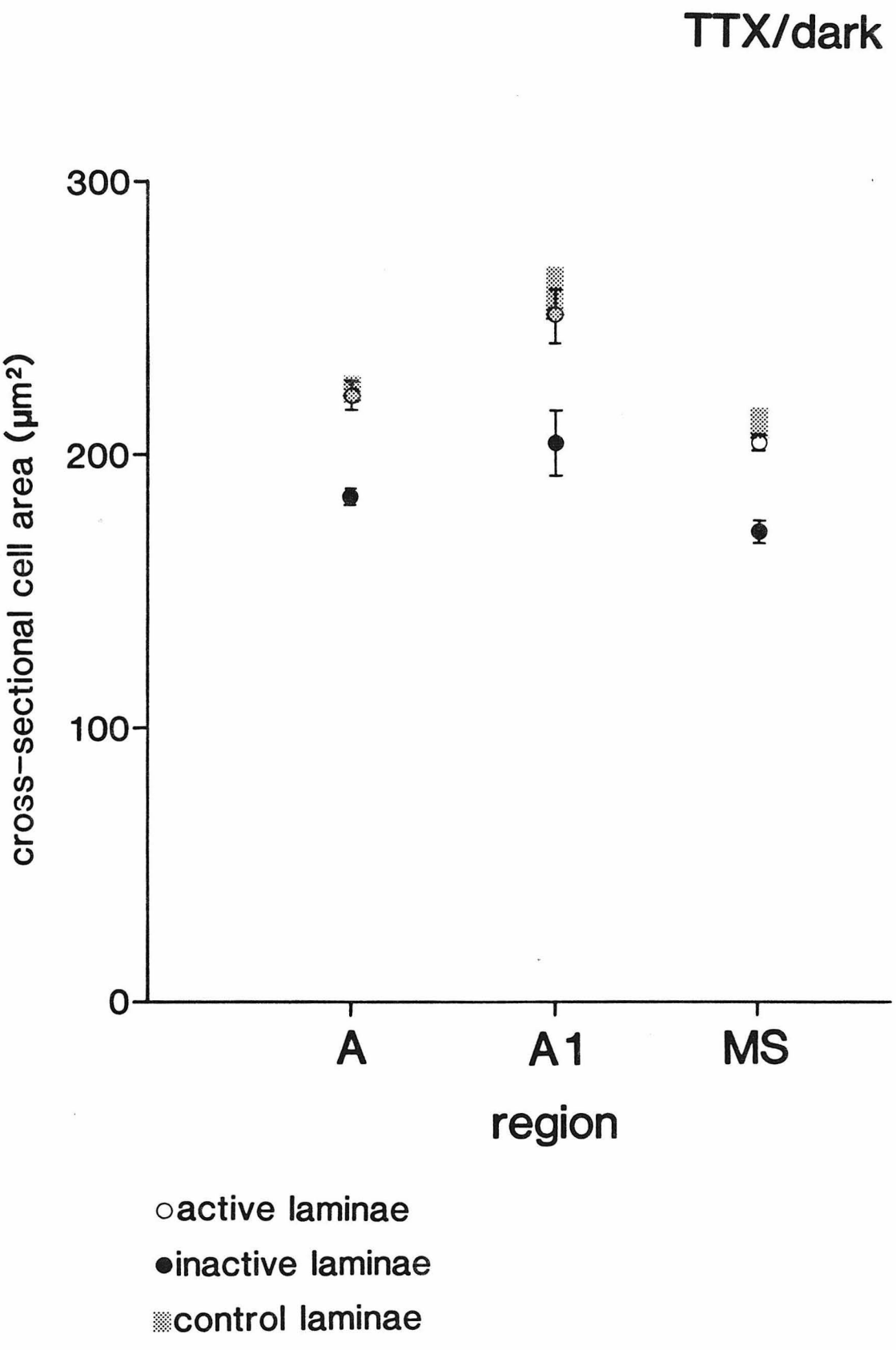

a right
←m
d
b left
d
m→

Figure 7. Changes in geniculate cell size after one week of monocular TTX treatment while in the dark. Mean cell areas ± standard error for active (open circles), inactive (filled circles) and control laminae (shaded) are shown. No expansion of cell size was observed in the active laminae, and a relatively uniform decrease of cell size was seen throughout the LGN. The results suggest that the decrease in cell size was due to the loss of afferent activity.


TTX/dark
300
200
100
0
cross-sectional cell area (µm²)
A
A1
MS
region
○active laminae
●inactive laminae
▒control laminae

16% smaller (range 13-19%) than that of active cells. In all regions of the geniculate of these animals, comparison with control kittens indicated that the active cells were not significantly different from normal; no expansion was evidenced ($U = 17\text{-}20$, $p > 0.1$). Cells in the inactive layers were, however, significantly smaller than normal in the binocular segment of lamina A ($U = 0$, $p < 0.001$), in lamina A1 ($U = 3$, $p < 0.01$) and in the monocular segment of lamina A ($U = 1$, $p < 0.01$).

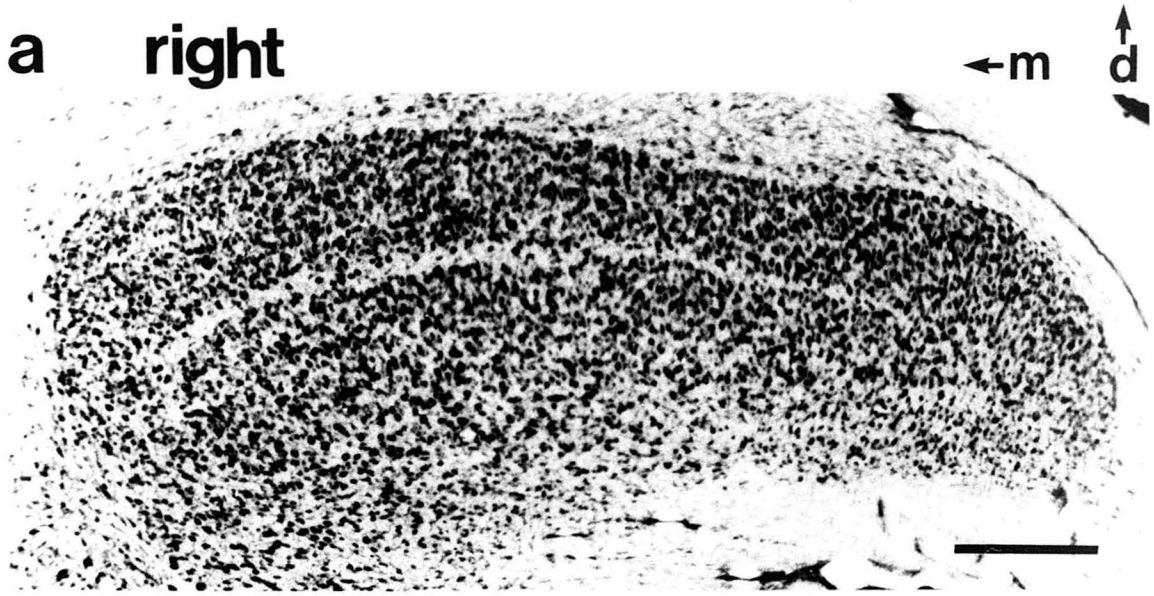
In summary, 7-week-old normal kittens were subjected to monocular TTX injection and placed in the dark for the week-long period of retinal blockade. Uniform shrinkage throughout the geniculate in laminae corresponding to the inactive eye was observed with no expansion of cell size in active laminae.

Recovery From the Effects of TTX

Are the morphological abnormalities observed in the LGN after 1 week of monocular TTX permanent, or is recovery possible after the pharmacological effect of TTX has worn off? Four kittens were reared identically to those in the first experiment described here: 7 weeks of normal rearing, followed by two monocular TTX injections during the eighth week. The animals were then allowed a three-week period of recovery from the TTX injections before being sacrificed and subjected to cell area analysis.

A sample LGN from one of these recovery animals is shown in Figure 8. The gross appearance of the LGN is very different from those geniculates seen earlier. Lamina A and A1 are uniformly stained, and cell areas appear to be similar. Cell area measurements, shown in Table 2, indicated a difference between TTX-treated and untreated laminae A cell areas of 10% (range 7-12%). Lamina A1 previously inactive and previously active cell areas showed no difference in cell size (range -5%-2%), while a difference of 7% (range 4-9%) was seen between treated and untreated monocular segment cell areas. Statistical comparison of these cell areas with normal animals (Table 1) showed no statistically significant difference ($U = 12\text{-}22$, $p > 0.15$)

Figure 8. Recovery from the effects of monocular TTX treatment. Within three weeks after the end of the week of TTX-induced retinal inactivity, the LGN is normal in appearance, in comparison to what was seen in Figure 2. Medial is to the left in (a) and to the right in (b). The scale bar corresponds to 500 μm.


a right
←m
d

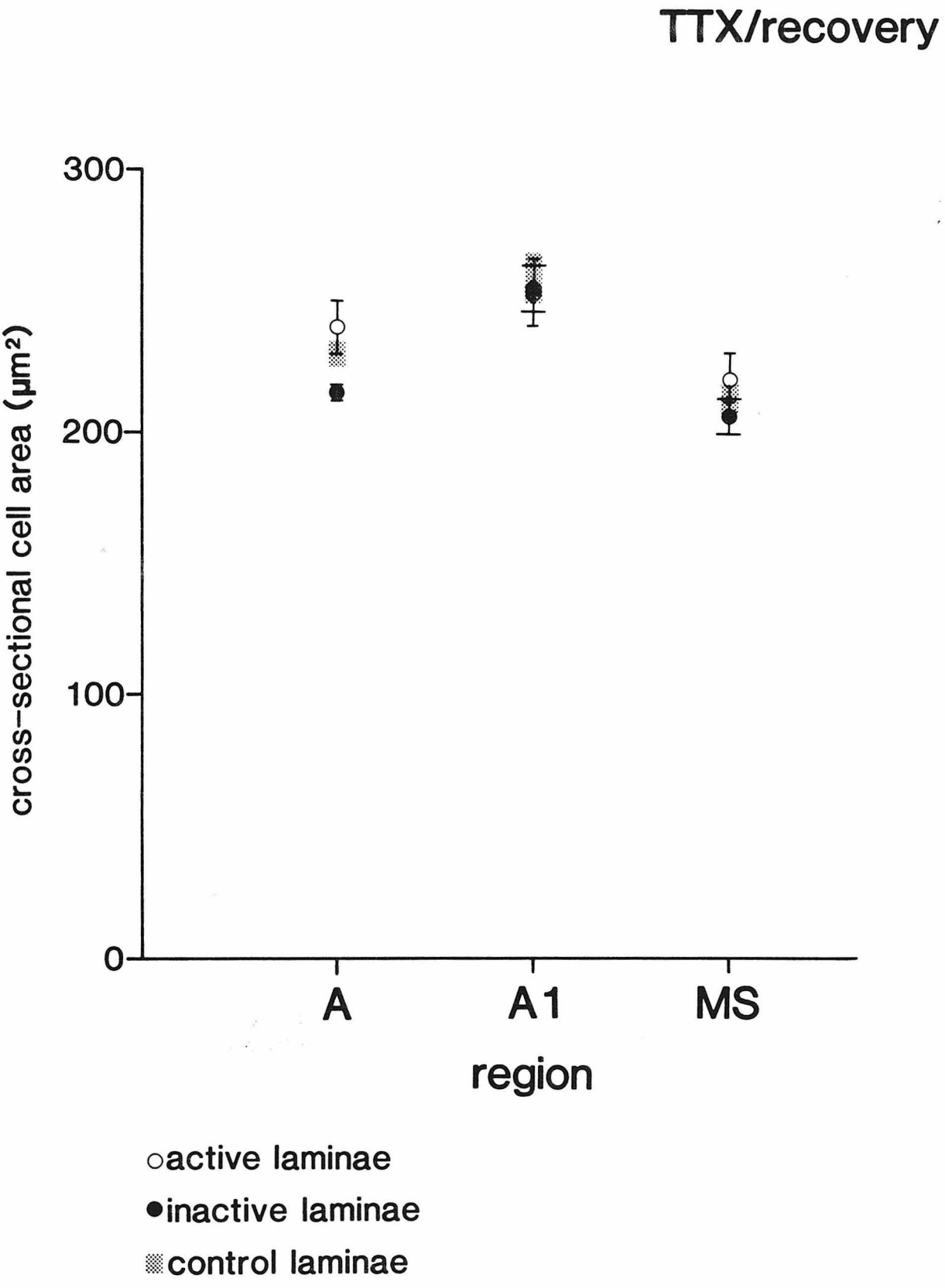

b left
d
m→

between any of the geniculate regions in the recovery animals and corresponding regions in the normals. A graphical comparison of these two groups is made in Figure 9. A statistical comparison of cell areas between TTX treated, recovery animals and TTX treated, non-recovery animals indicated a significant difference between corresponding laminae in these animals. Thus, mean cell areas that decreased due to TTX treatment increased during recovery ($U = 0$, $p < 0.015$ for all regions), while previously enlarged cell areas decreased in the recovery process (lamina A, $U = 1$, $p < 0.03$; lamina A1, $U = 0$, $p < 0.015$). While recovery was not complete at this point (note the difference in cell size in lamina A), the results indicate that the effects of intravitreal TTX were not degenerative, since a significant amount of recovery of cell size was possible within a short time after the end of the period of uniretinal blockade.

Effect of 1-Week Monocular Lid Suture

Studies of the effect of monocular lid suture on geniculate morphology typically have involved suture of one eyelid shortly after birth, with examination of the effects of the lid suture on LGN cell size some weeks or months later. The intent here was to determine whether changes in geniculate cell size could be observed in a lid suture paradigm analogous to the TTX treatment period. Four animals were reared normally for seven weeks, then had one eyelid sutured shut for one week. Cell area measurements from these animals are shown in Table 2. Small differences in cell size between corresponding deprived and nondeprived laminae were observed in the binocular portions of the geniculate: in lamina A, a difference of 8% (range 3-15%) between mean area in deprived and nondeprived laminae was measured, and as was a difference of 11% (range 7-15%) between corresponding A1 laminae. A very small difference of 3% (range -3-6%) was seen in the monocular segment of lamina A. None of these cell areas, however, was significantly different from normal when

Figure 9. Recovery of cell size after monocular TTX treatment. A comparison of the mean cell areas ± standard error between previously treated (filled circles), previously untreated (open circles), and control laminae (shaded) indicates that afer a 3-week period of recovery from the monocular TTX treatment, cell areas in all regions of the LGN were not significantly different from normal. The difference between previously active and inactive lamina A was significant, however, indicating that recovery was not complete.


TTX/recovery
300
200
100
0
cross-sectional cell area (µm²)
A
A1
MS
region
○active laminae
●inactive laminae
▒control laminae

compared with the control kittens (U = 11-21, p >0.1). The comparison is represented graphically in Figure 10. The results suggest that lid suture at this age and of this duration may produce a slight decrease of LGN cell size in deprived binocular lamina, with no expansion of cell size in binocular nondeprived laminae and no change in the monocular segment.

Changes in Cytochrome Oxidase Levels After TTX Treatment

Is the observed change in LGN cell size after monocular retinal blockade mirrored by a change in enzymatic activity of the geniculate neurons? Wong-Riley (1979) has shown that a five-month monocular lid suture from birth significantly reduces levels of the enzyme cytochrome oxidase, involved in electron transport and oxidative phosphorylation, in geniculate laminae receiving input from the deprived eye of the cat. Since one week of TTX-induced monocular inactivation affected cell areas in the LGN as dramatically as a many-month monocular lid suture, an analysis for a corresponding change in cytochrome oxidase levels was made. Seven geniculate blocks were stained for the presence of cytochrome oxidase. Of these seven animals, three received monocular TTX injections for one week, two received monocular citrate injections to control for the effects of injection alone, one was untreated, and one received a one-week lid suture, all starting at age 7 weeks. The cytochrome oxidase stain does not show selective staining of cell bodies, so the comparisons were restricted to inspection for possible density differences. As can be seen in Figure 11, the amount of variation of label between corresponding laminae was slight. The top pair (Figure 11a) of geniculates were subjected to retinal blockade of the left eye with TTX. The right (inactive) lamina A shows an overall reduction in staining intensity relative to the adjacent lamina A1, with a gradient in intensity from the binocular segment (lightest) to the monocular segment (heaviest). In the other (left) hemisphere, no difference in staining density can be seen between (inactive)

Figure 10. Changes in geniculate cell size after 1 week of monocular lid suture starting at 7 weeks of age. Comparisons of deprived (filled circles), nondeprived (open circles) and control (shaded) mean cell areas indicate modest amounts of shrinkage in the deprived lamina, and no growth in the nondeprived laminae. The effects are significantly smaller than those of monocular TTX injections at this age and duration of treatment.

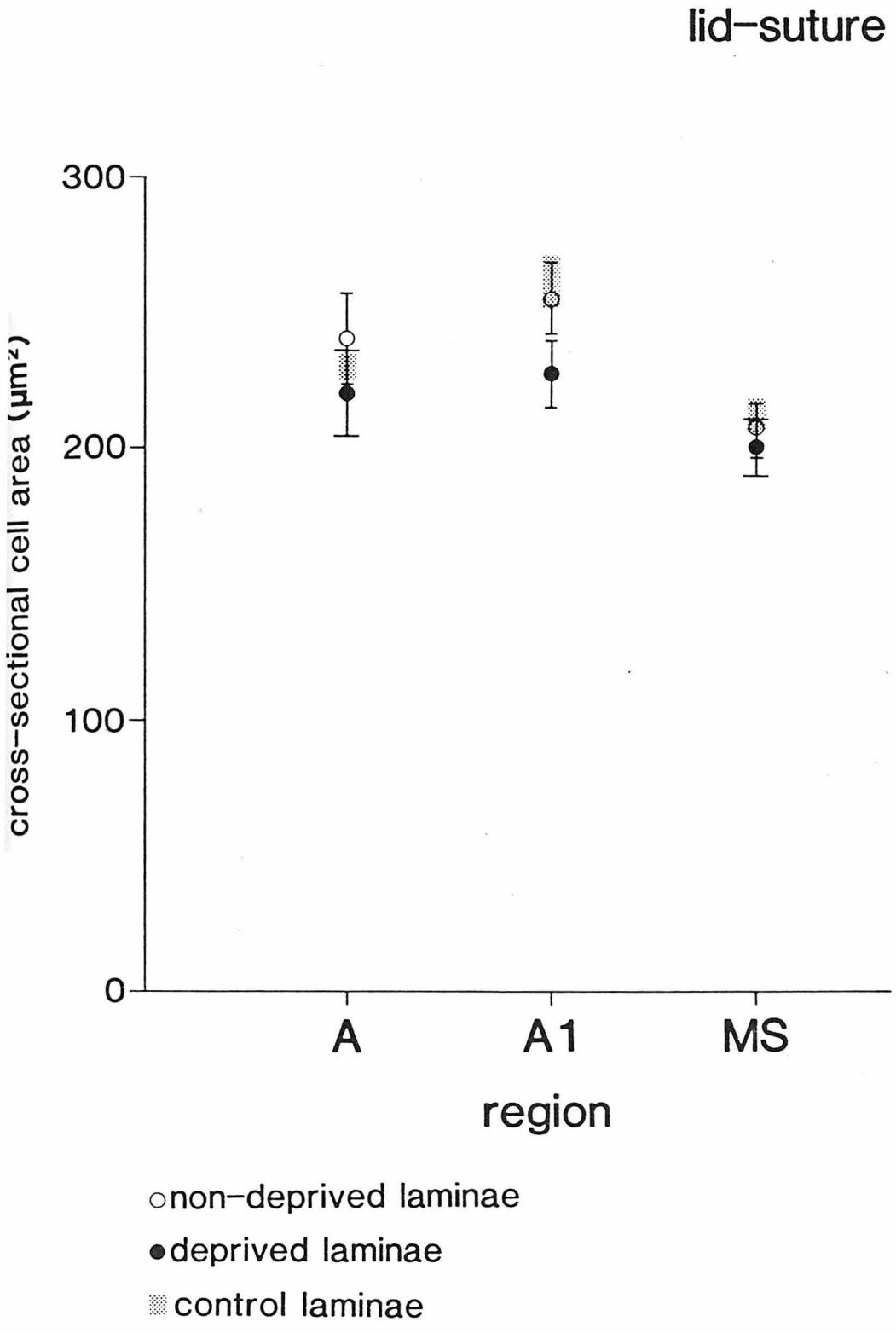
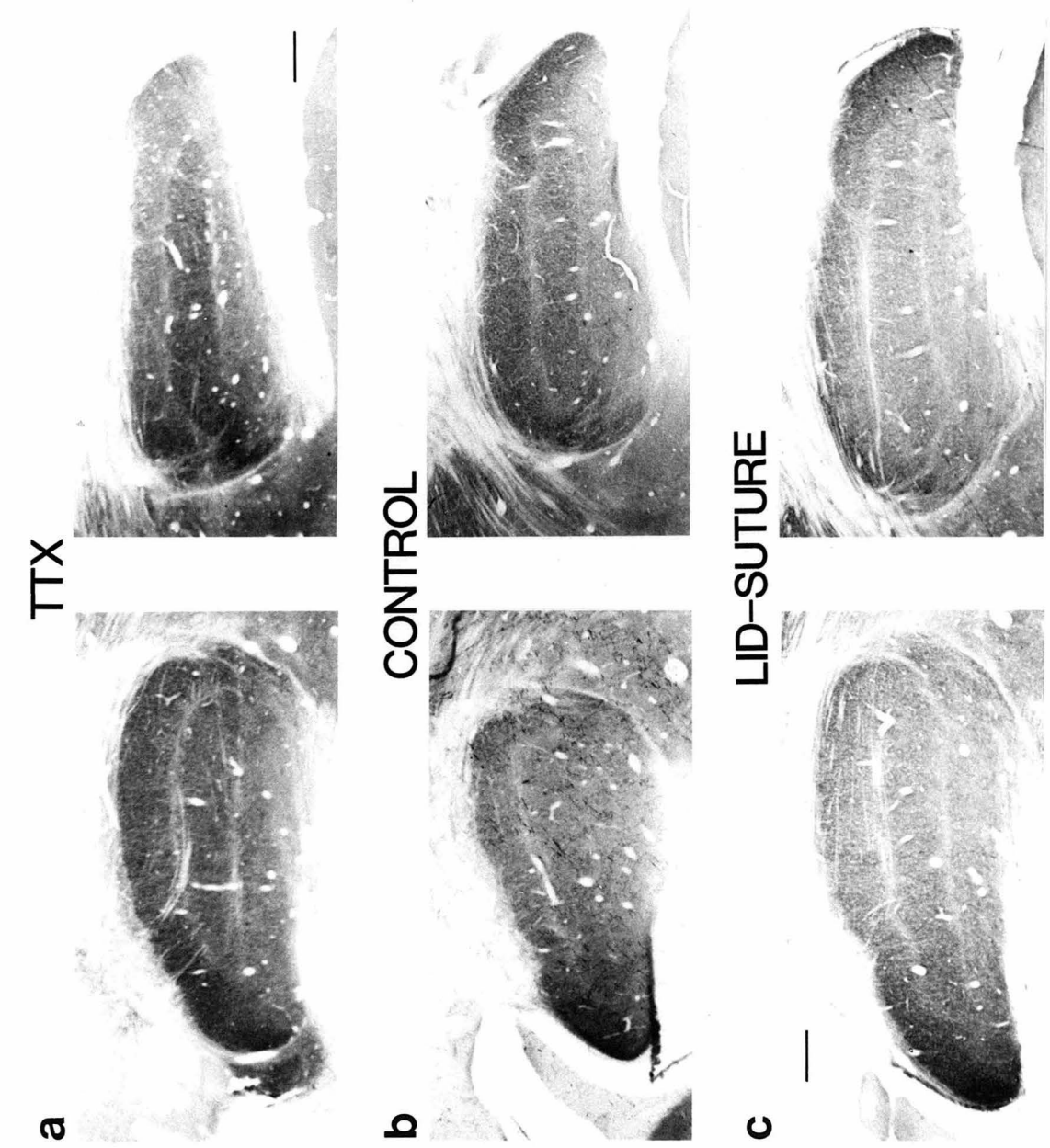

lid-suture
300
200
100
0
cross-sectional cell area (µm²)
A
A1
MS
region
○non-deprived laminae
●deprived laminae
▒control laminae

Figure 11. The effects of monocular TTX treatment in kittens on cytochrome oxidase activity. Comparison of the differential staining within each LGN between a one-week monocular TTX animal (a), a control animal (b), and a one-week monocular lid suture animal (c). In all animals the lateral third of lamina A stained more heavily than the more medial portions of the lamina. Only a slight reduction in staining is observed in the inactive (right) lamina A of the TTX animal, though larger differences in staining after longer periods of monocular TTX treatment of adult cats have been reported elsewhere (Wong-Riley and Riley, 1983). Scale bar corresponds in 500 μm.


TTX
CONTROL
LID–SUTURE
a
b
c

lamina A1 and (active) lamina A. The middle pair (Figure 11b) is from a normal animal. A gradient in stain intensity is again observed, with both left and right monocular segments and the lateral binocular segments more heavily stained than the medial binocular segments. The gradient (but not the overall reduction) in staining between the monocular and binocular segments of the TTX-treated kitten (Figure 11a) may be due to this normal difference. The bottom LGN (Figure 11c) are from a kitten given a left eyelid suture at age seven weeks for one week, and are not visibly different from that of a normal kitten. A report has recently been published investigating the effects of monocular TTX injections into adult cats on levels of cytochrome oxidase activity (Wong-Riley and Riley, 1983). The results of that study are in general agreement with the present findings, in that slight reductions in staining intensity in inactivated laminae were seen by them after one week of TTX treatment; longer periods of inactivation produced more obvious changes in cytochrome oxidase levels. The gradient in staining intensity between inactive binocular and monocular segments was also noted in that study. The presence of this gradient in staining in lamina A of TTX-treated adults, where a uniform change in cell size is observed (see next section), supports the suggestion that the differential stain across the extent of the inactive lamina A simply reflects an endogenous gradient in cytochrome oxidase levels. The significance of changes in cytochrome oxidase levels is not clear; these results serve mainly to indicate that large changes in geniculate cell size are not necessarily accompanied by correspondingly large changes in cytochrome oxidase levels.

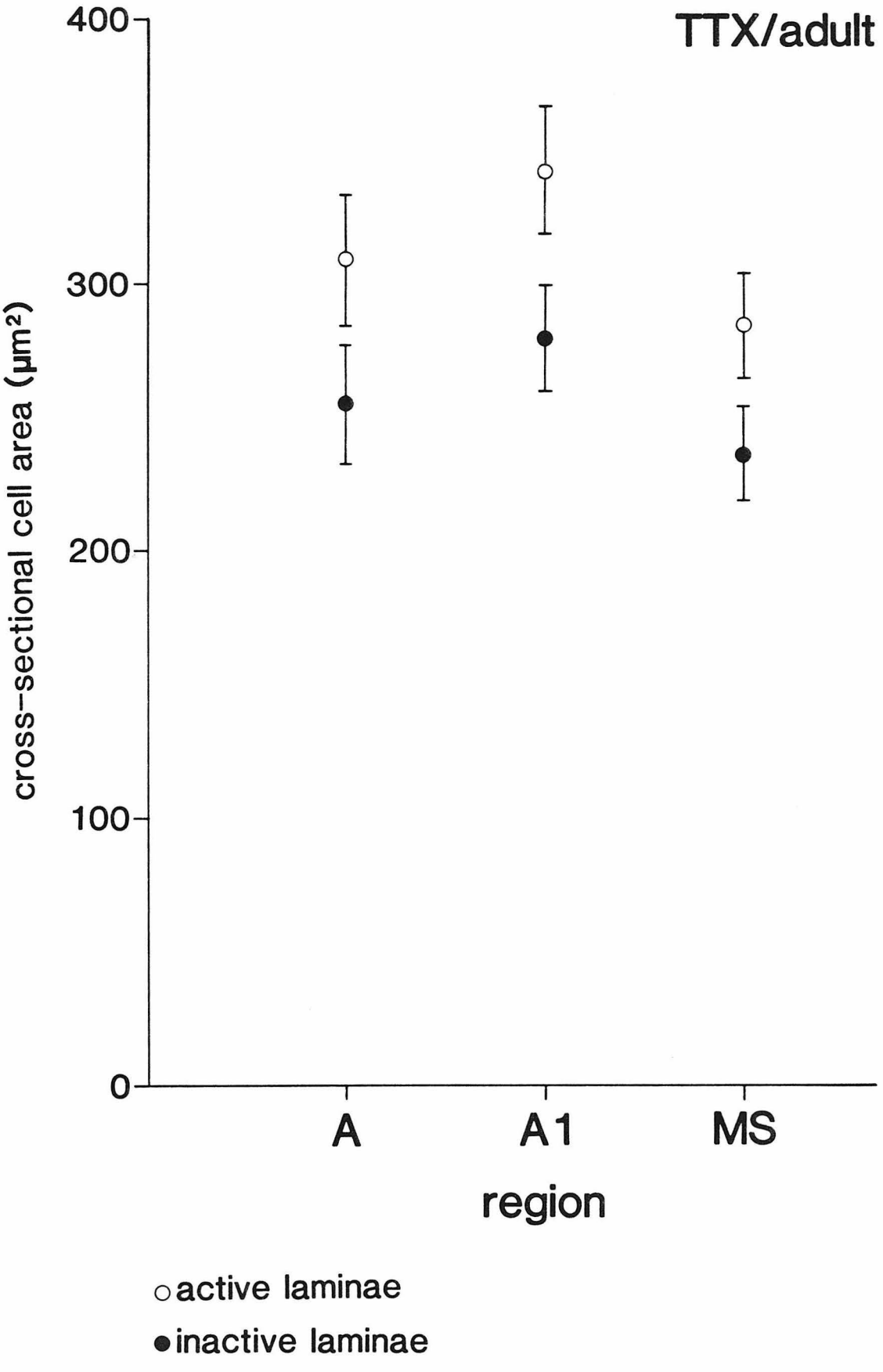
The Effect of Monocular TTX on Adult Cats

The critical period in cats of susceptibility to the effects of monocular suture on cortical physiology ends at roughly three months of age (Hubel and Wiesel, 1970; Blakemore and Van Sluyters, 1974). The effects of monocular lid suture on geniculate

neuron morphology are also restricted to a critical period (Wiesel and Hubel, 1965b; Sherman and Wilson, 1981). The morphological state of the geniculate at the end of the critical period, regardless of its degree of abnormality, becomes permanent at that time. An important exception to the permanence of post-critical period geniculate morphology is the experiment of Spear and Hickey (1979) where kittens were monocularly sutured at birth until adulthood, at which time the nondeprived eye was enuclated, leaving only the sutured eye. A full recovery of cell size was observed in those animals. Enucleation, however, is a particularly traumatic manipulation, resulting in the presence of degeneration products throughout the geniculate. Is a non-degenerative signal, such as unilateral retinal blockade with TTX, sufficient impetus for a change in LGN cell size in an adult?

Four cats were reared normally until either 6 months (574 and 628) or 1 year of age (502 and 549). At that time they were subjected to the same one-week period of monocular retinal blockade induced by TTX. The quantified results are presented in Table 2. The change in cell size between inactive and active corresponding laminae was uniform across the geniculate: in lamina A, 17% (range 13-20%); in lamina A1, 19% (range 16-20%); and in the monocular segment, 17% (range 13-22%). The results are presented graphically in Figure 12. The uniformity of the change in cell size throughout the geniculate and the magnitude of the change (roughly 20%) was very similar both to that of the TTX plus dark-reared animals, and also to the magnitude of the change in cell size in the monocular segment in the TTX plus light-reared animals. Those animals, in comparison with their controls, exhibited no expansion of cell size in the active laminae; rather the change in cell size was due exclusively to a decrease in cell size in the inactive laminae. This suggests that the change in cell size in adults was due to a shrinkage of cells in inactive laminae.

Figure 12. Changes in cell size after one week of monocular TTX treatment in adult cats. The difference in mean cell size ± standard error between active (open circles) and inactive (filled circles) laminae was uniform throughout the LGN, similar to what was seen after monocular TTX treatment in the dark. The absence of a larger effect in the binocular region suggests that the change in cell size was due to the loss of afferent activity, and not to the relative imbalance of activity from the two eyes.


TTX/adult
400
300
200
100
0
cross-sectional cell area (μm²)
A
A1
MS
region
○ active laminae
● inactive laminae

DISCUSSION

Differential Activity versus Absolute Activity in the Control of Geniculate Cell Size

The demonstration by Guillery and Stelzner (1970) that the effects on cell size induced by differential activity from the two eyes is restricted to the binocular segment of the LGN provides a basis for comparison with the present results. By examining the different effects in the binocular and monocular segments, it is possible to assess the roles of differential and total afferent activity on LGN cell size. 1) Kittens that were subjected to monocular TTX treatment and left in a normally lit environment exhibited a greater difference in cell size in the binocular segment than in the monocular segment, consistent with the binocular competition hypothesis (Guillery, 1972) that differential activity from the two eyes more strongly affects the binocular segment. However, the presence of a significant decrease in cell size in the monocular segment cannot be accounted for by binocular competition, since the monocular segment is a region devoid of binocular interaction. The total loss of afferent activity from the treated eye must be responsible for this reduction in cell size. While the net change in cell size was larger in the binocular segment than in the monocular segment of these animals, the decrease in cell size in the inactive laminae (as compared to normal controls) was uniform throughout the LGN. Since the loss of afferent activity was responsible for the reduction in the monocular segment, the implication is that the cell shrinkage seen throughout the LGN was also due to the abolition of afferent activity. The increase in cell size in the binocular segment is presumably due to the effects of binocular competition. In fact, the magnitude of the shrinkage was greater than that of the expansion (20% versus 10%), so the loss of afferent activity exerted a greater net effect on cell size than did the imbalance of activity from the two eyes. 2) The kittens that were placed in the dark during the week of monocular blockade of retinal ganglion cell activity exhibited

significant reductions in cell size throughout the LGN. However, no significant difference was observed between the binocular and monocular segments. This uniformity of response across the LGN suggests that the changes were due to the loss of afferent activity. The magnitude of the decrease in cell size in these animals was very similar to that of the TTX/light animals. In fact, the homogeneity of the mean cell sizes in corresponding inactive laminae throughout the TTX/light and TTX/dark animals (Table 2) is remarkable considering the normal variability of cell sizes. The imbalance of neuronal activity in the TTX/dark animals did not appear to contribute to the observed change in cell size, suggesting that an imbalance of tonic activity only (no visual activity in the dark) is not a strong competitive stimulus. 3) Adults subjected to the same one-week period of unilateral blockade of retinal ganglion cell activity exhibited significant changes in cell size, uniformly throughout the LGN. The extent of the critical period in the visual cortex, during which binocular competition can modify neuronal connectivity, is known to be restricted to the first 3-5 months (Hubel and Wiesel, 1970; Blakemore and Van Sluyters, 1974; Cynader and Mitchell, 1980). However, the critical period for the LGN is less clearly defined. Several studies report that cell sizes are no longer modifiable if one eye is sutured at 14 weeks of age (Wiesel and Hubel, 1965b; Cragg et al., 1976; Sherman and Wilson, 1981), though enucleation after the age of three months can result in LGN cell size changes (Garey and Dursteler, 1975; Spear and Hickey, 1979). Early monocular suture appears to reduce the number of Y cells encountered in the deprived laminae (Sherman et al., 1972; but see Shapley and So, 1980); reverse suturing at 10 months of age can reverse this physiological abnormality (Hoffmann and Cynader, 1977). Regardless, the results here in the adult showed that after the end of the cortical critical period, monocular TTX treatment affected geniculate size significantly. There was no significant difference in the extent of this change between the monocular and binocular segments, suggesting that the loss of afferent activity and

not the imbalance of activity from the two eyes was responsible for this change in cell size.

The Role of Afferent Activity on Neuronal Connectivity

The decrease in cell size after monocular impulse blockade in areas not involved in binocular interactions suggests that geniculate neurons are shrinking in response to either a general loss of activity or specifically in response to a loss in tonic afferent activity. A significant role for afferent activity has been demonstrated in a variety of processes including synapse maintenance and elimination in the mammalian neuromuscular junction (Benoit and Changeux, 1978; Thompson et al., 1979), presynaptic sprouting and terminal distribution in the neuromuscular junction (Brown and Ironton, 1977) and the development of normal geniculate physiology (Archer et al., 1982). However, the importance of afferent activity is not ubiquitous; studies have indicated that halting afferent activity does not affect the development of retinotectal connections in the salamander (Harris, 1980, but see Meyer, 1983 for effects in goldfish), or result in sprouting of heart ganglion cells (Roper and Ko, 1978).

Some recent studies on the role of visual and tonic activity in segregation of ocular dominance columns in the visual cortex are pertinent here. Cynader and Mitchell (1980) have shown that rearing a kitten from birth in the dark preserves the capability of the animal to undergo synaptic modification beyond the end of the normal critical period (see Part II of this thesis). Swindale (1981) has recently demonstrated by autoradiographic means the absence of ocular dominance columns in layer IV of the primary visual cortex in dark-reared cats, though there is some debate as to whether the segregation is completely absent or merely reduced from normal. A study by Stryker (1981) used a technique similar to that presented in this work, intravitreal TTX injections, to compare the rate of segregation of ocular dominance

columns in the cat between normal animals, dark-reared animals, and animals reared with binocular TTX-induced blockade of retinal ganglion cell activity. The results indicate a graded effect in the rate of segregation of ocular dominance, where segregation is reduced in dark-reared animals and completely halted in animals deprived of both visually evoked and spontaneous retinal activity.

The results presented here suggest similar graded effects of afferent activity on LGN cell size. In animals whose afferent activity is reduced but not completely blocked, such as occurs in binocular or monocular lid suture, the effects on cell size due to the loss and not the imbalance of visual activity are either small in magnitude or require a long time for development, or both. In monocularly sutured animals, for example, shrinkage of cell size in the monocular segment occurs to a significant degree only after 6 months of lid suture (Hickey et al., 1977) though recent reports indicate more rapid and pronounced changes in the monocular segments of some cats (Hickey, 1980; Kalil, 1980). Animals subjected to a more complete blockade of retinal activity, as were seen in this study, demonstrate rapid and large changes in geniculate size. The simplest hypothesis for this graded effect is that tonic and evoked activity contribute in equivalent fashion to the level of activity relevant to non-competitive interactions. If this were true, the total level of activity would be related in some (non-linear) manner to the reduction of geniculate cell size. A possible test for this hypothesis would be to slightly increase the level of afferent activity in a TTX-treated kitten through direct stimulation of the optic nerve and examine the resultant changes in LGN cell size. Alternatively, tonic firing may be carrying an activity-dependent trophic factor necessary for the maintenance of cell size. While it is likely that patterned activity is important for competitive interactions leading to cell hypertrophy, analogous to what is seen in the neuromuscular junction (Lømo and Westgaard, 1975), the specific role of tonic activity in the visual pathways remains unclear.

Neuronal Circuitry Responsible for LGN Cell Size Changes

One of the suppositions of the binocular competition hypothesis for the regulation of geniculate cell size (Guillery, 1972a) is that the site of competition is in the cortex. According to the hypothesis, the size of the geniculate cell body is related to the extent of its axonal arborization in layer IV of the visual cortex. The imbalance of visual activity produced by monocular lid suture would result in expansion or compression of the geniculo-cortical terminal field, and this synaptic modification would be reflected in a change in geniculate cell size. A quantitative relationship has, in fact, been established between geniculate cell size and the width of ocular dominance columns in the visual cortex of monkeys (LeVay et al., 1980). However, the presence of binocular inhibition within the LGN suggests the possibility that the competition may simply occur between local geniculate afferents, since ablation of the visual cortex does not reduce this inhibition (Rodieck and Dreher, 1979; Sanderson et al., 1971). To date, no experimental paradigm has been able to demonstrate the involvement of the cortex in the changes in LGN cell size observed after monocular deprivation.

An experimental procedure has been developed recently by the author which may provide the means of investigation of the question. By locally perfusing tetrodotoxin directly into one cortical hemisphere through a minipump/canula system (see Part II of this thesis), it is possible to completely silence a region of cortex roughly 6 mm in diameter. The perfusion does not affect either the LGN or the other cortical hemisphere. Within 12-24 hours after the end of the cortical TTX perfusion, cortical activity returns to normal. If the cortex is involved in geniculate cell size change due to binocular competition, then the expansion of cell size seen in the binocular segment of the kittens in the present study subjected to monocular TTX treatment might be reduced due to cortical inactivity. Preliminary results indicate that this is indeed the case, suggesting that the site of the competitive interactions

that regulate geniculate cell size is in the visual cortex. The continued reduction of cell size throughout the LGN seen in these kittens implies that the noncompetitive interactions regulating LGN cell size are retinogeniculate. Further investigations are necessary, however, to verify these points.

REFERENCES

Anker, R. L. The prenatal development of some of the visual pathways in the cat. J. Comp. Neurol. **173**:185-204, 1977.

Archer, S. M., Dubin, M. W. and Stark, L. A. Abnormal development of kitten retinogeniculate connectivity in the absence of action potentials. Science **217**:743-745, 1982.

Baker, F. H., Grigg, P. and von Noorden, G. K. Effects of visual deprivation and strabismus on the response of neurons in the visual cortex of the monkey including studies on the striate and prestriate cortex in the normal animal. Brain Res. **66**:185-208, 1974.

Barlow, H. B. and Levick, W. R. Changes in the maintained discharge with adaptation level in the cat retina. J. Physiol. Lond. **202**:699-718, 1969.

Benoit, P. and Changeux, J.-P. Consequences of blocking the nerve with a local anesthetic on the evolution of multi-inervation at the regenerating neuromuscular junction of the rat. Brain Res. **149**:89-96, 1978.

Bishop, P. O., Burke, W. and Davis, R. Activation of single lateral geniculate cells by stimulation of either optic nerve. Science **130**:506-507, 1959.

Blakemore, C. and Van Sluyters, R. C. Reversal of the physiological effects of monocular deprivation in kittens: further evidence for a sensitive period. J. Physiol. Lond. **237**:196-216, 1974.

Brown, M. C. and Ironton, R. Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials. Nature **265**:459-461, 1977.

Cragg, B., Anker, R. and Wan, Y. The effects of age on the reversibility of cellular atrophy in the LGN of the cat following monocular deprivation: A test of two hypotheses about cell growth. J. Comp. Neurol. **168**:345-354, 1976.

Garey, L. J. and Dursteler, M. R. Reversal of deprivation effects in the lateral geniculate nucleus of the cat. Neurosci. Lett. **1**:19-23, 1975.

Guillery, R. W. Binocular competition in the control of geniculate cell growth. J. Comp. Neurol. **144**:117-127, 1972a.

Guillery, R. W. Experiments to determine whether retinogeniculate axons can form translaminar collateral sprouts in the dorsal lateral geniculate nucleus of the cat. J. Comp. Neurol. **146**:407-420, 1972b.

Guillery, R. W. The effect of lid suture upon the growth of cells in the dorsal lateral geniculate nucleus of kittens. J. Comp. Neurol. **148**:417-422, 1973.

Guillery, R. W. and Stelzner, D. J. The differential effects of unilateral lid closure upon the monocular and binocular segments of the dorsal lateral geniculate nucleus of the cat. J. Comp. Neurol. **139**:413-422, 1970.

Harris, W. A. The effect of eliminating impulse activity on the development of the retinotectal projection in salamanders. J. Comp. Neurol. **194**:303-317, 1980.

Hickey, T. L. Translaminar growth of axons in the kitten dorsal lateral geniculate nucleus following removal of one eye. J. Comp. Neurol. **161**:359-382, 1975.

Hickey, T. L. Development of the dorsal lateral geniculate nucleus in normal and visually deprived cats. J. Comp. Neurol. **189**:467-481, 1980.

Hickey, T. L., Spear, P. D. and Kratz, K. E. Quantitative studies of cell size in the cat's dorsal lateral geniculate nucleus following visual deprivation. J. Comp. Neurol. **172**:265-282, 1977.

Hoffmann, K.-P. and Cynader, M. Functional aspects of plasticity in the visual system of adult cats after early monocular deprivation. Phil. Trans. R. Soc. Lond. **278**:411-424, 1977.

Hoffman, K.-P. and Holländer, H. Physiological and morphological changes in cells of the lateral geniculate nucleus in monocularly-deprived and reverse-sutured cats. J. Comp. Neurol. **177**:145-157, 1978.

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

Hubel, D. H., Wiesel, T. N. and LeVay, S. Plasticity of ocular dominance columns in monkey striate cortex. Phil. Trans. R. Soc. Lond. **278**:377-409, 1977.

Humason, G. L. Animal Tissue Techniques, pp. 79-81 (W. H. Freeman and Company, San Francisco, 1972).

Kalil, R. Development of the dorsal lateral geniculate nucleus in the cat. J. Comp. Neurol. **182**:265-292, 1978a.

Kalil, R. Dark rearing in the cat: effects on visuomotor behavior and cell growth in the dorsal lateral geniculate nucleus. J. Comp. Neurol. **178**:451-468, 1978b.

Kalil, R. A quantitative study of the effects of monocular enucleation and deprivation on cell growth in the dorsal lateral geniculate nucleus of the cat. J. Comp. Neurol. **189**:483-524, 1980.

Kuffler, S. W., FitzHugh, R. and Barlow, H. B. Maintained activity in the cat's retina in light and darkness. J. Gen. Physiol. **40**:683-702, 1957.

Kuppermann, B. and Kasamatsu, T. Cell shrinkage in geniculate neurons following brief unilateral blockade of retinal ganglion cell activity. Soc. Neurosci. Abstracts **6**:790, 1980.

LeVay, S., Stryker, M. P. and Shatz, C. J. Ocular dominance columns and their development in layer IV of the cat's visual cortex: A quantitative study. J. Comp. Neurol. **179**:223-244, 1978.

LeVay, S., Wiesel, T. N. and Hubel, D. H. The development of ocular dominance columns in normal and visually deprived monkeys. J. Comp. Neurol. **191**:1-51, 1980.

Lømo, T. and Westgaard, R. H. Further studies on the control of ACh sensitivity by muscle activity in the rat. J. Physiol. Lond. **292**:603-626, 1975.

Loop, M. S. and Sherman, S. M. Visual discrimination during eyelid closure in the cat. Brain Res. **128**:329-339, 1977.

Meyer, R. L. Tetrodotoxin inhibits the formation of refined retinotopography in goldfish. Dev. Brain Res. **6**:293-298, 1983.

Rakic, P. Genesis of the dorsal lateral geniculate nucleus in the rhesus monkey: Site and time of origin, kinetics of proliferation, routes of migration and pattern of distribution of neurons. J. Comp. Neurol. **176**:23-54, 1977.

Roper, S. and Ko, C.-P. Impulse blockade in frog cardiac ganglion does not resemble partial denervation in changing synaptic organization. Science **202**:66-68, 1978.

Rodieck, R. W. Maintained activity of cat retinal ganglion cells. J. Neurophysiol. **30**:1043-1071, 1967.

Rodieck, R. W. and Dreher, B. Visual suppression from the non-dominant eye in the lateral geniculate nucleus: a comparison of cat and monkey. Exp. Brain Res. **35**:465-477, 1979.

Sanderson, K. J. The projection of the visual field to the lateral geniculate and medial interlaminar nuclei in the cat. J. Comp. Neurol. **143**:101-118, 1971.

Sanderson, K. J., Bishop, P. O. and Darian-Smith, I. The properties of the binocular receptive fields of lateral geniculate neurons. Exp. Brain Res. **13**:178-207, 1971.

Seligman, A. M., Karnovsky, M. J., Wasserkrug, H. L. and Hanker, J. S. Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB). J. Cell Biol. **38**:1-14, 1968.

Shatz, C. J. The prenatal development of the cat's retinogeniculate pathway. J. Neurosci. **3**:482-499, 1983.

Shatz, C. J. and Stryker, M. P. Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J. Physiol. Lond. **281**:267-283, 1978.

Shapley, R. M. and So, Y.-T. Is there an effect of monocular deprivation on the proportion of X and Y cells in the cat lateral geniculate nucleus? Exp. Brain Res. **39**:41-48, 1980.

Sherman, S. M., Hoffman, K.-P. and Stone, J. Loss of a specific cell type from the dorsal lateral geniculate nucleus in visually deprived cats. J. Neurophysiol. **35**:532-541, 1972.

Sherman, S. M., Guillery, R. W., Kaas, J. H. and Sanderson, K. J. Behavioral, electrophysiological, and morphological studies of binocular competition in the development of the geniculo-cortical pathways of cats. J. Comp. Neurol. **158**:1-18, 1974.

Sherman, S. M. and Wilson, J. R. Further evidence for an early critical period in the development of the cat's dorsal lateral geniculate nucleus. J. Comp. Neurol. **196**:459-470, 1981.

Singer, W. Inhibitory binocular interaction in the lateral geniculate body of the cat. Brain Res. **18**:165-170, 1970.

Spear, P. D., Tong, L. and Langsetmo, A. Striate cortex neurons of binocularly deprived kittens respond to visual stimuli through the closed eyelids. Brain Res. **155**:141-146, 1978.

Spear, P. D. and Hickey, T. L. Postcritical period reversal of effects of monocular deprivation on dorsal lateral geniculate cell size in the cat. J. Comp. Neurol. **185**:317-328, 1979.

Stone, J. and Fukuda, Y. Properties of cat retinal ganglion cells: a comparison of W-cells with X- and Y-cells. J. Neurophysiol. **37**:722-748, 1974.

Stryker, M. P. Late segregation of geniculate afferents to the cat's visual cortex after recovery from binocular impulse blockade. Soc. Neurosci. Abstracts **7**:842, 1981.

Thompson, W., Kuffler, D. P. and Jansen, J. K. S. The effect of prolonged reversible block of nerve impulses on the elimination of polyneuronal innervation of newborn rat skeletal muscle fibers. Neuroscience **4**:271-281, 1979.

Wiesel, T. N. and Hubel, D. H. Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. J. Neurophysiol. **26**:978-993, 1963a.

Wiesel, T. N. and Hubel, D. H. Simple cell responses in striate cortex of kittens deprived of vision in one eye. J. Neurophysiol. **26**:1003-1017, 1963b.

Wiesel, T. N. and Hubel, D. H. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J. Neurophysiol. **28**:1029-1040, 1965a.

Wiesel, T. N. and Hubel, D. H. Extent of recovery from the effects of visual deprivation in kittens. J. Neurophysiol. **28**:1060-1072, 1965b.

Wong-Riley, M. Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res. **171**:11-28, 1979.

Wong-Riley, M. and Riley, D. A. The effect of impulse blockage on cytochrome oxidase activity in the cat visual system. Brain Res. **261**:185-193, 1983.

PART II

Control of the Critical Period in Dark-Reared Cats: New Evidence of a Dependence on Visual Activity

INTRODUCTION

In the normal cat visual cortex, most cells can be driven independently by visual stimulation through either eye (Hubel and Wiesel, 1962; Blakemore and Pettigrew, 1970). If one eye of a young kitten is sutured shut for a few days, however, the number of cells in the visual cortex which are influenced by the deprived eye decreases markedly, resulting in a shift in eye preference (ocular dominance) by the cortical cells towards the experienced eye (Wiesel and Hubel, 1963b). Normally this shift in ocular dominance after monocular suture is restricted to a critical period in development starting at age three weeks and extending to about 3-5 months of age (Hubel and Wiesel, 1970; Cynader and Mitchell, 1980), with a peak of sensitivity at 5-6 weeks of age and a gradual decline thereafter (Hubel and Wiesel, 1970; Blakemore and Van Sluyters, 1974; Movshon, 1976). However, Cynader and associates (Cynader et al., 1979; Cynader and Mitchell, 1980; Timney et al., 1980) have shown that if kittens are placed in a completely dark environment from birth for four or more months and subsequently monocularly sutured, visual cortical cells become driven primarily by the experienced eye. If instead of completely depriving an animal of visual input by placing it in the dark, both eyes are simply sutured closed from birth for several months (reducing but not completely blocking visual input) and subsequently one eye is opened to provide a period of monocular vision, no dominance by the experienced eye is observed (Mower et al., 1981). These results suggest that completely depriving an animal of visual input can prolong the critical period of susceptibility to monocular deprivation.

The prior demonstrations of cortical plasticity in dark-reared animals were based on a single experimental protocol: dark-rearing from birth followed by monocular deprivation. This paradigm is analogous to that used in early work demonstrating the plasticity of young kittens, where monocular deprivation was

initiated shortly after birth (Wiesel and Hubel, 1963b; 1965). Subsequent studies led to the development of various paradigms which demonstrated the extent of plasticity present in the visual cortex of young kittens. Primary among these manipulations were delaying the onset of monocular deprivation for several weeks after birth (Hubel and Wiesel, 1970) and performing a reversal of the initial monocular deprivation, wherein one eye is sutured closed from shortly after birth, and some time later that eye is opened and the other eye is sutured closed (Blakemore and Van Sluyters, 1974; Movshon, 1976). In much the same way, one aim of the present study was to investigate the extent of the plasticity preserved by dark-rearing using similar visual exposure protocols. Accordingly, animals were allowed either two weeks of binocular vision after dark-rearing and before monocular deprivation, or subjected to reverse-suturing upon removal from the dark.

Other studies have presented evidence demonstrating the importance of normal levels of norepinephrine for the maintenance of plasticity in response to monocular suture of kittens (Kasamatsu and Pettigrew, 1976; Kasamatsu et al., 1979). Since the available evidence suggests that the cortex of dark-reared adults resembles that of young kittens, the possibility of an analogous dependence on norepinephrine in the maintenance of dark-reared adults was investigated.

Finally, the role of visual activity on the development of the critical period was investigated by asking whether some visual experience after birth prior to dark-rearing would trigger the critical period to run its normal 3-5 month course in the dark. If so, one might expect that no plasticity should be observed six months later, upon removal from the dark. Accordingly, kittens were allowed several weeks of visual exposure prior to dark-rearing and subsequent monocular suture.

A preliminary account of this work has appeared elsewhere (Kuppermann and Ramachandran, 1981).

METHODS

The data presented here were obtained from fifteen cats, all from a partially inbred colony of Tabbies. A variety of rearing conditions were utilized in this study and are summarized in Table 1. A brief description of each condition is presented below.

1) Four cats were placed in a dark room prior to eye opening at 1 week of age. They were then reared in the dark for 6-10 months. Upon removal from the dark, they were immediately monocularly lid-sutured, one of them for 10 days, two for 25 days, and one for 5 months. The animals given 10 days and 5 months of monocular vision underwent no other rearing manipulation. The two animals exposed to 25 days of monocular vision were then reverse-sutured, wherein the previously deprived eye was opened and the non-deprived eye was sutured shut. The reverse-suture was maintained for 4-6 months.

2) Two animals were placed in the dark at age 1 week for 6 and 18 months. They were then removed from the dark and allowed 2 weeks of binocular exposure in a normally lit environment before being monocularly sutured for 6 and 11 weeks.

3) Three animals were placed in the dark at 1 week of age and maintained there for 6.5-7.5 months. One week prior to permanent removal from the dark they were briefly removed from the dark and the primary visual cortex of one hemisphere was implanted with a minipump/cannula system containing 6-hydroxydopamine (6-OHDA). The implantation procedure is described below. The animals were returned to the dark immediately after completion of the implantation surgery, which typically lasted 1 hour. Upon permanent removal from the dark 1 week after the implantation, one eye was sutured shut for 3-7 weeks.

4) Four animals were allowed several weeks in a normally lit environment before being placed in a dark room (two kittens into the dark at age 3 weeks, one at 4 weeks,

TABLE 1

Rearing conditions of all the cats in the present study

Animal	Age into Dark	Duration in Dark	Treatment after DR
CP1	1 week	6.5 months	10 days MD
508	1 week	10.0 months	5 months MD
CP5	1 week	6.5 months	2 weeks BE/6 weeks MD
CP14	1 week	1.5 years	2 weeks BE/11 weeks MD
CP2	1 week	6.0 months	25 days MD/4 months RS
CP11	1 week	6.5 months	25 days MD/6 months RS
CP6	1 week	7.0 months	7 weeks 6-OHDA+6 weeks MD
601	1 week	6.5 months	30 days 6-OHDA+23 days MD
CP12	1 week	7.5 months	7 weeks 6-OHDA+6 weeks MD
CP8	3 weeks	6.0 months	25 days MD
CP9	3 weeks	6.5 months	6 weeks MD
682	4 weeks	1.0 year	5 weeks MD
583	5 weeks	7.0 months	22 days MD
549	reared in lit environment for 6 months then 25 days MD		
550	reared in lit environment for 6 months then 25 days MD		

DR refers to rearing the animal in a completely dark room. MD refers to monocular deprivation induced by suturing one eyelid shut. RS refers to reverse suture, where after the period of monocular deprivation was over, the previously sutured eye was opened, and the previously experienced was sutured closed for the indicated period of time. BE refers to binocular exposure in a normally lit environment. 6-OHDA refers to local intracortical perfusion with 6-hydroxydopamine into one hemisphere (see Methods). Continuous perfusion with 6-OHDA was initiated one week prior to removal from the dark, at which time the animal was monocularly deprived for the indicated period. 6-OHDA perfusion was maintained throughout the period of monocular deprivation.

and one at 5 weeks). They were then reared in the dark for an additional 6-12 months. Upon removal from the dark they were immediately monocularly lid-sutured for 3-6 weeks.

5) Two control animals were reared in a normal lit environment for 6 months and then monocularly lid-sutured for 25 days.

All lid-sutured animals were monitored daily for signs of pinhole openings in the sutured eyelid; any holes were closed immediately upon discovery. In cases where openings were not found and repaired within a short time, the animal was dropped from the study.

Cortical Implant Procedure

The visual cortex of three dark-reared adult cats were chronically implanted (AP, -5 mm; L, 2 mm; 2 mm deep) with a stainless steel cannula (26 gauge) under ketamine anethesia (20 mg/kg, i.m.). The cannula was connected with plastic tubing to an osmotic minipump (Alzet 1701 or 2002) which was placed subcutaneously at the neck. The minipump/cannula system was filled with 4 mM 6-OHDA, an antagonist of norepinephrine, in 0.4% ascorbate-saline (pH = 3.0) and was capable of continuously delivering 0.5 µl of solution per hour for 14 days. The minipump was replaced every 14 days; the replacement surgery was performed under 2% fluothane anesthesia. In one animal a second minipump/cannula system containing only the vehicle solution (0.4% ascorbic acid in saline, pH = 3.0) was implanted into the corresponding location in the other hemisphere.

Recording Procedure

The animals were intubated endotracheally under gas anesthesia composed of 2% fluothane mixed with N_2O/O_2 (2:1), and a section of tubing was inserted into a radial vein for the delivery of infusion solutions. During recordings the animals were anesthetized with a gas mixture of $N_2O/O_2/CO_2$ in a ratio of 75:22.5:2.5 (Venes

et al., 1971) and hyperventilated to ensure sufficient oxidation. Animals were immobilized with an initial dose of gallamine triethiodide (Flaxedil, 20 mg) and maintained throughout the recording sessions with an infusion solution which contained Flaxedil (7.5 mg/kg/hr), dexamethasone (0.2 mg/kg/hr), glucose and saline. Body temperature was maintained at 37.5°C, and the heart rate was monitored continuously. Pupils were dilated by topical application of 1% cyclogyl. The corneae were protected from drying by placing contact lenses with zero power over them. Eye position was monitored by projecting the image of the optic disc onto the tangent screen several times throughout the course of the recording.

Visual stimuli were presented on a tangent screen 57 cm from the animal's eyes through a rear projection system, and were moved on the screen by a joystick. The intensity of visual stimuli was about 1-2 log units above background illumination, which was kept at the mesopic level during recordings.

Single units were recorded in the primary visual cortex with tungsten-in-glass microelectrodes (Levick, 1972). The electrode tracks were angled 5° medially and 10° anteriorly so that sampling of isolated single units was made across laminar and columnar boundaries. Cells were recorded at about every 100 μm. At least 30 visually responsive cells were isolated, typically in one or two penetrations. Recordings were usually made in the hemisphere contralateral to the sutured eye so that neither the natural bias of cortical cells towards the contralateral eye (Hubel and Wiesel, 1962; Blakemore and Pettigrew, 1970) nor the asymmetric, contralaterally biased cortical response to monocular deprivation (Sherman et al., 1974) would exaggerate the degree of modifiability observed in the dark-reared cortex. In the 6-OHDA treated cats, the recording track was roughly 2 mm anterior to the site of perfusion. Recording sessions lasted from 12 to 20 hours for a single hemisphere, and up to 30 hours for the bihemispheric recordings.

For each isolated cell the following receptive field features were characterized:

minimum response field (Barlow et al., 1967), response to stationary flashing spots and light slits, orientation and direction selectivity, velocity tuning, and preferred stimulus width and length. The responses of the units were assayed by qualitative methods. Eye preference data are presented in the form of ocular dominance histograms following the seven-group scheme of Hubel and Wiesel (1962), where cells driven exclusively by the contralateral eye are classified in group 1, cells with equal input from both eyes are placed in group 4, and cells dominated exclusively by the ipsilateral eye are classified in group 7. Cells which were unresponsive to any visual stimulus were noted and are indicated in a column adjacent to the histogram.

At the end of each track a small electrolytic lesion was made. Animals were anesthetized with a lethal dose of Nembutal (0.2 mg/kg, i.v.), perfused with warm saline followed by 10% formol-saline. Thirty-micron thick sections were cut on a freezing microtome, stained for Nissl substances, and later used for reconstruction of each microelectrode track.

Cell Size Measurement

The lateral geniculate nuclei (LGN) of two animals (CP14 and 508) were cut from the rest of the brain in a single block. The blocks were embedded with celloidin (Humason, 1972), sectioned at 40 μm, and stained for Nissl substances. The cross-sectional areas of 420 LGN neuronal somas were measured from each animal. The measurements were made from cells in the mediolateral extent of the binocular portion of laminae A and A1 and from the monocular segment of lamina A (the region that extends beyond lamina A1) at an anteroposterior plane corresponding to Sanderson's (1971) coronal 5, representing the central 10° of the visual hemifield. Seventy cells from each region (binocular lamina A, lamina A1, and the monocular segment of A) were measured from both hemispheres of each animal. Cell outlines were drawn at a total magnification of 900x through a camera lucida attached to a

Leitz microscope. These outlines were then processed on a Wang digitizing tablet coupled to a PDP 11/34 computer.

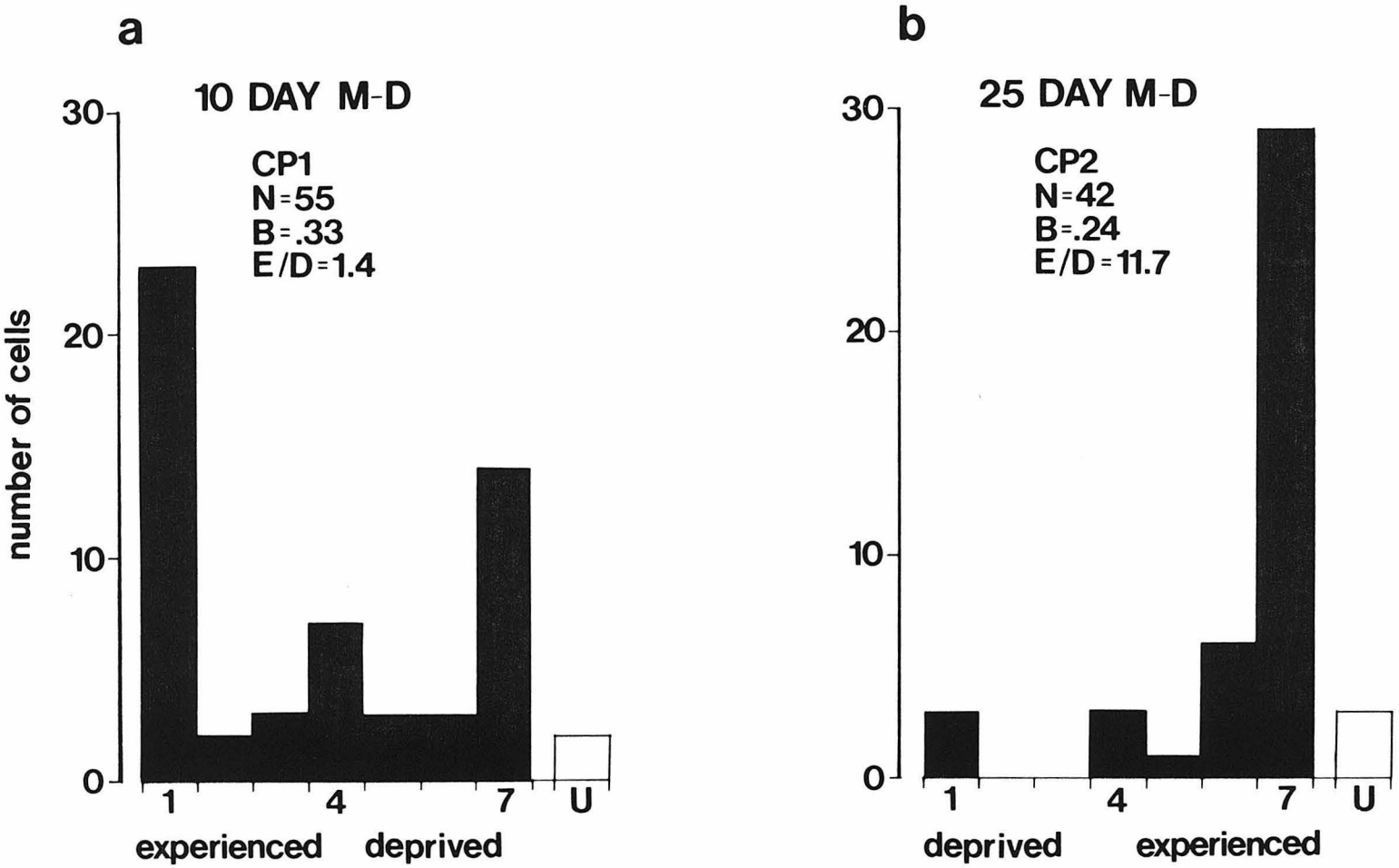
RESULTS

Previous studies of cortical plasticity in dark-reared cats utilized a single experimental paradigm: dark-rearing from shortly after birth for several months and subsequent closing of one eye lid immediately upon removal from the dark. The animals were then allowed from a few days to several months of monocular vision prior to cortical electrophysiological recording. Figure 1 shows the resultant ocular dominance histograms produced from our laboratory in which the same paradigm was used. In Figure 1a the animal (CP1) was reared in the dark for 6.5 months and then given 10 days of monocular exposure in a normally lit environment. The histogram shows an abnormal distribution of eye preference, with a proportion of cells with some input from both eyes (cells in groups 2-6) relative to all the cells (referred to as the binocularity index, or B) of 0.33, versus B = 0.80 for normal (Hubel and Wiesel, 1962; Blakemore and Pettigrew, 1970) and dark-reared adults (Cynader et al., 1976; Mower et al., 1981). The ten days of monocular deprivation, however, were not sufficient to produce a histogram strongly dominated by input from the experienced eye. The ratio (E/D) of the number of cells with dominant input from the experienced eye (groups 1-3 in this case, since the eye ipsilateral to the site of recording was sutured) to the number of cells dominated by the deprived eye (groups 5-7) was 1.4, compared to a ratio of contralaterally dominated to ipsilaterally dominated cells (C/I) of 1.6 in normal animals. This result is consistent with the findings of Cynader and Mitchell (1980), in which they showed that at least two weeks of monocular deprivation were needed after an extended period of dark-rearing before a strong dominance by the non-deprived eye was observed. Approximately 50% of the visually responsive cells encountered in this animal were orientation selective, a number also

Figure 1. Ocular dominance histograms from animals dark-reared for at least six months and monocularly deprived immediately upon removal from the dark. The animals' identification (i.e., CP1) is given in each histogram in this study for reference to Table 1, which gives a complete rearing description. Ocular dominance is assigned according to the seven-group scheme of Hubel and Wiesel (1962), with cells in group 1 responding exclusively to stimulus from the contralateral eye, cells in groups 2 and 3 dominated by the contralateral eye but with increasing input from the ipsilateral eye, cells in group 4 being driven equally well by either eye, cells in groups 5 and 6 exhibiting increasing dominance by the ipsilateral eye, and cells in group 7 being driven exclusively by the ipsilateral eye. N is the number of visually responsive units which comprise the histogram. B is an index of binocularity determined by taking the ratio of the number of cells with some input from both eyes (groups 2-6) to all of the visually responsive units in the histogram. E/D is a measure of the cortical effects of monocular deprivation determined by dividing the number of cells with dominant input from the experienced eye (either groups 1-3 or 5-7, depending on which eye was not closed) by the number of cells with dominant input from the deprived eye. Cells driven equally well by either eye (group 4) are excluded. The E/D index can be compared to normal values for C/I (cells dominated by the contralateral eye versus the cells dominated by the ipsilateral eye) of about 1.6 or normal values for I/C (cells in groups 5-7 divided by cells in groups 1-3, the inverse of C/I) of about 0.6, depending on which eye was deprived. In most, but not all of the histograms in this study, the contralateral eye was deprived. a) Upon removal from the dark, this animal (CP1) was subjected to 10 days of monocular deprivation (see Table 1). A strong decrease of binocularity was observed, with B=0.33 versus B=0.80 for normal animals. No dominance by the experienced (contralateral) eye was observed, with E/D=1.4 versus C/I=1.6 for normal adults. b) This animal (CP2) was dark-reared for a similar

period, but was allowed 25 days of monocular vision. A strong dominance by the experienced (ipsilateral) eye was observed, with E/D=11.7 versus a normal I/C of 0.6.

DARK REARING FOLLOWED BY MONOCULAR DEPRIVATION



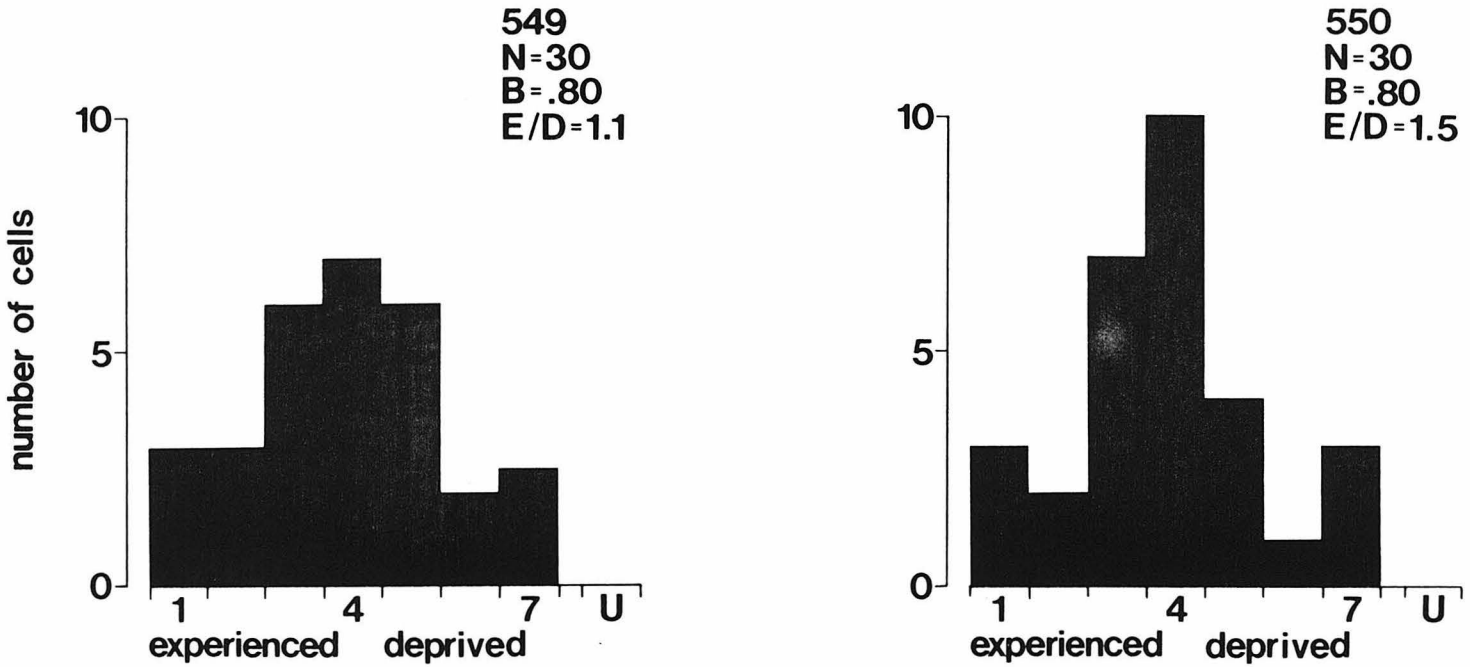
in agreement with that of Cynader and Mitchell (1980). Figure 1b shows the resultant histogram from another animal (CP2) which was darkreared for 6 months, and then monocularly sutured for 25 days upon removal from the dark. The histogram is strongly dominated by input from the experienced eye (in this case the eye ipsilateral to the site of recording) with a ratio of experienced eye dominant to deprived eye dominant cells (E/D) of 11.7 versus a ratio of ipsilaterally to contralaterally dominated cells (I/C, the inverse of C/I) of 0.6 in normal adults.

The purpose of providing at least six months of dark-rearing for each animal was to place it well beyond the critical period of susceptibility to monocular deprivation, which has been reported to end at three months of age (Hubel and Wiesel, 1970). However, recent reports have suggested that the critical period may extend to 5-8 months of age (Cynader and Mitchell, 1980). In order to ensure that the dominance by the experienced eye seen in Figure 1b was not due to normal residual plasticity available to animals at this age, regardless of rearing condition, two normal 6 month old animals were subjected to 25 days of monocular vision. The resultant histograms are shown in Figure 2 and exhibit no effects of the monocular deprivation, with both normal levels of binocularity, B=0.80, and roughly normal C/I ratios of 1.1 and 1.5. The observed dominance by the experienced eye in Figure 1b, therefore, was due to the combined effects of dark-rearing and subsequent monocular deprivation, and not to the monocular deprivation alone.

The experimental paradigm used above (dark-rearing followed immediately by monocular deprivation) does not give an indication of the extent of the critical period remaining after removal from the dark. Four dark-reared cats were therefore subjected to two different visual exposure protocols in order to investigate whether the dark-reared cortex remains plastic for a period after removal from the dark.

Figure 2. As a control for the amount of plasticity normally present in 6-month-old light-reared animals, two normally reared 6-month-old cats were subjected to 25 days of monocular deprivation. In both cases normal values of binocularity (B=0.80) were observed. No unusual dominance by the non-deprived (contralateral) eye was observed, with E/D values of 1.1 and 1.5 compared to a normal C/I of about 1.6. The naturally occurring bias towards the contralateral eye in normal untreated adults has been well documented (Hubel and Wiesel, 1962; Blakemore and Pettigrew, 1970), so that the contralateral dominance observed here is not a consequence of the monocular deprivation. For the use of conventions, please see the Figure 1 legend.

NORMAL REARING FOLLOWED BY MONOCULAR DEPRIVATION



Dark-rearing followed by binocular visual exposure prior to monocular deprivation

Two animals were placed in the dark prior to eye opening at one week of age. One animal (CP5) remained in the dark for 6.5 months and the other (CP14) for 1.5 years. Upon removal from the dark, both were allowed two weeks of binocular experience in a normally lit environment before being monocularly deprived. The duration of the binocular exposure was chosen on the basis of the demonstration that 1-2 weeks of visual experience after extended periods of dark-rearing were sufficient to restore cortical responsiveness and receptive field characteristics to near normal levels (Cynader and Mitchell, 1980). The subsequent periods of monocular deprivation differed for the two animals, with the younger one (CP5) subjected to six weeks of monocular vision, and the older one (CP14) receiving eleven weeks of monocular deprivation. The resultant ocular dominance histograms are shown in Figure 3, and in both indicate a strong dominance by the experienced eye, with E/D ratios of 2.1 and 3.0 versus a normal I/C (ipsilateral eye open during monocular vision in both animals) of 0.6. More than 70% of the visually responsive units encountered in each animal were orientation selective.

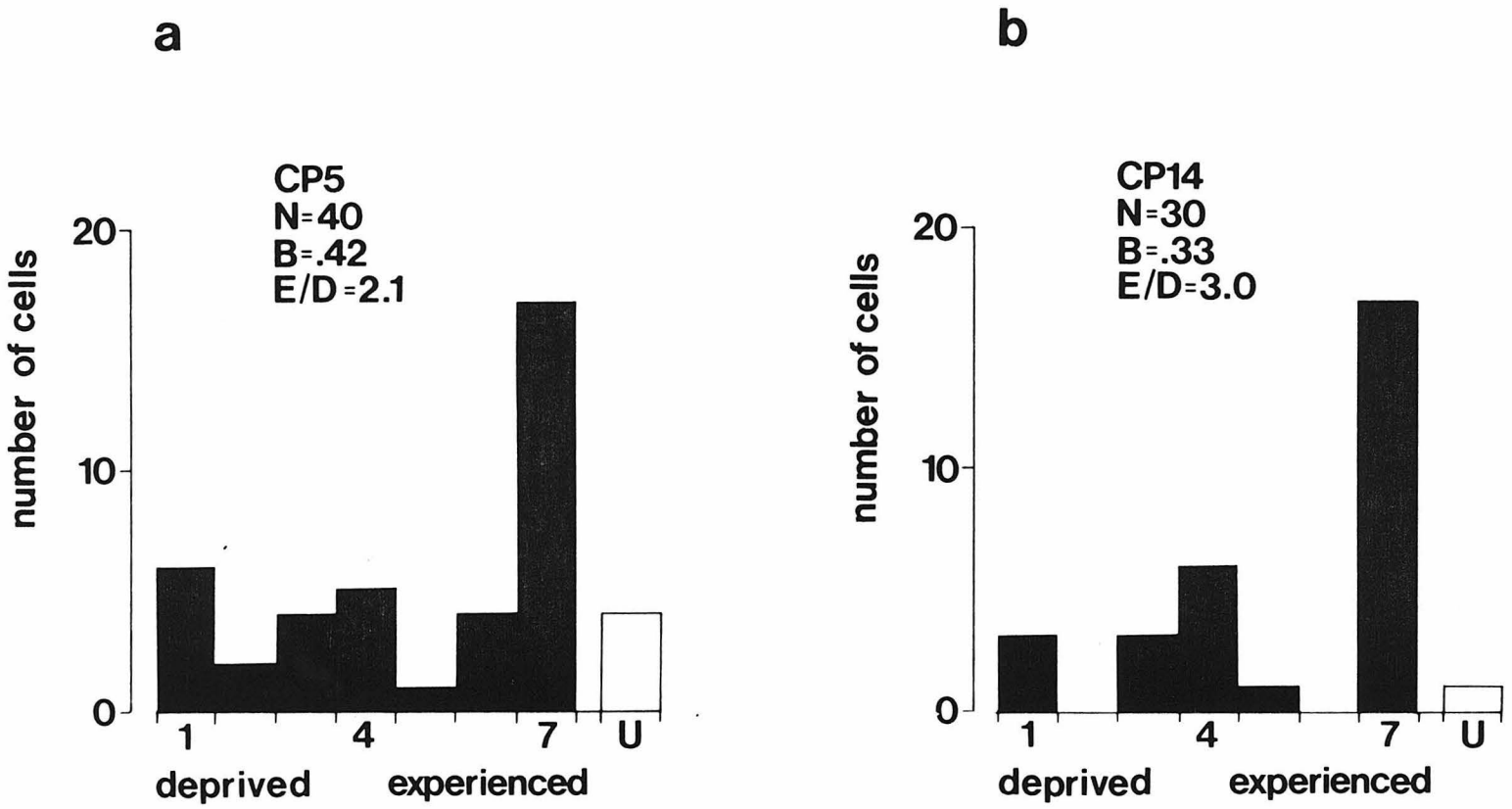
These results are quite similar to those in Figure 1b and in other reports where no binocular experience was allowed after dark-rearing and prior to monocular deprivation (Cynader and Mitchell, 1980; Mower et al., 1981). It appears, therefore, that the plasticity preserved by dark-rearing was sufficiently robust as to withstand two weeks of binocular experience before the monocular deprivation was initiated, even when the period of dark-rearing was as long as 1.5 years (CP14).

Reversal of the effects of monocular deprivation in dark-reared animals

One of the most striking demonstrations of the neuronal plasticity of the visual cortex of kittens is the ability of the cortex to undergo a reversal of the effects of monocular deprivation by suturing the previously open eye and allowing the initially

Figure 3. Dark-rearing followed by binocular exposure prior to monocular deprivation. a) This animal (CP5) was dark-reared, allowed 2 weeks of binocular exposure upon removal from the dark, and then monocularly deprived for 6 weeks. Binocularity is reduced relative to normal animals (B=0.42 versus 0.80 for normals). The experienced (ipsilateral) eye is dominant, with E/D=2.1 versus I/C=0.6 for normal animals. b) This animal (CP14) was kept in the dark for 1.5 years, then given 2 weeks of binocular exposure prior to monocular deprivation of 11 weeks. Both a reduction in binocularity (B=0.33 versus 0.80 for normals) and a dominance by the experienced eye (E/D=3.0 versus normal I/C=0.6) was observed. For conventions see Figure 1 legend.

BINOCULAR EXPOSURE PRIOR TO MONOCULAR DEPRIVATION



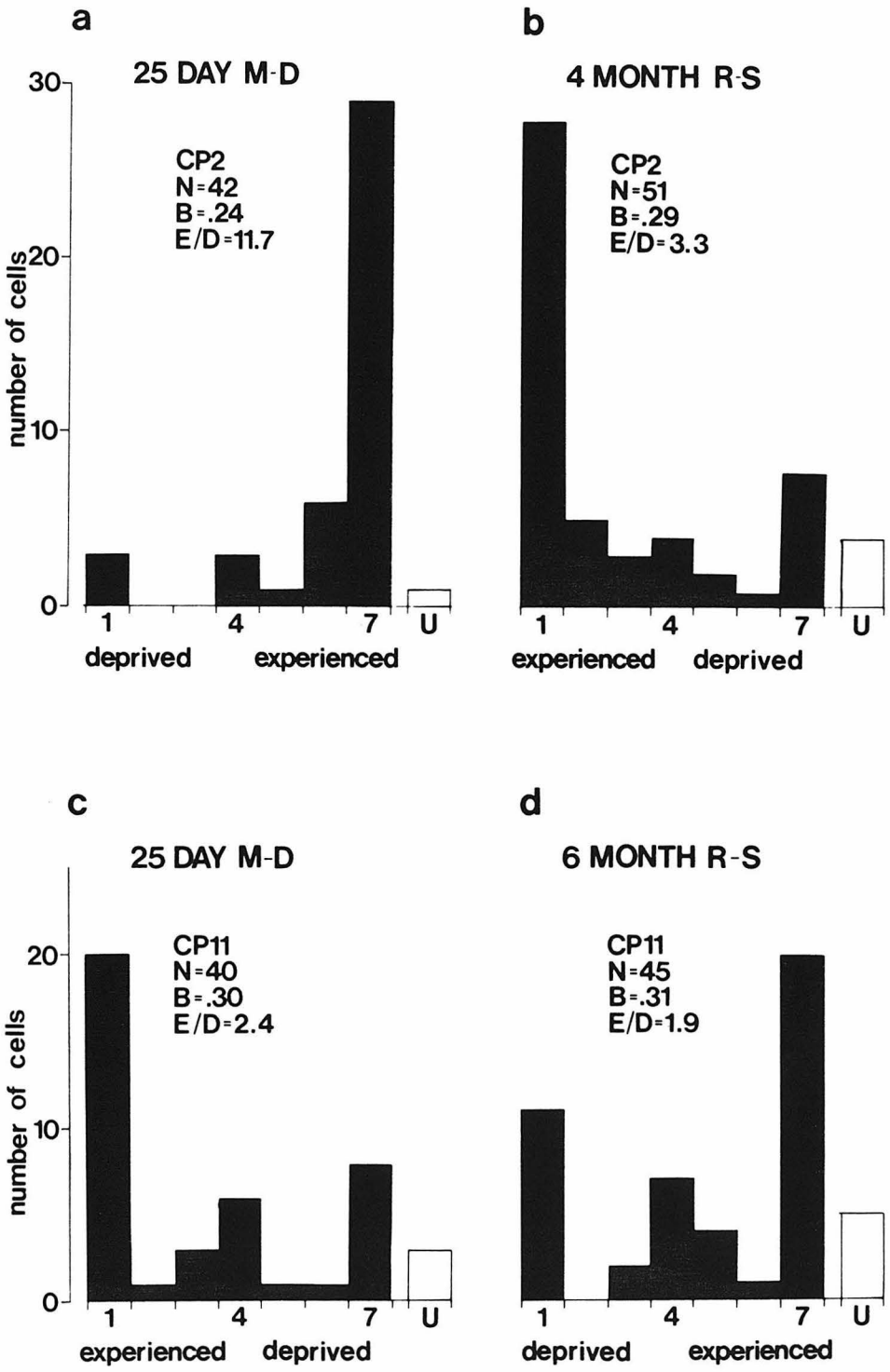
deprived eye a period of visual experience (Blakemore and Van Sluyters, 1974; Movshon, 1976; Blasdel and Pettigrew, 1978). Two cats were subjected to a reverse-suturing protocol by dark-rearing them from shortly after birth for 6 months (CP2) and 6.5 months (CP11), monocularly depriving them for 25 days immediately upon removal from the dark, and then reverse-suturing their eyelids for 4 months (CP2) and 6 months (CP11). Electrophysiological recordings were made in each animal after the initial period of monocular deprivation and again after the extended period of reverse suturing. The resultant histograms are shown in Figure 4 (Figure 4a is the same as Figure 1b). Both animals exhibited an initial histogram dominated by input from the experienced eye (Figures 4a and 4c), though the effect was stronger in one animal (CP2; E/D=11.7 versus I/C=0.6 in normal animals, ipsilateral eye was the experienced eye) than in the other animal (CP11; E/D=2.4 versus normal C/I=1.6, contralateral eye was non-deprived). After the period of reverse-suture, electrophysiological recording from the same hemisphere (Figures 4b and 4d) demonstrated a strong shift in ocular dominance from the previously experienced eye to the newly experienced eye, with E/D=3.3 for animal CP2 (versus C/I=1.6 for normal animals) and E/D=1.9 for animal CP11 (versus I/C=0.6 for normal animals and a prior I/C=0.4). It appears that dark-rearing preserves a high degree of cortical plasticity, in that it was possible to alter an established distribution of ocular dominance several weeks after removal from the dark.

Morphology of the LGN after Dark Rearing and Monocular Deprivation

In addition to cortical changes in ocular dominance, another prominent abnormality seen in the visual pathway of young kittens subjected to monocular deprivation is the change in soma size of neurons in the LGN: cells in the laminae of the LGN which receive input from the deprived eye shrink in size, while cells in laminae with input from the experienced eye increase their size (Wiesel and Hubel, 1963a; Guillery

Figure 4. Dark-rearing followed by monocular deprivation and subsequent reverse-suturing. a) This dark-reared animal (CP2) was subjected to 25 days of monocular deprivation immediately upon removal from the dark. A strong dominance by the experienced eye was observed. This histogram is the same as shown in Figure 1b. b) After the recording session from which the histogram in (a) was composed, the animal was reverse-sutured, whereby the initially deprived eye was left open, and the initially experienced eye was sutured closed for 4 months. The second recording was made in the same hemisphere at a site close to the prior recording location. A shift in ocular dominance towards the newly experienced eye was observed, with E/D=3.3 versus 1.6 for normal animals. c) A second animal (CP11) was similarly dark-reared and subjected to monocular deprivation. A strong reduction of binocularity was observed (B=0.30) but the dominance by the experienced eye was less strong than usual for dark-reared adults (E/D=2.4). c) Even though the shift in dominance towards the initially experienced eye was not large, after six months of reversal in the same animal a strong shift towards the newly experienced (ipsilateral) eye was observed, with E/D=1.9 compared with both I/C=0.6 for normals and I/C=0.42 prior to reverse-suturing. For conventions see Figure 1 legend.

DARK REARING FOLLOWED BY REVERSE-SUTURE

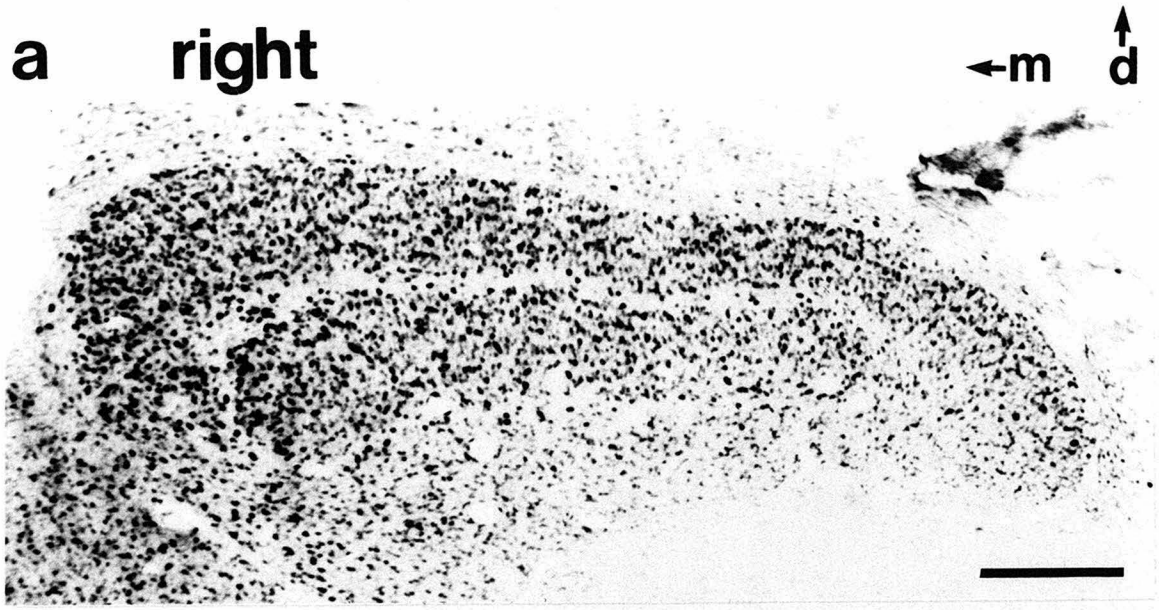


and Stelzner, 1970; Hickey et al., 1977). These changes are seen primarily in the binocular segment of the LGN, which corresponds to the central visual field, and are thought to be due to competition between intracortical afferents originating from the LGN (Guillery, 1972). The morphological response by the LGN to the effects of monocular deprivation is therefore another indication of the plasticity of young kittens, and suggests another comparison with the plasticity of dark-reared adult cats. Since extended periods of monocular deprivation are most suitable for observing significant amounts of LGN cell size change, only two animals in the study were examined for geniculate cell size abnormalities (CP14: dark-reared/2 weeks binocular exposure/11 weeks MD; and 508: dark-reared/5 months monocular deprivation). No obvious changes in appearance were seen upon inspection of the LGN (Figure 5), with a relatively even distribution of stain throughout the full extent of laminae A and A1. Young kittens subjected to an equivalent period of monocular deprivation exhibit gross changes in LGN appearance when viewed at the same magnification. A comparison of the mean cell sizes between corresponding deprived and non-deprived laminae revealed no significant size difference in any region of the LGN of either animal (Table 2). Unlike young kittens, this small sample of dark-reared adults indicated no response by the LGN somas to the effects of monocular deprivation.

Local depletion of norepinephrine in dark-reared cats

Norepinephrine (NE) has been shown to play a significant role in the maintenance of neuronal plasticity of the visual cortex of young kittens. Depletion of NE by local perfusion of 6-hydroxydopamine (6-OHDA), a NE antagonist, reduces the susceptibility of the visual cortex to monocular deprivation (Kasamatsu and Pettigrew, 1979); subsequent replacement of NE by exogenous microperfusion restores the sensitivity to deprivation (Kasamatsu et al., 1979). Since the ability of

Figure 5. Coronal section through the right (a) and left (b) LGN of animal CP14, whose left eye was sutured closed for 11 weeks. A, A1 and MS refer to the binocular segment of lamina A, lamina A1 and the monocular segment of lamina A, respectively. No obvious difference in either staining intensity or cell size can be observed in either the deprived laminae (lamina A and MS in the right LGN, lamina A1 in the left) or the experienced laminae (lamina A and MS in the left LGN, lamina A1 in the right). The scale bar corresponds to 500 μm.


a
right
←m
d

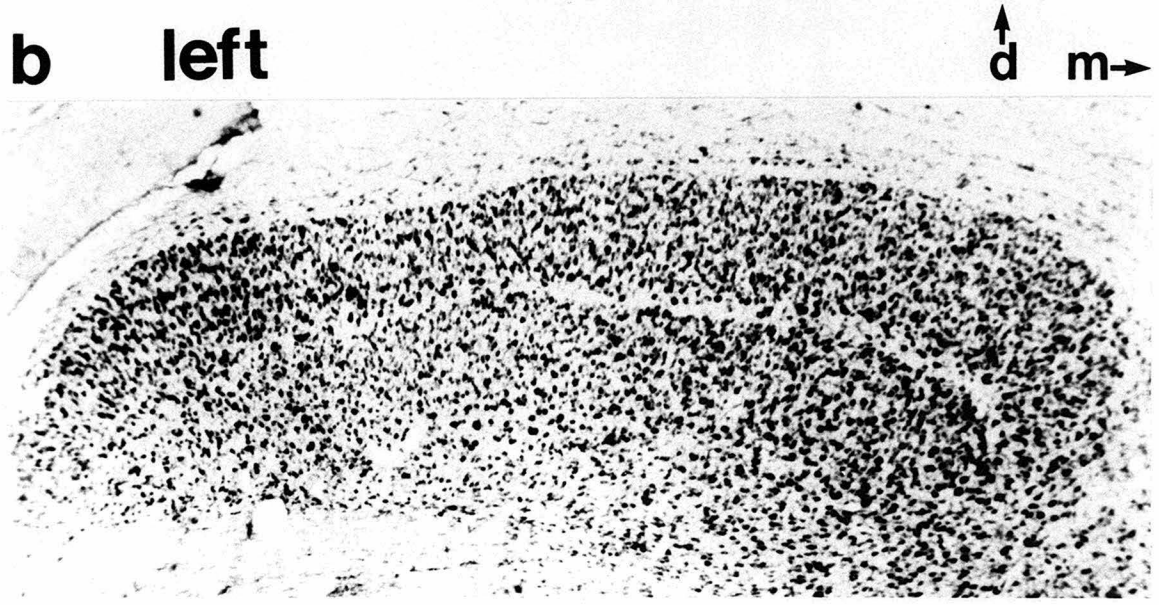

b
left
d
m→

TABLE 2

Mean ± S. E. (μm^2) of LGN laminae cell sizes

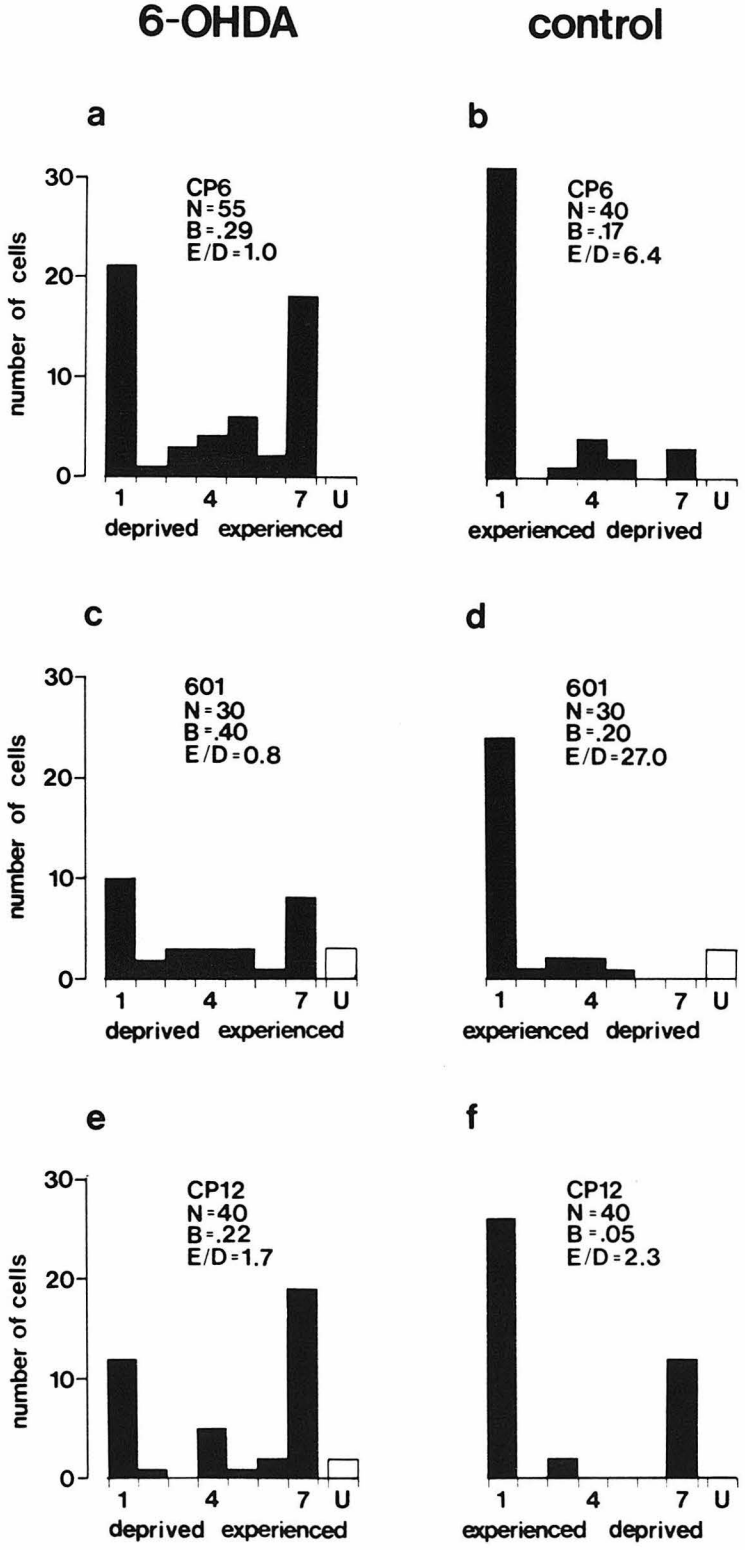
	A			A1			MS		
Animal	Deprived	Experienced	%D	Deprived	Experienced	%D	Deprived	Experienced	%D
CP14	213±7	210±7	-1%	227±8	226±8	0%	190±5	198±5	4%
508	224±8	221±7	-1%	231±8	245±9	6%	210±6	215±5	2%

Each value is the mean ± standard error of 70 cell areas from the indicated lamina of a single animal, given in μm^2. Deprived refers to laminae with input from the sutured eye; experienced refers to lamina with normal input. The %D was calculated by dividing the difference in mean cell area between corresponding deprived and experienced laminae by the mean cell area of the experienced lamina. None of the %D was significantly different at $p<0.1$.

the dark-reared adult visual cortex to undergo modification as a result of altering visual input closely resembles that of young kittens, the possibility of a similar effect of 6-OHDA on the visual cortex of these cats was investigated. Three cats were placed in the dark shortly after birth for either 6.5 months (601), 7 months (CP6), or 7.5 months (CP12). One week before removal from the dark for the period of monocular vision, the animals were briefly brought into the light to be implanted with an intracortical minipump/cannula system containing 4 mM 6-OHDA (see Methods section). This concentration of 6-OHDA has been shown to deplete NE from an area of cortex roughly 10 mm in diameter within several days (Kasamatsu et al., 1981). The animals were subjected to local perfusion of 6-OHDA for one week prior to removal from the dark, therefore, to ensure adequate depletion of NE prior to monocular deprivation. The perfusion of 6-OHDA continued throughout the duration of the monocular deprivation. One animal (601) was implanted with a minipump/cannula system containing vehicle solution (0.4% ascorbic acid in saline) in the corresponding location of the other hemisphere as a control for the perfusion process. Each animal was subjected to electrophysiological recording from corresponding locations in both the experimental (6-OHDA) and control (no treatment or vehicle solution) hemispheres. The resultant histograms are shown in Figure 6. In two of the animals (CP6 and 601) a smaller shift in ocular dominance was observed in the NE-depleted hemisphere than in the control hemisphere. Animal CP6 exhibited an E/D ratio of 1.0 in the 6-OHDA treated hemisphere (Figure 6a) versus E/D=6.4 in the control, untreated hemisphere (Figure 6b). Similarly, in animal 601 the E/D ratio was 0.8 in the 6-OHDA treated hemisphere (Figure 6c) and 27.0 in the control, ascorbate-saline treated hemisphere (Figure 6d). In the third animal, CP12, a similarly small E/D ratio of 1.7 was observed in the 6-OHDA treated hemisphere (Figure 6e), but the control hemisphere (Figure 6f) was only modestly affected by the monocular deprivation (E/D=2.3 versus a normal C/I=1.6). This may have been due to

Figure 6. These dark-reared animals were depleted of NE in one hemisphere by intracortical perfusion with 6-OHDA, a NE antagonist, starting one week before permanent removal from the dark (see Methods). Upon removal from the dark, one eye was sutured closed for several weeks; 6-OHDA perfusion was maintained throughout the period of monocular vision. a) After six weeks of monocular deprivation, electrophysiological recording from the NE-depleted hemisphere of this animal (CP6) indicated a strong loss of binocularity (B=0.29) but no apparent shift in ocular dominance towards the experienced (ipsilateral) eye, with E/D=1.0 versus about 0.6 for normal cats. b) Recording made from the control, untreated hemisphere of the same animal as in (a). A strong dominance by the experienced (contralateral) eye was observed (E/D=6.4). c) This animal (601) was dark-reared for 6.5 months, treated with 6-OHDA in one hemisphere as described above, and subjected to concurrent monocular deprivation for 23 days. A loss of binocularity relative to normal was observed (B=0.40), but the cortical units were not dominated by the experienced (ipsilateral) eye, with E/D=0.8 compared to 0.6 for normal adults. d) Recording from the control hemisphere of the same animal as in (c). This hemisphere was subjected to intracortical perfusion of a vehicle solution containing 0.4% ascorbic acid in saline. A strong shift in response to the monocular deprivation was observed. Electrode tract reconstructions from both hemispheres of this animal are shown in Figure 7. e) This animal (CP12) was reared in the dark for 7.5 months, treated with 6-OHDA in one hemisphere as described above, and monocularly deprived for 7 weeks. A loss of binocularity (B=0.22) was observed, as in the other two 6-OHDA treated cortices, but in this case a slight dominance by the experienced eye was also observed, with E/D=1.7 versus I/C=0.6 for normal cats. f) Recording from the control, untreated hemisphere of the same animal as in (e). A very strong loss of binocularity was observed, with only two cells out of 40 receiving any input from both eyes. However, only a small degree of dominance by

the experienced eye was observed (E/D=2.3 versus 1.6 for normal adults). For conventions see Figure 1 legend.


6-OHDA
control
a
b
CP6
N=55
B=.29
E/D=1.0
CP6
N=40
B=.17
E/D=6.4
number of cells
30
20
10
0
1
4
7
U
deprived experienced
experienced deprived
c
d
601
N=30
B=.40
E/D=0.8
601
N=30
B=.20
E/D=27.0
e
f
CP12
N=40
B=.22
E/D=1.7
CP12
N=40
B=.05
E/D=2.3

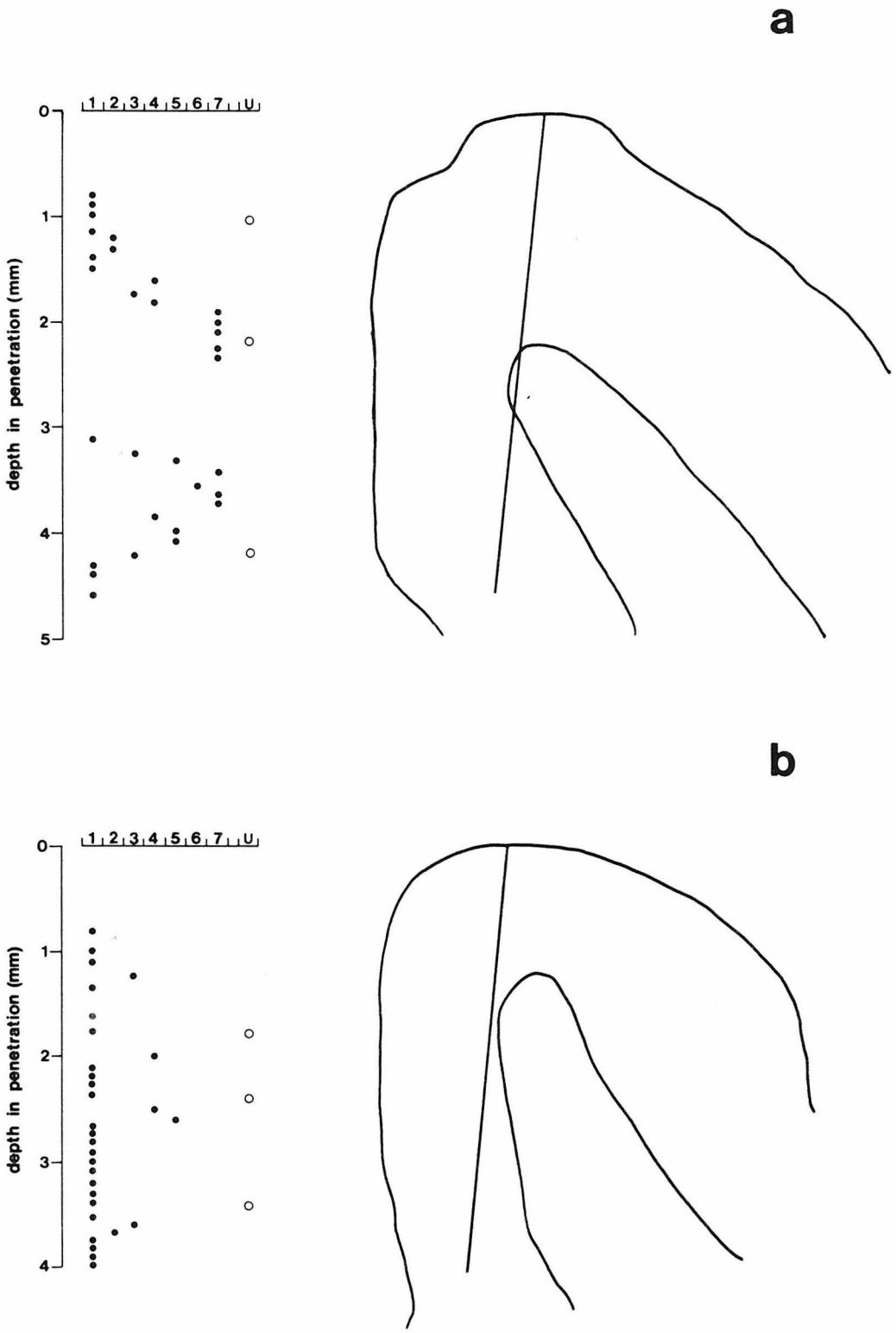
some leakage of 6-OHDA into the control hemisphere or to a variability in the effectiveness of dark-rearing between animals. In fact, this animal (CP12) was a littermate of CP11, who exhibited a smaller response to reverse-suturing than was seen in the other reverse-sutured animal. In all three cases a severe reduction of binocularity from normal levels of 80% to a range of 22-40% in the 6-OHDA treated hemispheres was observed. The reconstruction of the electrode tracks from both hemispheres of animal 601 is shown in Figure 7.

While depletion of NE did not remove the susceptibility of the visual cortex to monocular deprivation, in that a strong reduction in binocularity was observed, the 6-OHDA treatment did significantly reduce the shift in dominance towards the experienced eye. Normal levels of NE, therefore, may play a role in maintaining the preserved plasticity of dark-reared cats, but to a smaller degree than that seen in young kittens.

Visual experience prior to dark-rearing and monocular deprivation

The above results have shown that the sensitivity of the visual cortex to monocular deprivation is preserved to a high degree by dark-rearing started at one week of age. The critical period for susceptibility to the effects of monocular deprivation, however, does not start until the kitten is three weeks old (Hubel and Wiesel, 1970). The results do not, therefore, address the issue of whether the onset of the critical period is delayed by dark-rearing and once initiated runs a predetermined course, or whether the control of the critical period is dependent on activity. Four kittens were allowed either three weeks (CP8 and CP9), four weeks (682), or five weeks (583) of normal visual experience from birth before placing them in the dark for an additional 6 months (CP8) and (CP9), 7 months (583), or one year (682). Upon removal from the dark they were monocularly deprived for 22 days (583), 25 days (CP8), 5 weeks (682), or 6 weeks (CP9). The resultant ocular dominance histograms

Figure 7. Electrode track reconstructions from both hemispheres of a dark-reared/monocularly-deprived animal (601) subjected to intracortical perfusion with 6-OHDA in one hemisphere (a) and perfusion with a vehicle solution into the other hemisphere (b). The histogram corresponding to (a) is shown in Figure 6c, and (b) corresponds to Figure 6d. One millimeter on the ordinate scale corresponds to one millimeter of cortex. The abscissa denotes ocular dominance, with visually unresponsive units indicated by open circles in column u.


a
1 2 3 4 5 6 7 U
0
1
2
3
4
5
depth in penetration (mm)
b
1 2 3 4 5 6 7 U
0
1
2
3
4
depth in penetration (mm)

are shown in Figure 8. In all four cases, a strong shift in ocular preference towards the experienced eye was observed, with a range of E/D from 2.5 to 8.7, versus a normal I/C of 0.6 (contralateral eye sutured in all cases). The degree of modification was reduced somewhat in the animals kept 4 and 5 weeks in a normally lit environment from birth prior to dark-rearing, but was still quite significant (E/D=2.5 and 3.3 versus I/C=0.6 for normal animals). Two to four weeks of visual experience prior to dark-rearing, therefore, did not strongly reduce the sensitivity of the visual cortex to subsequent monocular deprivation.

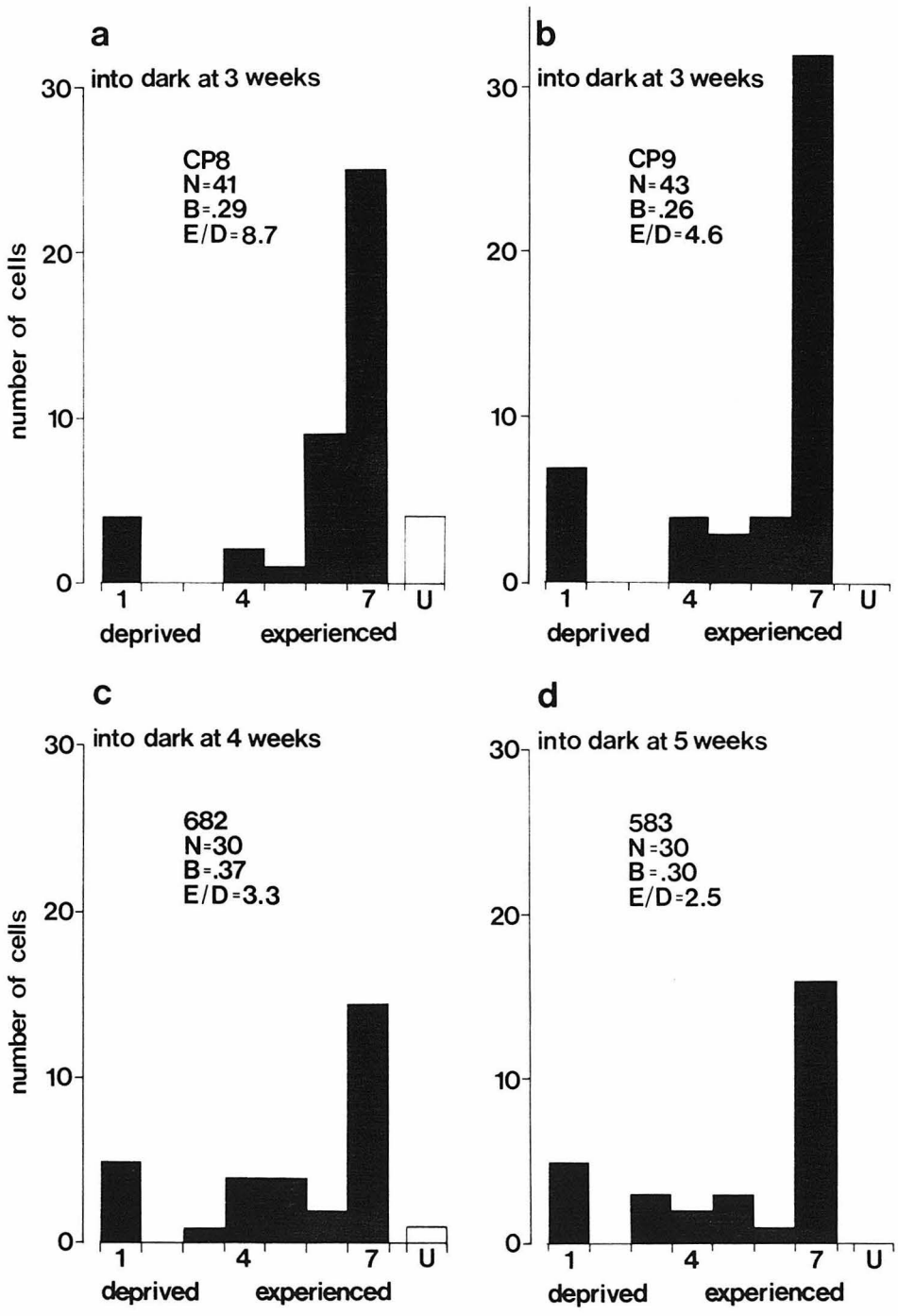
DISCUSSION

New evidence for the preservation of cortical plasticity in the dark

The results of this study indicate that there is a remarkable amount of plasticity in the visual cortex of cats after prolonged dark-rearing. The sensitivity of the cortex to monocular deprivation seemed only slightly reduced if the animal was allowed two weeks of binocular experience between removal from the dark and subsequent monocular deprivation. More striking was the ability of the cortex to undergo a reversal of ocular dominance several weeks after removal from the dark. The ability of the visual cortex of young kittens to respond to reverse suturing is strongly dependent on the age of the animal: reverse suturing after five weeks of age does not produce a complete reversal of dominance towards the newly experienced eye, and after six weeks of age the reversal is quite limited (Blakemore and Van Sluyters, 1974; Movshon, 1976). The strength of the reversal seen in the dark-reared cats suggests that the visual cortex at the time of reverse suturing (25 days after removal from the dark) had not undergone serious reduction of its sensitivity to alterations in visual environment. The duration of the postponed critical period of dark-reared adults was not determined but the reversal result indicates that it lasts at least several weeks.

Figure 8. Binocular exposure prior to dark-rearing and subsequent monocular deprivation. a) This animal (CP8) was placed in the dark at three weeks of age then dark-reared for 6 months. Upon removal from the dark, one eye was sutured closed for 25 days. The histogram indicates a strong dominance by the experienced (ipsilateral) eye with E/D=8.7. b) Another animal was reared similarly: into the dark at three weeks for 6.5 months, and subsequent monocular deprivation for 6 weeks. Again, a strong shift in connectivity towards the experienced eye was observed, with E/D=4.6. c) This animal (682) was left in a normally lit environment for 4 weeks from birth, placed in the dark for one year, and then monocularly deprived for 5 weeks. A shift in ocular dominance towards the experienced eye is evident, with E/D=3.3. d) This animal (583) was placed in the dark at 5 weeks of age for 7 months, and then monocularly deprived for 22 days. The results indicate a strong loss of binocularity and a dominance by the experienced eye (E/D=2.5 versus I/C=0.6 for normals), though the shift in ocular dominance was somewhat less than was seen in (a) and (b). For conventions see Figure 1 legend.

BINOCULAR EXPOSURE PRIOR TO DARK-REARING AND MONOCULAR DEPRIVATION



While the dark-reared adult visual cortex is capable of an impressive degree of plasticity, important differences exist in its sensitivity to visual alterations when compared to that of young kittens. One such difference is the speed with which the visual cortex responds to monocular deprivation. Three days of monocular deprivation in a young kitten are sufficient to produce a visual cortex dominated by the experienced eye, while two weeks are necessary for an equivalent response from a dark-reared adult. A major portion of these two weeks, however, may be spent in restoring general responsiveness and receptive field properties to the visual cortical neurons, which deteriorate after extended periods of dark-rearing (Blakemore and Van Sluyters, 1975; Buisseret and Imbert, 1976; Cynader et al., 1976; Cynader and Mitchell, 1980; Leventhal and Hirsch, 1980; Mower et al., 1981). This period of time may be analogous to the preplastic condition of newly born kittens, where the cortical responsiveness and receptive field features are somewhat immature (Hubel and Wiesel, 1963; Blakemore and Van Sluyters, 1975). It would be of interest to determine how quickly a dark-reared adult allowed one week of binocular exposure upon removal from the dark would respond to subsequent monocular deprivation. The speed of takeover by the experienced eye in such a case would provide a better indication of the sensitivity of the adult dark-reared cortex.

A second difference in the susceptibility to visual alterations between young kittens and dark-reared adults is the lack of a morphological response by the dark-reared LGN to monocular deprivation. The primary hypothesis for the significance of cell size changes in the LGN after monocular deprivation is that they are a manifestation of competition by geniculate afferents for post-synaptic sites in the cortex. The size of a geniculate neuronal soma in such a hypothesis is related to the extent of its axonal arborization in layer IV of the visual cortex; expansion or contraction of the terminal field would be reflected in a corresponding increase or decrease in LGN cell size (Guillery, 1972; Sherman et al., 1974; LeVay et al., 1980).

Since an anatomical expansion is implied in this hypothesis, it may be that the changes in ocular dominance seen after dark-rearing and subsequent monocular deprivation are not structural changes in connectivity, but rather functional changes in synaptic efficacy. Alternatively, if changes in connectivity occurred outside of layer IV, one would not expect to see them reflected on LGN cell size. An autoradiographic analysis of ocular dominance stripe width in a dark-reared/monocularly deprived adult might help to resolve the question.

The results obtained from the NE depleted hemisphere of dark-reared/monocularly deprived animals suggest that at least some component of the plasticity preserved by dark-rearing is dependent on the presence of active noradrenergic afferents, similar to what is seen in young kittens (Kasamatsu and Pettigrew, 1976; Kasamatsu et al., 1979). Although the NE depleted hemisphere was abnormal in its distribution of ocular dominance, the primary effect of the manipulation was not to shift ocular towards the non-deprived eye, but rather to induce a strong loss of binocularity. This result is similar to that seen in normally reared adults subjected to exogenous intracortical NE perfusion and concomitant monocular deprivation (Kasamatsu et al., 1979). That study suggested that NE may play a more important role in the maintenance of binocularity than in the takeover by the experienced eye. The results from the NE-depleted dark-reared cortex are consistent with such a suggestion.

Effects of early visual experience on prolongation of the critical period

A particularly interesting result of the present study was the preservation of plasticity in the dark even after a period of visual exposure prior to dark rearing. The typical procedure for dark-rearing is to place the kittens in the dark prior to eye opening at roughly seven days of age. As a result, kittens are deprived of visual stimulation before the rapid phase of cortical synaptogenesis begins at about 8 days

of age (Cragg, 1975), before segregation of afferents to layer IV of the primary visual cortex begins at two weeks of age (LeVay et al., 1978), and before the inception of the period of susceptibility to monocular suture at three weeks of age (Hubel and Wiesel, 1970). It was of interest, therefore, whether visual experience prior to dark-rearing affected the subsequent sensitivity to monocular deprivation. The two animals which were placed in the dark at three weeks of age (CP8 and CP9) may not yet have been plastic, since the critical period starts at roughly that age (Hubel and Wiesel, 1970). Even at that age, however, there is a demonstrable difference in cortical development detween dark-reared and normal kittens (Blakemore and Van Sluyters, 1975). The other two animals (583 and 682) were within the critical period at the time of placement into the dark and the demonstration of plasticity there suggests that the maintenance of the critical period may be dependent on activity.

It has been suggested that the convergence of thalamic inputs in the visual cortex provides the basis for competition to occur between those afferents for the limited number of post-synaptic sites (Wiesel and Hubel, 1965; Ganz et al., 1968; Guillery and Stelzner, 1970). As the segregation of the inputs to the visual cortex proceeds, the possibility for competition decreases since fewer terminals from the two eyes are in close contact, and the sensitivity to monocular deprivation declines. The segregation of the thalamic inputs to the visual cortex of young kittens, however, does not normally proceed to completion; even at its full extent (reached by 6-7 weeks of age) some overlap remains at the boundaries of the columns devoted to each eye (LeVay et al., 1978). In fact, the normal critical period persists for some time after layer IV reaches a mature level of segregation, so that a process different from the terminal field modification seen early in the critical period may be involved in its later stages. The demonstration of reduced or absent segregation of the geniculo-cortical afferents in dark-reared animals (Swindale, 1981; however, see Stryker, 1981) suggests by analogy that reduced segregation may be partially

responsible for the preservation of plasticity of the visual cortex in the dark. The kittens in the present study which were allowed binocular exposure prior to dark-rearing, therefore, may have been plastic due to their placement into the dark when the normal process of segregation was incomplete and the cortex was in the early stages of the critical period. Whether brief exposure to light later in the critical period provides a signal for the critical period to run its course was not tested in the present study, although a recent preliminary report suggests that this may indeed be the case (Mower et al., 1983).

In summary, this group of experiments demonstrated the strong sensitivity of the dark-reared visual cortex to various alterations of visual environment. The ability of the dark-reared cortex to withstand two weeks of binocular experience after removal from the dark and before monocular deprivation, as well as its ability to respond to reversal of a previously established ocular dominance distribution are functional manifestations of plasticity similar to what is seen in very young kittens. The lack of a shift in ocular dominance in response to monocular deprivation in the NE-depleted cortex is consistent with this functional similarity. The absence of a corresponding change in LGN cell size suggests that the preserved plasticity of dark-reared adults may be due to alterations in synaptic efficacy of intracortical circuitry and not to any actual retraction or expansion of the geniculo-cortical terminal fields in the cortex. The degree of plasticity seen in cats placed in the dark shortly after the onset of the critical period indicates a strong activity dependence to the duration of the critical period: once initiated, the critical period does not run an inexorable course.

REFERENCES

Barlow, H. B., Blakemore, C. and Pettigrew, J. D. The neural basis of binocular depth discrimination. J. Physiol. Lond. **193**:327-342, 1967.

Blakemore, C. and Pettigrew, J. D. Eye dominance in the visual cortex. Nature **225**:426-429, 1970.

Blakemore, C. and Van Sluyters, R. C. Reversal of the physiological effects of monocular deprivation in kittens: further evidence for a sensitive period. J. Physiol. Lond. **237**:196-216, 1974.

Blakemore, C. and Van Sluyters, R. C. Innate and environmental factors in the development of the kitten's visual cortex. J. Physiol. Lond. **248**:663-716, 1975.

Blasdel, G. G. and Pettigrew, J. D. Effect of prior visual experience on cortical recovery from the effects of unilateral eyelid suture in kittens. J. Physiol. Lond. **274**:601-619, 1978.

Buisseret, P. and Imbert, M. Visual cortical cells; their developmental properties in normal and dark-reared kittens. J. Physiol. Lond. **255**:511-525, 1976.

Cragg, B. C. The development of synapses in the visual system of the cat. J. Comp. Neurol. **160**:147-166, 1975.

Cynader, M., Berman, N. and Hein, A. Recovery of function in cat visual cortex following prolonged deprivation. Exp. Brain Res. **25**:139-156, 1976.

Cynader, M. and Mitchell, D. E. Prolonged sensitivity to monocular deprivation in dark-reared cats. J. Neurophysiol. **43**:1026-1040, 1980.

Cynader, M., Mustari, M. J. and Gardner, J. C. Modification of cortical ginocular connectivity. Soc. Neurosci. Symp. **4**:99-120, 1979.

Fregnac, Y. and Imbert, M. Early development of visual cortical cells in normal and dark-reared kittens: Relationship between orientation selectivity and ocular dominance. J. Physiol. Lond. **278**:27-44, 1978.

Ganz, L., Fitch, M. and Satterberg, J. A. The selective effect of visual deprivation on receptive field shape determined neurophysiologically. Exp. Neurol. **22**:614-637, 1968.

Guillery, R. W. Binocular competition in the control of geniculate cell growth. J. Comp. Neurol. **144**:117-127, 1972.

Guillery, R. W. and Stelzner, D. J. The differential effects of unilateral lid closure upon the monocular and binocular segments of the dorsal lateral geniculate nucleus in the cat. J. Comp. Neurol. **139**:413-422, 1970.

Hickey, T. L., Spear, P. D. and Kratz, K. E. Quantitative studies of cell size in the cat's dorsal lateral geniculate nucleus following visual deprivation. J. Comp. Neurol. **172**:265-282, 1977.

Humason, G. L., Animal Tissue Techniques, San Francisco: W. H. Freeman and Co., 1972, pp. 79-81.

Hubel, D. H. and Wiesel, T. N. Receptive fields, binocular interaction, and functional architecture in the cat's visual cortex. J. Physiol. Lond. **160**:106-154, 1962.

Hubel, D. H. and Wiesel, T. N. Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. J. Neurophysiol. **26**:994-1002, 1963.

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

Imbert, M. and Buisseret, P. Receptive field characteristics and plastic properties of visual cortical cells in kittens reared with or without visual experience. Exp. Brain Res. **22**:25-36, 1975.

Kasamatsu, T., Itakura, I. and Jonsson, G. Intracortical spread of exogenous catecholamines: effective concentration for modifying cortical plasticity. J. Pharm. Exp. Ther. **217**:841-850, 1981.

Kasamatsu, T. and Pettigrew, J. D. Depletion of brain catecholamines: failure of ocular dominance shift after monocular occlusion in kittens. Science **194**:206-209, 1976.

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

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

Kuppermann, B. and Ramachandran, V. S. Prolonging the critical period for plasticity in the cat visual cortex. ARVO Abstracts **20**:71, 1981.

LeVay, S., Stryker, M. P. and Shatz, C. J. Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study. J. Comp. Neurol. 179: 223-244, 1978.

LeVay, S., Wiesel, T. N. and Hubel, D. H. The development of ocular dominance columns in normal and visually deprived monkeys. J. Comp. Neurol. **191**:1-51, 1980.

Leventhal, A. G. and Hirsch, H. V. B. Receptive-field properties of different classes of neurons in visual cortex of normal and dark-reared cats. J. Neurophysiol. **43**:1111-1132, 1980.

Levick, W. R. Another tungsten microelectrode. Med. Biol. Engng. **10**:510-515, 1972.

Movshon, J. A. Reversal of the physiological effect of monocular deprivation in the kitten's visual cortex. J. Physiol. Lond. **261**:125-174, 1976.

Mower, G. D., Berry, D., Burchfiel, J. L. and Duffy, F. H. Comparison of the effects of dark rearing and binocular suture on development and plasticity of cat visual cortex. Brain Res. **220**:255-267, 1981.

Mower, G. D., Christen, W. G. and Caplan, C. J. Visual input is a switch for cortical plasticity. ARVO Abstracts **22**:139, 1983.

Sanderson, K. J. The projection of the visual field to the lateral geniculate and medial interlaminar nuclei in the cat. J. Comp. Neurol. **143**:101-118, 1971.

Sherman, S. M., Guillery, R. W., Kaas, J. H. Sanderson, K. J. Behavioral, electrophysiological, and morphological studies of binocular competition in the development of the geniculo-cortical pathways of cats. J. Comp. Neurol. **158**:1-18, 1974.

Stryker, M. P. Late segregation of geniculate afferents to the cat's visual cortex after recovery from binocular impulse blockade. Soc. Neurosci. Abstr. **7**:842, 1981.

Swindale, N. V. Absence of ocular dominance patches in dark reared cats. Nature **290**:332-333, 1981.

Timney, B., Mitchell, D. E. and Cynader, M. Behavioral evidence for prolonged sensitivity to effects of monocular deprivation in dark-reared cats. J. Neurophysiol. **43**:1041-1054, 1980.

Venes, J. L., Collins, W. F. and Taub, A. Nitrous oxide: an anesthetic for experiments in cats. Am. J. Physiol. **220**:2028-2031, 1971.

Wiesel, T. N. and Hubel, D. H. Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. J. Neurophysiol. **26**: 978-993, 1963a.

Wiesel, T. N. and Hubel, D. H. Single cell responses in striate cortex of kittens deprived of vision in one eye. J. Neurophysiol. **26**:1003-1017, 1963b.

Wiesel, T. N. and Hubel, D. H. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J. Neurophysiol. **28**:1029-1040, 1965.

APPENDIX

Enhanced Binocular Interaction in the Visual Cortex of Normal Kittens Subjected to Intracortical Norepinephrine Perfusion

B. D. Kuppermann and T. Kasamatsu

(Submitted to Brain Research)

ABSTRACT

It was previously proposed that norepinephrine (NE)-containing nerve terminals in the visual cortex are important for the maintenance of cortical plasticity. Observations at that time indicated that local microperfusion of exogenous NE for one week directly into kitten visual cortex, with no alteration of the visual environment, resulted in an unexpected bias in ocular dominance toward the contralateral eye. The proportion of binocular cells, however, remained close to normal. In the present study, we examined this contralateral bias in visual cortical neurons addressing the following two issues: the time needed before the change in ocular dominance becomes obvious, and its dependence on visually evoked activity. We found no bias in ocular dominance toward the contralateral eye when the continuous local perfusion of 48 μM NE lasted for three days. Such change became obvious after one week. However, if the animal was placed in the dark during the period of NE perfusion, no change whatsoever in ocular dominance was observed. These results suggest that NE itself does not affect ocular dominance circuitry directly, since both high levels of NE and an extended period of visually evoked activity are necessary for the observed change in ocular dominance to occur. We conclude that the present results are consistent with the previously proposed role for NE in the modulation of visual cortical plasticity.

INTRODUCTION

In 1976, Kasamatsu and Pettigrew presented evidence that the catecholamine (CA)-containing system in the brain is necessary for maintaining neuronal plasticity in the kitten visual cortex. It was observed that the usual shift of ocular dominance did not occur in monocularly lid-sutured kittens if CA-containing nerve terminals in the striate cortex had been destroyed specifically by 6-hydroxydopamine (6-OHDA), a CA-related neurotoxin (Kasamatsu and Pettigrew, 1976, 1979). When exogenous norepinephrine (NE) was perfused directly into the 6-OHDA-treated cortex, neuronal plasticity was restored; the striate cortex became susceptible again to monocular deprivation, resulting in a shift of ocular dominance in favor of the non-deprived eye (Pettigrew and Kasamatsu, 1978; Kasamatsu et al., 1979). It was then proposed that NE does not directly alter the neuronal circuitry responsible for binocular cells, but rather increases the ease with which changes in neuronal connectivity are attained in response to the animal's visual experience (Kasamatsu et al., 1981).

If NE itself does not directly alter the connectivity of visual cortical neurons, the perfusion of NE into the visually normal cortex would not be expected to result in any changes in ocular dominance. When this premise was tested, however, the resultant ocular dominance distribution showed a strong bias to the contralateral eye, despite the presence of many binocularly driven cells (Figure 9, Kasamatsu et al., 1979). Since this change in ocular dominance occurred with no alteration of visual experience, it apparently imposed a difficulty for our NE hypothesis of visual cortical plasticity. In the present study we examine this issue. As will be described below, exogenous NE perfused into the normal striate cortex seemed to allow the naturally occurring contralateral bias in ocular dominance to become exaggerated, leading to an ocular dominance distribution skewed toward the contralateral eye. Present results showed that this change in ocular dominance required one week in a lit environment to occur. These results indicate that the exaggerated contralateral bias

in ocularity observed in animals treated with intracortical NE can be interpreted within the framework of our NE hypothesis. NE did not directly alter cortical connectivity of binocular cells but rather NE and concomitant evoked activity in the visual afferents were necessary for changes in ocular dominance circuitry to occur. A preliminary account was presented elsewhere (Kuppermann and Kasamatsu, 1979).

METHODS

Eight kittens at 5-7 weeks of age were used in this study (Table 1). The visual cortex of each animal was implanted (AP, -5 mm; L, 2 mm) under ketamine anesthesia (20 mg/kg, i.m., repeated if necessary), with a metallic cannula through which 10^{-2} mg/ml (48 μM) l-norepinephrine hydrochloride in 0.4% ascorbate-saline (pH 3.0) was continuously perfused at the rate of 1 μl/hr. The cannula was connected through plastic tubing (PE50) to an osmotic minipump (Alzet 2001) which was placed subcutaneously at the neck. We have shown that exogenous NE perfused directly and continuously into the cortex in this fashion can reach an area up to 10 mm away from the site of cannulation and that the lowest effective concentration of NE, as stored in the cannula-minipump system, for enhancing neuronal plasticity is about 48 μM (Kasamatsu et al., 1979, 1981). The geometry of the perfusion and recording sites was such that the point of closest approach by the electrode to the perfusion site was 2 mm and the furthest distance was approximately 3.5 mm, producing a concentration gradient that was less than 4-fold (Figure 6, Kasamatsu et al., 1981a). The actual concentration of NE at these distances has been shown to be approximately 3 x 10^{-7} M or less, which is in physiological range (Kasamatsu et al., 1981a). After recovering from anesthesia, five animals were returned to our cat colony for various time periods (3, 5 and 8 days) and three animals to a dark room for a week before physiological recordings. A total of twelve recordings were made from these eight animals (see Table 1). In animals which were recorded from twice, the cannula-

TABLE 1

Summary of experimental conditions and ocular dominance data

animal	age at start of NE perfusion	light/dark	days of perfusion at recording	B	C/I
225	6w	light	3	.63	0.9
348	5w+3d	light	3	.77	1.1
498	6w	light	5	.73	1.3
506	6w+2d	light	5	.60	1.6
816	6w+2d	light	5	.70	3.3
225	6w	light	8	.73	20.0
498	6w	light	8	.70	6.3
506	6w+2d	light	8	.57	6.3
225	*	light	*	.73	1.1
237	5w	dark	7.5	.77	1.2
247	5w	dark	7.5	.83	1.6
493	5w+1d	dark	7.5	.70	0.8

*Control, non-perfused hemisphere of animal subject to 8 days of local NE perfusion in the experimental hemisphere.

Experimental treatment and ocular dominance data from each animal and each recording session. Age is given in weeks (w) + days (d). B is a measure of binocularity determined by taking the ratio of the number of cells with any binocular input to all visually responsive cells recorded from in that session. C/I is the ratio of the number of cells dominated by input from the contralateral eye to those dominated by input from the ipsilateral eye, excluding those cells driven equally well by either eye.

minipump system remained implanted during the first recording session. These animals were revived from anesthesia and paralysis and returned to the colony for subsequent recording.

Single unit recordings were carried out following the standard procedures in our laboratory (Kasamatsu et al., 1979, 1981b). Briefly, the animal was intubated endotracheally under gas anesthesia with Halothane (~3%) mixed with N_2O/O_2 (2:1), and polyethylene tubing was inserted into a radial vein for the delivery of the infusion solutions. During recordings the animal was anesthetized with a gas mixture of $N_2O/O_2/CO_2$ with ratios of 75/22.5/2.5 (Venes et al., 1971) and maintained under hyperventilation in order to ensure enough oxidation. The animal was immobilized with an initial dose of Flaxedil (20 mg) and maintained throughout the recording session with an infusion solution which contained Flaxedil (7.5 mg/kg/hr) and dexamethasone (0.2 mg/hr). Body temperature was maintained at 37.5°C by means of a heating pad and an electric mesh blanket with a feedback circuit. The heart rate was monitored continuously. Pupils were dilatated by topical application of 1% cyclogyl. The corneae were protected from drying by placing contact lenses with zero power over them.

Visual stimuli, light slits and dark bars on a light background, were presented on a tangent screen 57 cm from the animal's eyes through a rear projection system, and were moved on the screen by a joystick. The intensity of visual stimuli was at about 1-2 log units above the background illumination, which was kept at the mesopic level during recordings.

Tungsten-in-glass microelectrodes (Levick, 1972) were introduced in the postlateral gyrus, usually 1.5 mm from the midline and 2 mm anterior to the site of cannulation in the NE-perfused hemisphere or at the corresponding site in the other, control hemisphere. The electrode tracks were angled by 5° medially and 10° anteriorly so that sampling of isolated single units was made across laminar and

columnar boundaries. When an electrode was advanced through the medial bank, cells were recorded at about every 100 μm. At least 30 visually responsive cells were isolated, typically in one or two penetrations. Recording sessions lasted from 12 to 20 hours, except for the animal subjected to bihemispheric recordings, which lasted 30 hours.

For each isolated cell the following features of visual receptive field were characterized: minimum response field (Barlow et al., 1967), response to stationary flashing spots and light slits, orientation and direction selectivity, velocity tuning, and preferred stimulus width and length. In this way, the optimal stimulus was presented for the determination of the ocular preference of each cell. Results are presented in the form of ocular dominance histograms following the seven-group scheme of Hubel and Wiesel (1962).

At the end of each track a small electrolytic lesion was made by passing 5-8 μA direct current for 10 sec with negativity at the tip of the electrode. Animals were anesthetized with a lethal dose of Nembutal, perfused with warm saline followed by 10% formol-saline. Thirty-micron thick sections were cut, stained for Nissl substances, and later used for reconstruction of each microelectrode track.

RESULTS

Normal kittens whose visual cortex was perfused with NE for one week exhibited an obvious bias in ocular dominance toward the contralateral eye. An example is shown in Figure 1a (225, Table 1). We found only one cell, out of a sample of 30 visually active cells, which received stronger excitatory input from the ipsilateral than the contralateral eye. Thus, the ratio (C/I) of the number of cells predominated by the contralateral eye to that by the ipsilateral eye, excluding cells which receive equal input from the two eyes, strongly favored the contralateral eye (C/I=20.0). This result was compared to the usual distribution of ocular dominance

Figure 1. A change in the ocular dominance distribution of a six-week-old kitten (225) whose visual cortex was subjected to local continuous perfusion of 48 μM l-norepinephrine through an implanted cannula connected to an osmotic minipump for one week. Each of the seven ocular dominance groupings correspond to that originally defined by Hubel and Wiesel (1962). The number of unresponsive units (u) and geniculate fibers (gl) is also shown. B is a measure of binocularity determined by taking the ratio of cells with any binocular input (groups 2-6) to all the visually active cells in the histogram. C/I is the ratio of contralaterally dominated (groups 1-3) over the ipsilaterally dominated cells (groups 5-7). Recordings were made in an area from the hemisphere perfused with NE at a site roughly 2 mm from the perfusion site. Note that no lid-suture was performed in this or any other animal in this paper. Thirty cells, collected from one penetration, were recorded to construct each ocular dominance histogram. a) Results from the NE-perfused hemisphere. A bias towards the contralateral eye was observed in this animal, with a C/I ratio of 20.0. In spite of this strong contralateral bias, a high degree of cortical binocularity remained. b) Results from the non-perfused hemisphere of the same animal at the same recording session. The recording site corresponds to the same stereotactic coordinates as that for the perfused hemisphere. No exaggerated bias in ocularity was observed, with a C/I ratio of 1, close to the normal value.

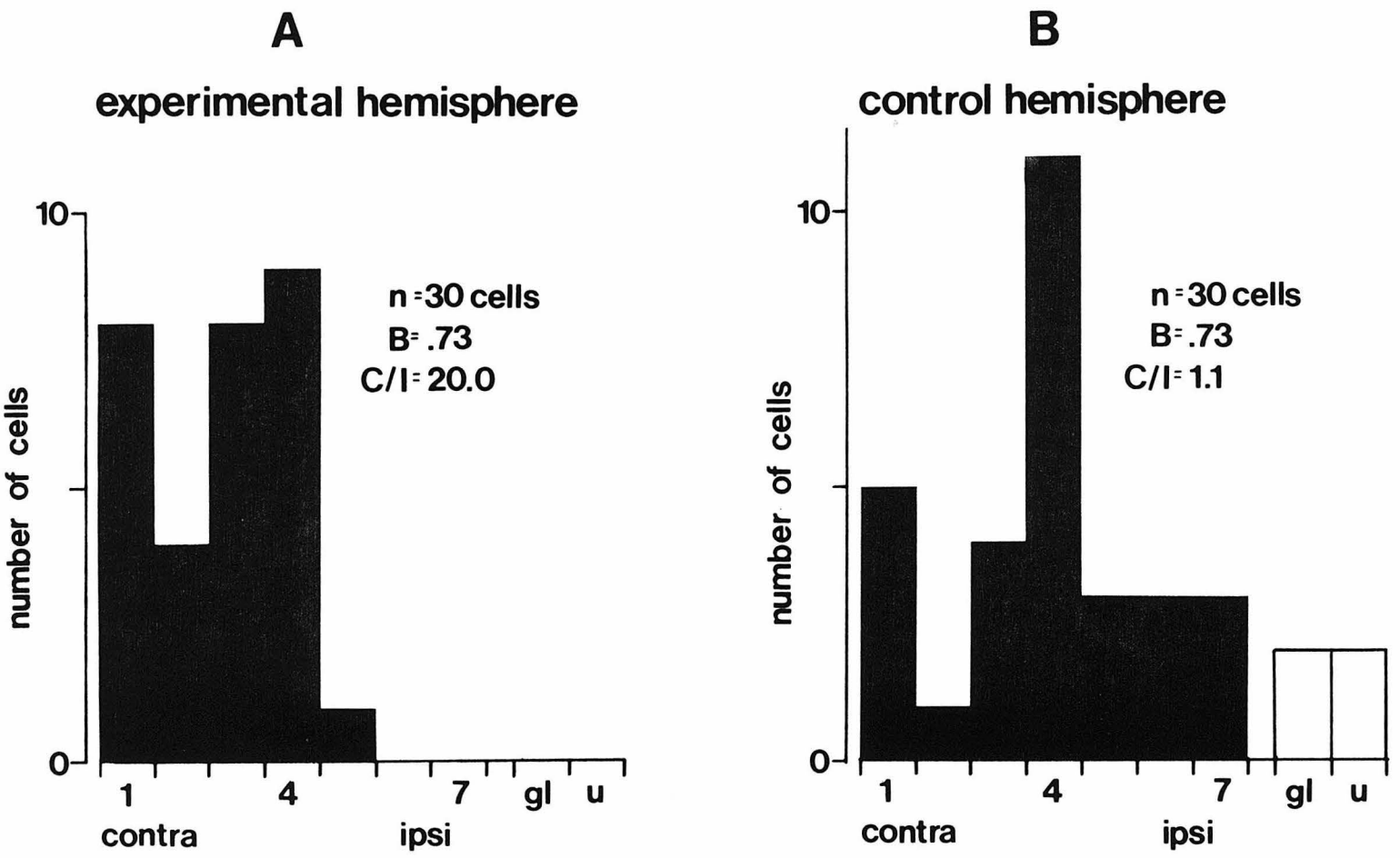

A
experimental hemisphere
10
0
number of cells
n = 30 cells
B = .73
C/I = 20.0
1
4
7
gl
u
contra
ipsi
B
control hemisphere
10
0
number of cells
n = 30 cells
B = .73
C/I = 1.1
1
4
7
gl
u
contra
ipsi

fig1

obtained from the other, untreated hemisphere of the same kitten (Figure 1b). Despite this apparent absence in the NE-perfused cortex of visual cortical cells which received stronger excitatory input from the ipsilateral eye than the contralateral eye, no obvious change was noted in the proportion of binocular cells to the total number of visually active cells (binocularity, B=0.73).

In searching for plausible explanations for this marked contralateral eye dominancy in the NE-perfused, otherwise normal visual cortex, two series of experiments were carried out. First, we determined how long a period of NE perfusion was necessary before the change in ocular dominance became obvious. Second, we examined whether visually evoked activity was necessary for the change to take place.

Time Course of Changes in Ocular Dominance

Five kittens were used: one animal was recorded 3 and 8 days after the start of NE perfusion (225), two animals at days 5 and 8 (498 and 506), and in two animals a single recording was made 3 days (348) or 5 days (816) after the start of NE perfusion. The NE perfusion was maintained during the recording sessions on days 3 and 5 except for one animal (816) recorded from on day 5, in which the cannula-minipump system was disconnected immediately before the start of recording. The NE-containing minipumps were disconnected before recording on day 8 in all three animals (225, 498, 506).

In animals perfused with NE for only 3 days, we obtained an ocular dominance distribution which did not differ substantially from normal, with a C/I ratio that was close to 1.0 (Figure 2a). After 5 days of NE perfusion, there was a slight increase in the C/I ratio (2.1), though it was not as obvious as seen later (Figure 2b). By the eighth day of continuous NE perfusion, visual cortical cells which were predominated by the contralateral input strongly outnumbered those receiving input primarily from

Figure 2. The time course of the change in ocularity towards the contralateral eye during continuous perfusion of NE. One animal was recorded from on days 3 and 8 of NE perfusion (225), two animals were recorded from on days 5 and 8 (498 and 506), and two animals were recorded from at one session only, either on day 3 (348) or day 5 (816). The initial individual histograms were composed of data taken from 30 visually responsive cells. The composite histograms shown here were produced by averaging the number of cells from each ocular dominance group from all the animals in a single experimental condition. The composite binocularity (B) and C/I ratio were calculated by averaging the individual ratios from each animal (see Table 1 for individual ratios). a) After 3 days of continuous NE perfusion, an essentially normal ocular dominance distribution was observed. There was a somewhat higher than normal number of ipsilateral monocular cells; this may be due to the effects of acute paralysis squint during recording (see Discussion). NE was being continuously perfused during the recording sessions which produced the data in this figure. The histogram is based on the results from two animals (225 and 348). b) After 5 days of continuous NE perfusion, a slight bias towards the contralateral eye was observed. There is an increase in the number of contralaterally dominated cells in comparison to the day 3 histogram (C/I = 2.1), but the histogram is roughly normal. This histogram is based on data from three animals (498, 506, and 816). Here again, there is an elevated number of monocular cells, both contralateral and ipsilateral, perhaps due to the ocular divergence induced by paralysis. NE was being perfused continuously during the recording sessions of two of these animals (498 and 506). c) After 8 days of continuous NE perfusion, a dramatic bias in ocular dominance towards the eye contralateral to the site of perfusion is observed. The C/I ratio (10.9) is much greater than normal (1.7, see Hubel and Weisel, 1962; Blakemore and Pettigrew, 1970), and binocularity (67%) is slightly lower than normal ($\sim$80%). This histogram is a composite of the results from three animals (225, 498, and 506).

Figure 2 (continued)

Vertical bars on the top of each column show either the range (a) or the standard deviation (b and c) between animals of the numbers of cells for each ocular dominance group. The absence of a vertical bar at the top of a column indicates no difference in the number of cells in that group between animals.

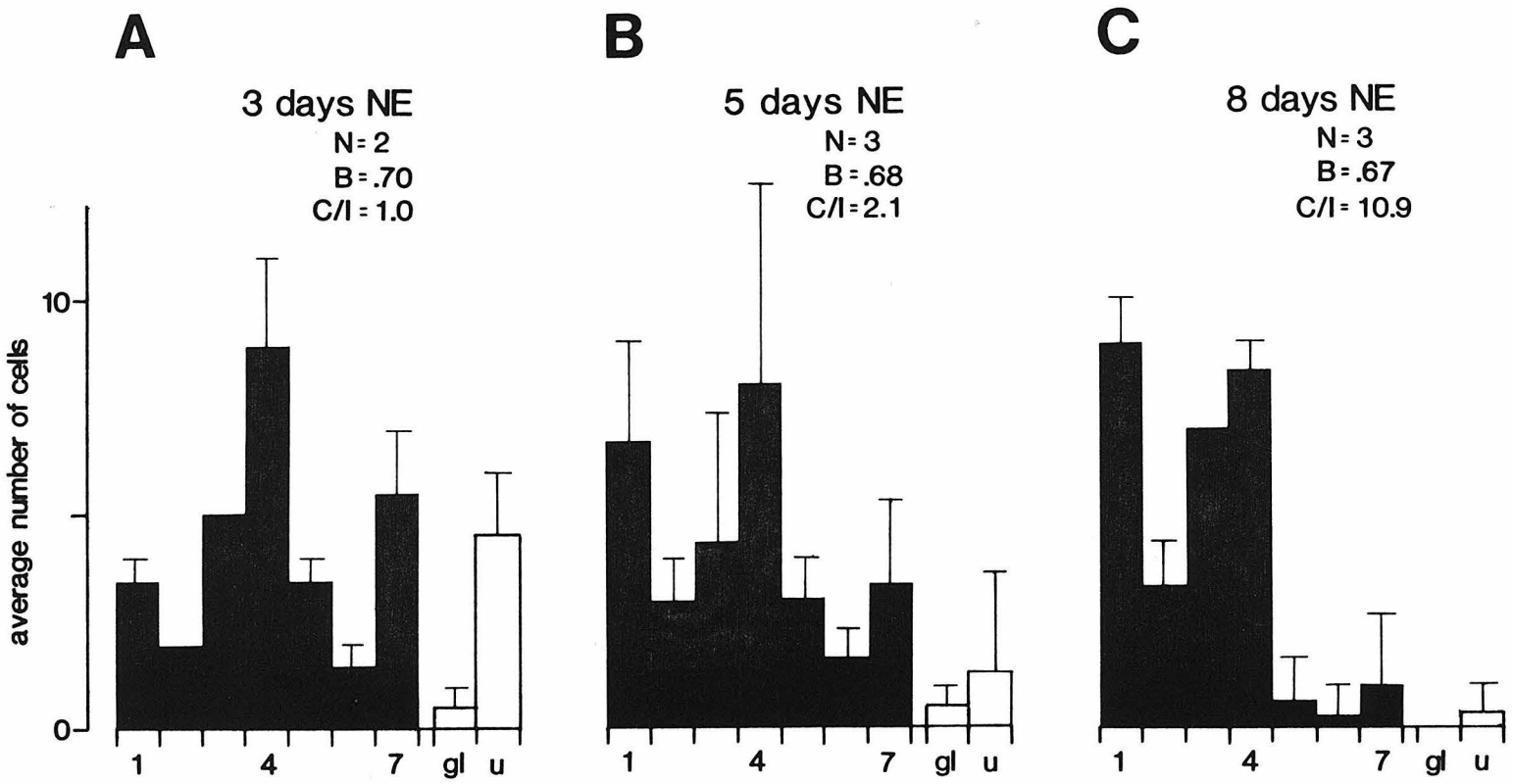

A
3 days NE
N = 2
B = .70
C/I = 1.0
B
5 days NE
N = 3
B = .68
C/I = 2.1
C
8 days NE
N = 3
B = .67
C/I = 10.9
average number of cells
10
0
1
4
7
gl
u

fig 2

the ipsilateral eye, with a C/I ratio of 10.9 (Figure 2c). Throughout this gradual increase in contralateral dominance, the levels of binocularity were reduced only slightly from normal (B = 0.57-0.77 versus ↶0.80 for normal kittens). We conclude that the NE-related change in the ocular dominance distribution in the visual cortex of normal kittens takes about one week before it becomes obvious.

Dependence on Visual Evoked Activity

If NE directly affects ocular dominance circuitry, one might expect the observed change in ocular dominance to occur independent of the presence or absence of visually evoked activity. To test this premise, we placed three animals (237, 247, 493) in total darkness during the period of intracortical NE perfusion of one week. When we recorded from the NE-perfused hemisphere of these three animals immediately after the end of the NE perfusion period, we found no change in ocular dominance. Binocularity (0.77) and the C/I ratio (1.2) both stayed close to normal (B ↶0.80; C/I, 1.0 ↶2.0). These results are shown in Figure 3b, and are compared to those in Figure 3a which reproduces the results in light reared animals. It appears, therefore, that the presence of visually evoked activity is necessary for changes in ocular dominance to occur in the NE-perfused, otherwise normal visual cortex.

DISCUSSION

NE Does Not Directly Alter Connectivity

The present results showed that the change in ocular dominance related to exogenous NE perfused into the cortex of visually normal kittens takes at least 5 days in a lit environment before it becomes even slightly noticeable. By comparison, the pharmacological action of NE in the cortex is known to occur quickly. When NE is iontophoretically delivered into the visual cortex, for example, the excitability of visual cortical neurons, if they are responsive to NE, is rapidly modified, and lasts for

Figure 3. The dependence on visually evoked activity for the development of changes in ocular dominance in the NE perfused cortex. a) Three 6-week-old kittens subjected to NE perfusion for 8 days and left in a light environment. The histogram in Figure 2c is reproduced here for direct comparison to Figure 3b. b) Three 5-week-old kittens subjected to NE perfusion for one week and kept in the dark during the period of perfusion. The resultant ocular dominance distribution is normal, with both a normal percentage of binocular cells (77%) and a normal ratio of contralaterally dominated to ipsilaterally dominated cells (1.2). For conventions, see captions in Figures 1 and 2.

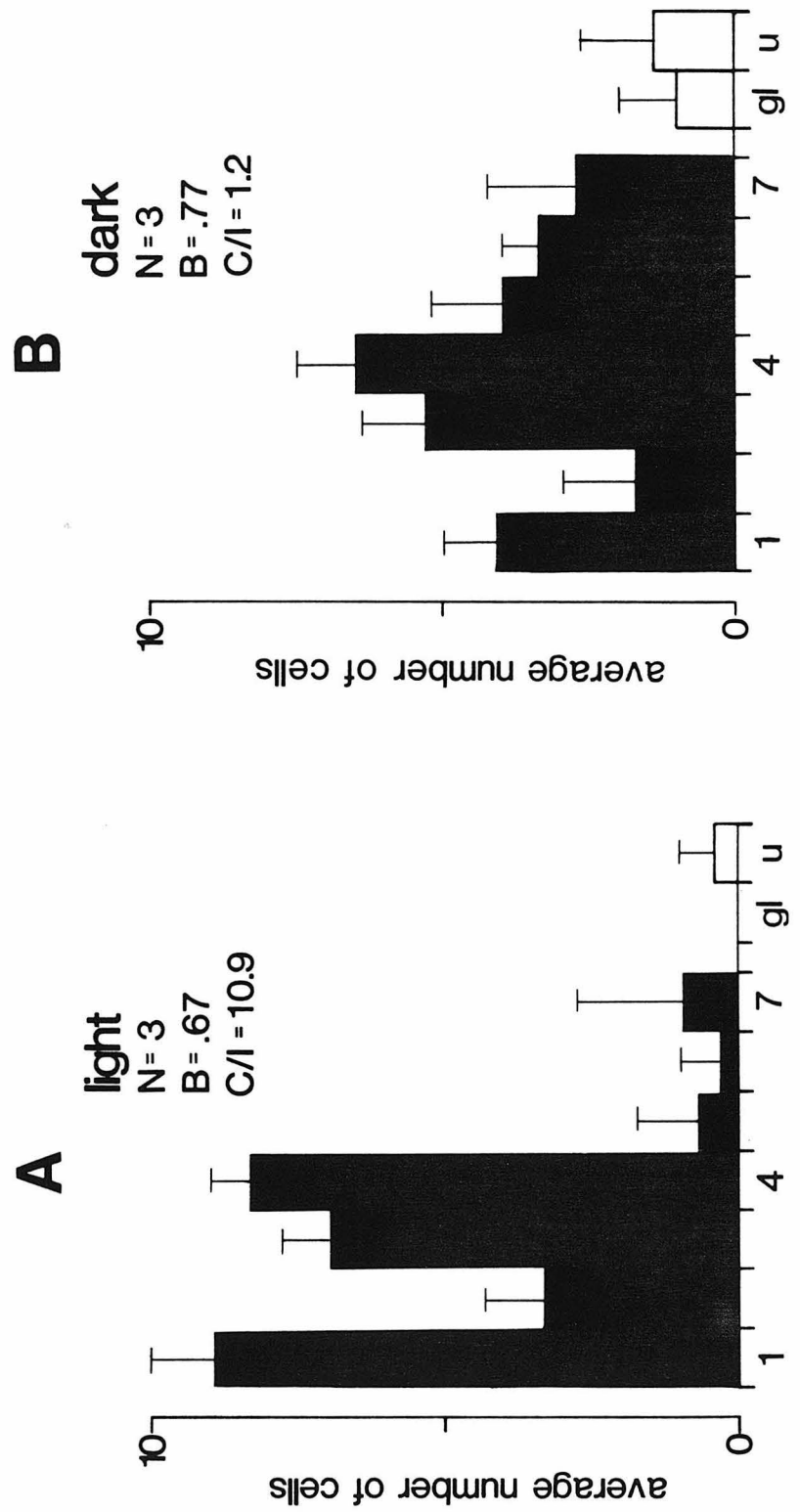

A
light
N=3
B=.67
C/I=10.9
average number of cells
10
0
1
4
7
gl
u
B
dark
N=3
B=.77
C/I=1.2
average number of cells
10
0
1
4
7
gl
u

fig 3

a few minutes after the end of iontophoresis (Kasamatsu and Heggelund, 1982). The specific uptake of exogenous NE by NE-containing terminals starts rapidly and is accomplished within a few hours (Jonsson et al., 1969). Therefore, if NE has a direct chemical effect on the cortical circuitry responsible for binocularity, the unique change observed in ocular dominance in the present study would probably be obtained earlier than reported here. The absence of such a change in ocular dominance after the week-long NE perfusion, if the animal is kept in the dark, further strengthens this thesis, since the observed change in ocular dominance required ongoing visual activity. We conclude that the contralateral bias in ocular dominance seen in the presence of exogenous NE is not due to a direct effect of NE itself on cortical circuitry, but due to a process which is dependent on visual experience. How then do changes in ocular dominance occur in the visually normal, NE-treated cortex?

Factors Involved in Changes in Ocular Dominance

Changes in ocular dominance are thought to be due primarily to competition between afferents from the two eyes for the limited number of postsynaptic sites on cortical cells (Wiesel and Hubel, 1965). The effectiveness of the binocular competition in producing changes in connectivity, however, is dependent on both the amount of plasticity in the system, and the amount of time that the competitive condition is maintained. Animals subjected to similar visual experience but with different degrees of plasticity could therefore exhibit different patterns of ocular dominance connectivity. Previous studies from this laboratory indicate that this is indeed the case. Kittens subjected to local intracortical NE perfusion and brief, 24 hour, monocular suture exhibit greater shifts in ocular dominance in the NE-perfused hemisphere, than in the control, non-perfused hemisphere of the same animal (Heggelund and Kasamatsu, 1981). A similar result was observed for the recovery of binocularity after monocular suture: more binocular cells were observed

during recovery in the NE-treated hemisphere than in the control hemisphere (Kasamatsu et al., 1981). In these cases, where the imbalance of visual input is either short-lived (as in a 24 hour monocular deprivation) or subtle (as in recovery, where both eyes are open after the prior monocular lid suture), the ability of the cortical circuitry to reflect the balance of incoming activity is highly dependent on the level of plasticity in the system.

An analogous situation was observed in the present study. Normal kittens have a slight innate bias of the contralateral projections to the visual cortex, as evidenced by the fact that the majority of binocular cells receive stronger excitatory inputs from the contralateral eye than the ipsilateral eye (Hubel and Wiesel, 1962; Blakemore and Pettigrew, 1970). In the presence of exogenous NE, the advantage exhibited by the contralateral afferents is further increased by the nature of the proposed action of NE on ocular dominance circuitry: binocular interactions are enhanced, since synaptic connections are more easily modified. We suggest that the observed skewed distribution of ocular dominance is primarily due to normal ongoing binocular interactions which are enhanced only under continuously high levels of plasticity. This hypothesis is not only consistent with the results presented here, but also it accounts for all the results obtained by this laboratory in its series of experiments on the role of NE in cortical plasticity.

Effects of Acute Paralysis Squint

In addition to an obvious deviation of the ocular dominance distribution from what is usually seen in the normal visual cortex, when we examined the ocular dominance histograms individually, we noted a small but consistent increase in the number of monocular cells (in both groups 1 and 7), which was especially noticeable 3 and 5 days after the onset of NE perfusion (Figures 2a and b). This result is particularly interesting in that increased numbers of ipsilateral monocular cells were

observed during a process that eventually produces a contralaterally biased pattern of connectivity. It is possible that this small increase in monocularity may have been due to the effects of increased plasticity during NE perfusion in conjunction with the loss of normal convergence of the two visual axes, which is inevitable in the acutely anesthetized and paralyzed state of the animal during recording (Heggelund and Kasamatsu, 1981). In fact, we did not obtain an increase in ipsilateral monocular cells in any animal whose pump was removed prior to the start of the recording session, including one kitten recorded from 5 days after the onset of NE perfusion (816).

Plasticity During Normal Development

We suggested above that the results presented here were due to an increase of plasticity produced by the perfusion of exogenous NE. However, the visual cortex of six-week-old kittens is known to be extremely plastic, even without the addition of exogenous NE. How does increasing the level of NE further enhance the plasticity of a system that has already manifested a high level of plasticity? The level of plasticity is known to change with age in the normal cortex (Hubel and Wiesel, 1970). It may be that it is not saturated at a given age, since, for example, the sensitivity of the adult visual cortex to monocular deprivation can, at least in part, be restored by exogenous NE (Kasamatsu et al., 1979). It is plausible that the presence of exogenous NE does in fact raise the level of plasticity of a non-saturated system beyond the usual levels seen in the normal cortex, even in young animals. The ability of exogenous NE to accelerate modification of ocular dominance in response to monocular lid suture and recovery from monocular lid suture, as mentioned earlier, is consistent with this idea. We recently suggested that β adrenoreceptors within the visual cortex may be involved in enhancing visual cortical plasticity (Kasamatsu, 1979; 1980). The activation of β adrenoreceptors, whose total number is limited

depending on the animal's age (Jonsson and Kasamatsu, 1983), may become more efficient with the increased availability of NE in the visual cortex perfused with NE for one week. If a higher percentage of β adrenoreceptors are occupied more of the time, more synaptic modification than normal could occur in response to the incoming visual activity.

A second paradox is why, despite the enhanced binocular interaction in the NE-perfused, otherwise normal cortex, the resultant ocular dominance histogram is not composed primarily of monocular cells. A binocular but ipsilaterally dominated group 5 cell, for example, when primed by the enhanced plasticity due to the perfusion of NE might be expected to become a monocular ipsilateral group 7 cell. The fact that this did not occur suggests that the neuronal circuitry through which these changes in ocular dominance occur somehow integrates the preponderance of contralateral inputs in the visual cortex. Binocular interactions may include competition among groups of cells, in addition to the competition by presynaptic terminals for postsynaptic sites on a single cell as proposed originally by Wiesel and Hubel (1965). This competition among groups of cells would probably require an inhibitory mechanism. It is not clear, however, whether the GABAergic inhibitory mechanism demonstrated by Sillito et al. (1981) contributes to this binocular interaction.

In conclusion, levels of NE do not appear to change the competitive balance of incoming visual activity, or to alter connectivity directly. Rather NE enhances the ability of binocular afferents to modify neuronal connectivity, which eventually leads to changes in ocular dominance (for further discussion see Kasamatsu, 1983). How NE mediates plasticity is not yet well understood, but current research in our laboratory on β adrenoreceptors and cyclic nucleotides may give us insight on its cellular mechanisms.

ACKNOWLEDGEMENTS

We thank J. D. Pettigrew and D. C. Van Essen for their valuable comments on the manuscript. We are also grateful to C. Hochenedel for typing the manuscript and to Lederle Laboratories for donating the Flaxedil. This work was supported by USPHS grant EY-03409. B.K. was supported by NIH training grant 5 T32 GM07737.

REFERENCES

Barlow, H. B., Blakemore, C. and Pettigrew, J. D. The neural basis of binocular depth discrimination. J. Physiol. Lond. **193**:327-342, 1967.

Blakemore, C. and Pettigrew, J. D. Eye dominance in the visual cortex. Nature **225**:426-429, 1970.

Heggelund, P. and Kasamatsu, T. Exogenous noradrenaline increases the neuronal plasticity in cat visual cortex: localized, continuous microperfusion and iontophoresis. In: Adv. Physiol. Sci. Vol. 36, Cellular Analogues of Conditioning and Neural Plasticity, edited by O. Fehér and F. Jóo. Budapest: Akadémiai Kiadó, Oxford: Pergamon Press, 1981, p. 233-242.

Hubel, D. H. and Wiesel, T. N. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J. Physiol. Lond. **160**:106-154, 1962.

Hubel, D. H. and Wiesel, T. N. Binocular interaction in striate cortex of kittens reared with artificial squint. J. Neurophysiol. **28**:1041-1059, 1965.

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

Jonsson, G., Hamberger, B., Malmfors, T., and Sachs, C. Uptake and accumulation of ^{3}H-noradrenaline in adrenergic nerves of rat iris. Effect of reserpine, monoamine oxidase, and tyrosine hydroxylase inhibition. Eur. J. Pharmacol. **8**:58-72, 1969.

Jonsson, G. and Kasamatsu, T. Maturation of monoamine neurotransmitters and receptors in cat occipital cortex during postnatal critical period. Exp. Brain Res., 1983, (in press).

Kasamatsu, T. Involvement of the β-adrenergic receptor in cortical plasticity. ARVO Abstracts **18**:135, 1979.

Kasamatsu, T. A possible role for cyclic nucleotides in plasticity of visual cortex. Soc. Neurosci. Abstracts **6**:494, 1980.

Kasamatsu, T. Neuronal plasticity maintained by the central norepinephrine system in the cat visual cortex. In: Prog. Psychobiol. Physiol. Psych. Vol. 10, edited by J. M. Sprague and A. N. Epstein. New York: Academic Press, 1983, p. 1-112.

Kasamatsu, T. and Heggelund, P. Single cell responses in cat visual cortex to visual stimulation during iontophoresis of noradrenaline. Exp. Brain Res. **45**:317-327, 1982.

Kasamatsu, T., Itakura, T. and Jonsson, G. Intracortical spread of exogenous catecholamines: effective concentration for modifying cortical plasticity. J. Pharm. Exp. Ther. **217**:841-850, 1981a.

Kasamatsu, T. and Pettigrew, J. D. Depletion of brain catecholamines: failure of ocular dominance shift after monocular occlusion in kittens. Science **194**:206-209, 1976.

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

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

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

Kuppermann, B. and Kasamatsu, T. Binocular competition: its enhancement by norepinephrine. Soc. Neurosci. Abstr. **5**:792, 1979.

Levick, W. R. Another tungsten microelectrode. Med. Biol. Engng. **10**:510-515, 1972.

Pettigrew, J. D. and Kasamatsu, T. Local perfusion of norepinephrine maintains visual cortical plasticity. Nature **271**:761-763, 1978.

Sillito, A. M., Kemp, J. A., and Blakemore, C. The role of GABAergic inhibition in the cortical effect of monocular deprivation. Nature **291**:318-320, 1981.

Venes, J. L., Collins, W. F., and Taub, A. Nitrous oxide: an anesthetic for experiments in cats. Am. J. Physiol. **220**:2028-2031, 1971.

Wiesel, T. N. and Hubel, D. H. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J. Neurophysiol. **28**:1029-1040, 1965.