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# Early experience, binocular competition, and the sculpting of relay cell morphology in the cat lateral geniculate nucleus

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# EARLY EXPERIENCE, BINOCULAR COMPETITION, AND THE SCULPTING OF RELAY CELL MORPHOLOGY IN THE CAT LATERAL GENICULATE NUCLEUS

by

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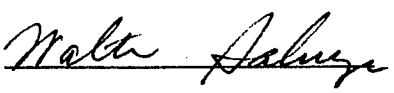
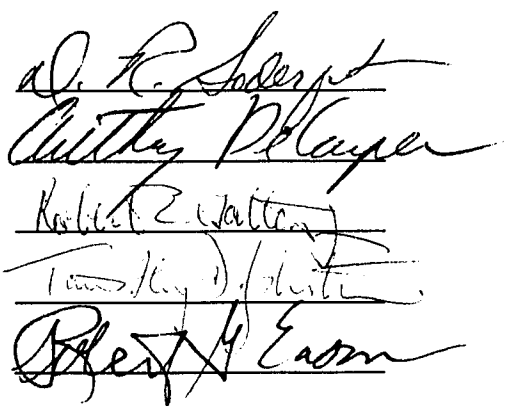
**Dissertation Adviser** 

**Committee Members** 

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Perturbations of early visual experience imposed by either monocular deprivation (MD) or artificially induced strabismus (squint) affect the morphological development of cells in the lateral geniculate nucleus (LGN) of cats. These morphological anomalies are thought to arise from competitive mechanisms which control many features of LGN cell development. Competition between the axon terminals of LGN cells innervated by the right and left eyes for establishing and maintaining synaptic contacts with cells in the visual cortex (i.e., binocular competition) is known to be a very important mechanism for controlling the development of LGN cells. Much of our present understanding regarding the importance of binocular competition is based on the effects of MD and squint on LGN somata. The objective of the present study was to further explore the role of binocular competition in the somatic development of LGN cells and extend these observations to determine the role of binocular competition in the dendritic development of LGN cells. Binocular competition was eliminated in some of the animals reared with either MD or squint by concomitant sagittal transection of the optic chiasm (OX), performed on either postnatal day 27 or 28. The morphology of LGN cells was studied using an ***in vitro*** procedure which allowed for intracellular injections of lucifer yellow into identified LGN relay cells. Using blind

coded LGN samples, 76 LGN relay cells were drawn with camera lucida techniques at a total magnification of 1500X. The morphology of each cell was quantified using Sholl ring procedures (see Sholl, 1955), aided by a morphometric software package. Each of the LGN cells included in our sample were classified according to a slightly modified version Guillery's (1965) class 1, 2, and 3 criteria. Information regarding each cell's rearing history (e.g., MD, squint, etc..) was obtained, and a series of analyses of variance was performed on a number of different somatic and dendritic characteristics. In animals reared with intact optic chiasms, both MD and squint were shown to produce significant changes in the somatic and dendritic profiles of LGN relay cells. Moreover, data from animals reared with MD or squint combined with sagittal transection of the OX (i.e., this combination of manipulations allowed for an assessment of the effects of deprivation while preventing the opportunity for abnormal binocular competition) demonstrated that OX transection protected cells in the deprived or squinted layer from many of the effects induced by either MD or squint (alone). These findings indicate that binocular competition exerts a powerful influence on the somatic and dendritic development of LGN cells. These observations also underscore the importance of early visual experience in the development of sensory nervous system and provide insights into the possible developmental etiology of visual amblyopias.

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# CHAPTER I

## INTRODUCTION

Early postnatal development is marked by a host of changes in the central nervous system which are responsible for the development of an organism's sensory, cognitive, and behavioral competence. Moreover, the extent to which the development of brain structure and function depends on early experience is of central interest to broad range of disciplines, not the least of which is that of psychology. One way in which experience is believed to influence the development of nervous system is through activity-dependent, competitive processes, which are said to represent the self-organizing properties of the nervous system (Hebb, 1949; Guillery, 1988; Kalil, 1989; Prestige and Wilshaw, 1975; Purves and Lichtman, 1980; Purves, 1980).

The nature of activity-dependent processes and their relationship to early visual experience has been studied extensively in the development of the visual system (for review see, Barlow, 1975; Kalil and Dubin, 1988; Shatz, 1988; Kalil, 1989; Movshon and Van Sluyters, 1981; Sherman and Spear, 1982; van Sluyters et al., 1990). Research on the development of central and peripheral visual pathways include a wide range of topics, however, studies which have focused on the development of neurons in lateral geniculate nucleus (LGN) have proven to be particularly fruitful in identifying the competitive mechanisms believed

responsible for self-organization in this thalamic nucleus (for review see, Sherman and Spear, 1982; Sherman, 1985). These studies have shown that much of the physiological and morphological development of LGN cells depends on competitive interactions either among retinogeniculate axon terminals (Friedlander, Stanford and Sherman, 1982; Garraghty, Roe, Chino and Sur, 1989; Sur, Humphrey and Sherman, 1982; Garraghty, Sur and Sherman, 1986) or geniculocortical axon terminals (Garraghty, Salinger and Hickey, 1984; Guillery and Stelzner, 1970; Guillery, 1972; Sherman, Guillery, and Kaas, 1974; Kratz and Spear, 1976; Hickey, Spear and Kratz, 1980) for establishing and maintaining synaptic contacts on LGN or cortical cells, respectively.

The present study was designed to assess the role of competitive interactions among geniculocortical axons (referred to as binocular competition) as visual projections from the two eyes compete for synaptic space in the visual cortex. The results obtained here offer insights into the significance of binocular competition as a mechanism involved in the self-organization of the LGN. Moreover, the present findings have implications for the clinical maintenance of visual amblyopias and suggest the possibility that subcortical structures may be involved in producing the visual dysfunctions observed in human and non-human amblyopes.

The LGN of the Adult Cat

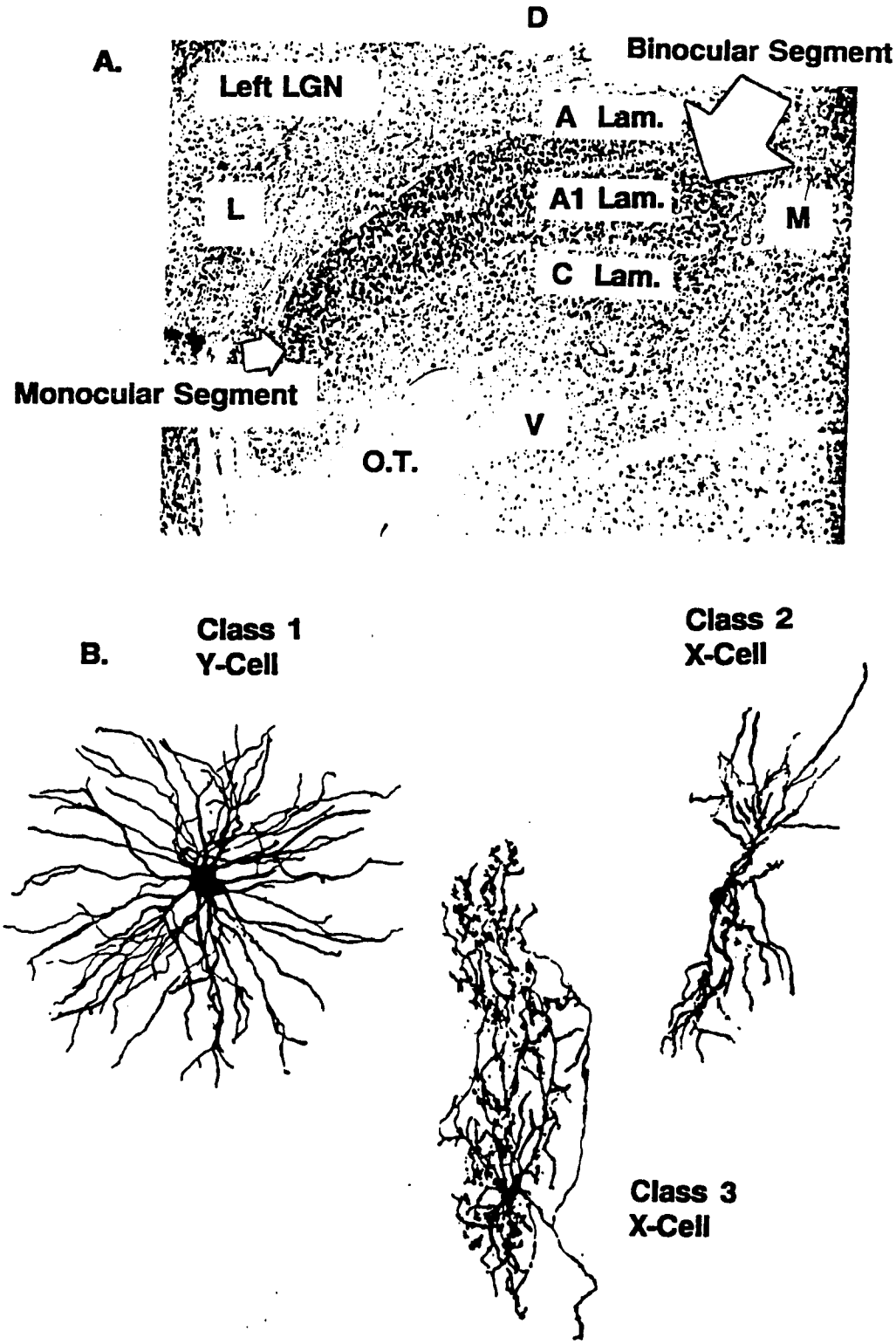
The LGN is located bilaterally in the thalamus of all mammals (Diamond & Hall, 1969). In the cat, each nucleus represents a retinotopically organized

registration of the entire contralateral hemifield (Sanderson, 1971; Kaas et al., 1972). Furthermore, the cat LGN is a laminated structure containing three primary laminae (Hickey and Guillery, 1974; Guillery,1970). The dorsal two layers are of primary concern for this study. The top, A layer receives retinal input from the contralateral eye and the adjacent A1 layer receives input from the ipsilateral eye. Figure 1 shows a sagittal section through the middle of the cat LGN that has been labeled with cressyl violet (taken from Hubel and Wiesel, 1963). The A lamina contains a complete neural representation of the contralateral visual hemifield, whereas the A1 representation only covers the central 40° of visual space (Sanderson, 1971). The central 40° of the A lamina and the entire A1 lamina are referred to as the binocular segment, because both the right and left eyes maintain a visual representation of these areas (see figure 1A). The remaining visual representation of the A layer is known as the monocular segment, because only one eye is sensitive to this region of the visual field (see figure 1B).

LGN cells in the A and A1 layers of the LGN have been characterized by both morphological and physiological criteria. Using Golgi labeled material, Guillery (1966) noted three classes of LGN cells in these laminae as determined by differences in their cell morphology, which he termed class 1, class 2, and

**Figure 1.** Figure 1A demonstrates a sagittal section through the middle of the cat LGN taken at Horsely Clarke atlas coordinates anterior 5.5 (taken from Hubel and Wiesel, 1963). The axes are marked by the letters M (medial), L (lateral), D (dorsal), V (ventral). The laminae are labeled A, A1 and C. Both the binocular and monocular segments of the nucleus are labeled and revealed by the arrows. Figure 1B shows and example of Guillery's class 1, class 2, and class 3 cells (taken from Sherman and Koch, 1986).

**Figure 1.**


D
A.
Binocular Segment
Left LGN
A Lam.
A1 Lam.
L
M
C Lam.
Monocular Segment
V
O.T.
B.
Class 1
Y-Cell
Class 2
X-Cell
Class 3
X-Cell

class 3 cells. Class 1 and class 2 cells were characterized as relay or projection neurons because they possessed axons which exited the nucleus and innervated the visual cortex. Class 3 cells were characterized as LGN interneurons because of their axons terminated within the nucleus. Figure 1B shows examples of these three classes of LGN neurons (see Sherman and Koch, 1986). Friedlander, Lin, Stanford, and Sherman (1981) used intracellular recordings combined with horseradish peroxidase staining and demonstrated that Guillery's (1966) morphologically classified cell classes roughly correspond to physiologically classified LGN X and Y cells. These researchers determined that LGN X cells were either class 2 or class 3 cells and that LGN Y cells exhibited morphological characteristics mostly like class 1 cells and on occasion like class 2 cells. These anatomical and physiological correlates are important for two reasons: (1) LGN X and Y projection cells possess different receptive field characteristics and therefore performs different visual functions (Blake, 1979; Lennie, 1980); and (2) changes in morphological characteristics following early visual perturbations offer insights into the functional consequences of the visual perturbations. This later point is important for the present study because morphological analysis will be used to assess the effect of monocular deprivation or squint on the development of LGN cell classes.

## LGN Development

***Normal Sequence of LGN Cell Development.*** At birth the cat LGN is a laminated structure having its retinal inputs segregated into eye-specific layers

(Kalil, 1978; Shatz, 1983). Although much of the general adult-like cytoarchitecture of this nucleus is present by birth, roughly two-thirds of its adult size can be attributed to growth occurring during the first postnatal month, after which there is a steady but significant decrease in the rate of somatic growth and nuclear expansion (Kalil, 1978). The normal growth of geniculate cells is essentially a linear process which occurs between birth and 56 days postnatal, at which time somatic development begins to slow down and become more variable in various areas within the nucleus. Moreover, this linear trend in somatic growth corresponds to both the general synaptic development in the LGN (Cragg, 1975; Mason, 1982b; Winfield, Headon and Powell, 1976), and to the completion of the critical period for cortical ocular dominance (Hubel and Wiesel, 1970; LeVay et al., 1978), but does not seem to reflect such events as eyelid opening, functional gradients in the retinal maturation, or the emergence of mature response properties (Hickey, 1978).

Retinal innervation of the LGN occurs prenatally at about embryonic day 37 (Shatz, 1983); however, much of the adult-like organization of retinal arbors does not emerge until the second month postnatal (Friedlander, Martin and Vahle-Hinz, 1983; 1985; Mason, 1982a; 1982b). The earliest reported descriptions of postnatal retinal arbors come from eight-day-old kittens which are noted for their numerous growth cones located at their fiber endings (Mason, 1982a). These growth cones, although still present at 14-16 days postnatal, are much reduced in number and fiber endings begin to take on a crenelated shape

similar to that of synaptic boutons (Mason, 1982a). Maturational changes in the retinal axons during the $3^{rd}$-$5^{th}$ week are distinguished by slight increases in length of crenelated branches and a further reduction in the number of growth cone containing appendages. The second postnatal month is marked by a number of changes in the ultrastructure of these retinal terminals (Mason, 1982b). Specifically, these changes include the development of glomeruli, the formation of glial processes around retinogeniculate synapses, the loss of axosomatic synapses which are common in younger animals, and the proliferation of axodendritic contacts located at or near dendritic branch points. The postnatal maturation in retinal arbors appears to occur just before, or in tandem with changes in the dendrites of LGN neurons (see below for details), thus suggesting the possibility that these changes result from a complex series of interactions among pre- and post- synaptic processes (Mason, 1982a).

Using Golgi impregnation, Mason investigated the sequence of dendritic maturation for LGN neurons (Mason, 1983). Her findings indicate that LGN neurons undergo a sustained development between their birth at approximately 4-5 weeks prenatal and the onset of dendritic extension occurring at 2 weeks postnatal. At birth and during the first postnatal week of life thin dendritic processes project from the somata; however, this immature state is rapidly modified by growth occurring during the $2^{nd}$-$4^{th}$ postnatal weeks when these cells undergo a period of elaborate dendritic extension. During the second postnatal month, the changes in LGN cell dendrites appear to be minor

modifications in shape. Although these changes may be minor in scale they are extremely important for synapse formation with retinal (Mason, 1982b) and extraretinal (Weber and Kalil, 1987) terminals. For example, dendritic spines proliferate between 3-6 weeks postnatal at a time of intense synapse formation. Furthermore, these dendritic spines decrease in number between 6-8 weeks postnatal, thereby leaving the familiar grape-like appendages at dendritic branch points characteristic of most class 2 and some class 1 adult geniculate neurons (for review of cell class profiles, see Guillery, 1966; Friedlander, 1981).

It appears, then, that the majority of dendritic growth is complete by the $4^{th}$ week after birth and that changes occurring during the second postnatal month are merely elaborations in form rather than length. This pattern, for the most part, corresponds to that observed in the retinal arbors which innervate the LGN. In contrast, somatic development has a prolonged pattern of development which extends up to the $140^{th}$ postnatal day (Kalil, 1978). Taken together, these data suggest that the mechanisms guiding dendritic growth operate at about the same time as those guiding the development of retinal arbors within the LGN (Mason, 1982a; 1982b; Mason, 1983), whereas the factors controlling somatic growth in the LGN appear to have a more delayed development (Kalil, 1978). In fact, data from animals reared with monocualar deprivation (reviewed below) suggest that somatic growth is likely to depend on the ability of LGN cells to establish a large number of synaptic contacts in visual cortex (Wiesel and Hubel, 1963). This possibility of a different mechanism controlling somatic and

dendritic development seems likely, however, little or no experimental evidence has been generated to support this idea. The present experiment addresses this question using experimental manipulations known to effect the anatomy and physiology of LGN cells.

One final consideration regarding normal development in the LGN is the timing of the development of the different functional classes of geniculate neurons and retinogeniculate arbors. Preliminary observations by Friedlander (1982) indicate that the physiological and morphological development of some LGN Y cells precedes that of LGN X cells, but Y cells, unlike X cells, are extremely sensitive to the effects of early monocular lid suture. This latter implies a relatively protracted maturation for LGN Y cells (see the discussion below for details). Friedlander (1982) proposes two possibilities to account for this paradox. First, it is possible that some class 1 (Y cells) neurons may be so immature as not to generate action potentials and, therefore, would be missed by the micropipettes. Alternatively, some of the X-like LGN projection cells with immature morphology may develop into Y-cells later.

By contrast, the morphological profiles of physiologically classified X and Y retinal afferents indicate that they mature in an opposite fashion from that predicted from Friedlander's sample of geniculate neurons (Sur, Humphrey and Sherman, 1982; Sur, Weller, and Sherman, 1982). In other words, the arbors of X retinal ganglion cells develop before the arbors of Y retinal ganglion cells. In fact, it appears that X arbors undergo a period of exuberant growth followed by

retraction, while the arbors of Y axons undergo a protracted pattern of growth which appears to correspond to the same period of X cell retraction (Garraghty et al., 1986; Sherman, 1985; Sur et al., 1984).

Finally, a growing body of data (see below) in animals reared with MD indicates that competitive interactions among these retinal X and Y cells are responsible for the changes which occur in these immature retinogeniculate projections during early postnatal life (Friedlander et al., 1982; Garraghty et al., 1986; Garraghty et al., 1987; Sur et al., 1982; Sur et al., 1984). Moreover, these changes in the competitive interactions among X and Y retinal axons induced by MD may have important consequences for the dendritic and/or somatic development of LGN cells.

***MD Induced Modifications of LGN Cell Morphology.*** Much of the research dealing with mechanisms which control somatic development in the thalamus have focused on the effects of MD rearing on cells in the LGN (for review see Sherman and Spear, 1982). MD causes a reduction in the cell body size for layers of the LGN innervated by the deprived eye (Wiesel, and Hubel, 1963; Guillery and Stelzner, 1970; Hickey, Spear and Kratz, 1977). This effect is thought to arise from competitive interactions occurring among LGN cell axon terminals for trophic substances obtained at geniculocortical synapses (see Guillery, 1988; Sherman and Spear, 1982). Under normal rearing conditions geniculate projections from each eye initially innervate striate visual cortex with an overlapping distribution (Shatz, Lindstrom and Wiesel, 1977). During the

first two postnatal months, however, the geniculate projections representing each eye segregate their terminals into eye-specific columns within the retinal recipient zone of striate visual cortex (Levay et al., 1978; van Sluyters, et al., 1990). Animals reared with MD, on the other hand, demonstrate a reduction in the amount of cortical innervation obtained from geniculate projections represented by the deprived eye (Shatz and Stryker, 1978) thus suggesting that this abnormal pattern of ocular dominance results from competitive interactions among geniculate terminals for cortical space (for review see Sherman and Spear, 1982).

Evidence from several sources supports the contention that the soma size in MD animals depends, in part, on the outcome of competitive interactions between LGN axons in cortex. First, animals reared with **binocular deprivation**, a manipulation which **does not** bias the competitive interactions in favor of either eye, results in a comparatively modest reduction (5-10%) in soma size for LGN cells (Guillery, 1973a; Hickey et al., 1977). Second, MD has no effect on the soma size of cells in the monocular segment whereas MD produces a significant reduction in soma size of cells in the binocular segment (Guillery and Stelzner, 1970). This is a significant observation because geniculate afferents in the monocular segment of the A layer do not compete with projections from the other eye for synaptic space in the visual cortex. Therefore, the lack of an MD effect on soma size in the monocular segment and the robust effects of MD in the binocular segment suggests that reductions in cell body size are likely to result

from binocular, competitive interactions among LGN afferents in the visual cortex. Finally, experimental rearing paradigms which eliminate competitive interactions among geniculate terminals in visual cortex (Garraghty et al., 1984; Guillery, 1972; Sherman et al., 1974) demonstrate that only a modest reduction in soma size occurs when deprivation takes place without binocularly mediated, competitive interactions among LGN axon terminals.

In addition to these changes in LGN soma size for animals reared with MD, there are also morphological modifications in the dendritic structure of LGN Y cells (Friedlander et al., 1982). Abnormalities in dendrite morphology have important implications for the efficacy of retinogeniculate transmission. In fact, these dendritic pathologies in LGN Y cells are thought, in part, to explain physiological observations which suggest a reduced encounter rate for Y cells in the deprived layers of the LGN (Sherman, Hoffmann and Stone, 1972; Eysel, Grusser and Hoffman, 1979; Hoffman and Hollander, 1978; Mower, Burchfiel and Duffy, 1981; Friedlander and Stanford, 1984). Moreover, these pathologies in LGN Y cell dendrites are thought to underlie many of the visual deficits observed in MD cats (Wiesel and Hubel, 1965; Dews and Wiesel, 1970; Movshon, 1976; Giffin and Mitchell, 1978). Whereas experimental tests of the mechanisms mediating soma development have been well worked out (see above), a corresponding series of experiments has not been performed for the dendrite development of LGN cells. This lack of information stands as an

important gap in our understanding of the competitive mechanisms which contribute to LGN cell organization in MD animals.

Two competitive developmental mechanisms have been postulated as possible causes of the dendritic pathologies (e.g., sinuous dendrites with tortuous dendritic paths) found in the deprived layers of animals reared with MD (Garraghty et al., 1986; Friedlander et al., 1982; Sherman, 1985). The first mechanism is competitive interactions among geniculocortical projections originating from the two eyes (i.e, binocular competition). Arbors of LGN Y cells in normally reared animals innervate several different ocular dominance columns, making them much more susceptible to binocularly mediated, competitive interactions than LGN X cells. The latter cells have arbors that typically innervate only one ocular dominance column (Bullier and Henry, 1979; Ferster and Levay, 1978; Gilbert and Wiesel, 1979). Hence, the first mechanism suggests that the observed effects on Y cell dendrites from the deprived layers in MD cats result from an inability of the Y cells to establish a sufficient number of cortical synapses.

Alternatively, it is possible that the abnormal dendrite development (see Friedlander et al, 1982) for deprived LGN Y cells results from problems in retinogeniculate innervation (Garraghty et al., 1986; Friedlander et al., 1982; Sherman and Spear, 1982; Sherman, 1985; Sur et al., 1982). As described earlier, the pattern of development for retinal X arbors undergoes an initial period of exuberance followed by a period of retraction which corresponds to the

time in which retinal Y arbors are expanding (Sur et al., 1984). This pattern of development suggests that the X arbors have an initial advantage over Y arbors during early development. This initial advantage for retinal X arbors can, however, be maintained by rearing animals with monocular lid suture during the first two postnatal months so that X arbors retain their exuberent growth (Sur et al., 1982). Moreover, in these conditions the arbors of retinal Y cells appear normal in the ventral (C) layers where X arbors are not found, but are abnormal in dorsal (A & A1) layers where retinal X arbors are present (Sur et al., 1982). These findings suggest that X and Y retinal arbors compete for synaptic space on LGN cells in the A and A1 layers but not in the C layer and that retinal Y cell terminals are at a competitive disadvantage in the A and A1 layers of MD animals. Thus, if retinal axon terminals guide LGN dendrite development, then it seems likely that these modifications in retinal X and Y arbors, which innervate the LGN cells, could account for the abnormalities in Y cell dendrites observed in the deprived A and A1 laminae of the LGN in the MD cat.

***Squint Induced Modifications of LGN Cell Morphology.*** Like MD, artificially induced strabismus redudes somatic growth of LGN cells innervated by the strabismic eye (Ikeda, Plant and Tremain, 1977; Tremain and Ikeda, 1982; by contrast see Crewther, Crewther and Cleland, 1985). One issue to be considered in squint reared animals is to determine if the effects of squint on soma development are found in layers innervated by the normal eye as well as those innervated by the misaligned eye. Three lines of evidence suggest this

possibility. First, data taken from electrophysiological studies on the spatial properties of cortical cells innervated from the normal eye (Chino, Shansky, Jankowski and Banser, 1983) and behavioral studies on the contrast sensitivity (Holopigian and Blake, 1983) and spatial tuning (Holopigian and Blake, 1984) of the normal eye suggest that the amblyopic deficits in cats are not simply restricted to representations of the misaligned eye. Second, LGN cells in cats reared with monocular paralysis were smaller than normal throughout the binocular segments of the A and A1 laminae in both hemispheres whether the innervating eye was paralyzed or mobile (Garraghty, Salinger and MacAvoy, 1985). Finally, the third line of evidence which suggest that squint induced modifications may produce a binocular effect in the LGN stems from data on the terminal arbors of retinal Y cells which innervate the LGN. Morphological estimates of terminal volume for retinogeniculate Y axons in the dorsal layers of the LGN suggest that axons originating from both the squinted and normal eye are equally affected by squint rearing (Garraghty, 1989). These three lines of results indicate that the effects of squint within the LGN are likely to be found in laminae innervated by both the normal and squinted eye.

To date, assessments of the morphological consequences of squint rearing have focused on somatic development. An important goal of the present study is to consider the effects of squint on dendritic development in the LGN. Unlike MD, the effects of squint rearing on LGN physiology appear to be focused on the X cell pathway (Jones, Kalil, and Spear, 1984; Tremain and Ikeda, 1982; Ikeda

and Tremain, 1979; Ikeda, Tremain, and Einon, 1978). It has been suggested that the loss of spatial acuity observed in LGN X cells innervated by the squinted eye results from accommodation errors. LGN (Ikeda and Tremain, 1979) and retinal (Ikeda et al., 1978) X cells are thought to be more susceptible to defocused images produced by squint rearing because of their relatively small receptive field and linear response properties (Blake, 1979; Lennie, 1980; Sherman and Spear, 1982). Like animals reared with squint, data from one animal reared with unilateral long-term application of atropine (beginning at 3 weeks of age) suggest that chronic defocusing yields smaller LGN somata and acuity deficits for LGN cells innervated by the defocused eye (Tremain and Ikeda, 1982). Researchers from other labs (Cleland, Crewther, Crewther and Mitchell, 1982; Crewther et al., 1985; Jones et al., 1984) have disputed some of Ikeda's results, claiming that the aggressive surgical procedures used to elicit strabismus may have contributed to some of the visual deficits noted in Ikeda's data.

Apart from providing the first description of the anatomical effects of squint on LGN cell dendrites, data from squinted animals combined with results from MD animals allowed for further elucidation of the relationship between somatic and dendritic development in the LGN. For instance, if squint or MD affect somatic development differently than dendrite development then this would suggest that the developmental mechanisms mediating dendritic and somatic growth are different.

Finally, it is important to consider the developmental mechanisms responsible for producing squint induced modifications in the morphology of LGN neurons. Unlike MD, squint establishes a situation in which inputs from the two eyes engage in competition for neural representation in which **neither** eye enjoys a competitive advantage. This mechanism of competition without advantage was first described by Hubel and Wiesel (Hubel and Wiesel, 1965) to account for the effects of strabismus in the visual cortex. In normal animals, activity originating from the two eyes is highly correlated. This correlated activity is thought to be important for the development of binocular cells in the visual cortex (Hubel and Wiesel, 1965). In squinted animals, however, the visual activity coming from the two eyes is not correlated because the ocular misalignment causes a loss in cortical binocularity. According to this mechanism of competition without advantage, poorly correlated patterns of activity between LGN projections originating from each eye cause an exaggerated retraction in the arbors of geniculocortical afferents. Therefore, previous observations in squint reared animals which demonstrate a reduced soma size in the LGN may be caused by this exaggerated retraction of the geniculocortical arbors. In other words, small arbors may only need small somata (Hubel and Wiesel, 1965; Tremain and Ikeda, 1982).

Alternatively, it is possible that squint rearing produces its morphological consequences in the LGN by virtue of a more peripheral mechanism. For example, recent experimental reports suggest that the arbors of both retinal X

and Y axons in the LGN are affected by squint rearing (Garraghty, et al., 1989; Roe, Garraghty, Chino, and Sur 1990). If retinal innervation is an important factor in controlling the somatic and dendritic development of LGN cells, it is possible that disruptions in the innervation pattern of the retinal inputs may result in abnormalities in the morphological organization of cells in the LGN. Hence, determining the relevant locus of the competitive interactions that give rise to squint-induced modifications in LGN cells has important developmental as well as clinical implications.

Electrotonic Consequences of Experience-Related Changes in LGN Cells

An understanding of the integrative performance of neurons requires an adequate description of the passive electrical cable properties of a cell's dendritic field (Bloomfield, Hamos and Sherman, 1987). These properties are important because they, in conjunction with synaptic location, play an important role in determining the postsynaptic response to incoming excitatory and inhibitory input (for review, see Jack, Noble and Tsien, 1975; Rall, 1959, 1970, 1977). Rall's (1959) equivalent cylinder model is widely used in many areas of nervous system for predicting the passive spread of electrical current from synaptic events (i.e., post-synaptic potentials) within a neuron's dendritic arbor.

This model is based on several assumptions regarding the morphological and physiological attributes of neurons (for review see Rall, 1959; Rall, 1977). Previous studies on the cable properties of geniculate cells from normally reared animals have been executed by Bloomfield, Hamos and Sherman (1987). Their

data demonstrate that LGN cells satisfy many of the morphological and physiological assumptions of Rall's model and, therefore, provide a biophysical substrate for the symmetrical and efficient spread of electrical current throughout the dendritic network. Because the focus of this study is directed at determining the role of early visual experience in controlling the competitive interactions which regulate LGN cell development, part of the ensuing analysis addresses the possibility that abnormal experience might yield LGN cell morphology which would challenge certain of Rall's morphological assumptions.

Focus of the Present Study

The present study is designed to fill gaps in our current understanding regarding the competitive mechanisms underlying LGN cell development. One of the important gaps in the MD literature is determining the role of binocular competition in the development of LGN dendrites. In order to fill this gap, a subset of the animals were reared with monocular eyelid suture combined with sagittal transection of the optic chiasm (MD-OX). MD-OX eliminates retinally mediated competitive interactions among LGN cell axons in the visual cortex, thereby affording the opportunity to assess the effects of MD on LGN cell dendrites in the absence of this type of competitive interaction (Garraghty, et al., 1984). Moreover, because the present study involves an analysis of both somata and dendrites, it was possible to explore the development of these two morphological components of LGN cells across the different visual rearing conditions in MD and MD-OX reared animals.

The second phase of the study assessed the contribution of binocular competition in the development of LGN somata and dendrites in animals reared with squint. The present study, therefore, provided the first systematic attempt to determine the contribution of binocular competition to the development of LGN cells in animals reared with squint. The present study also addressed the possibility that squint produces abnormalities in the dendritic development of LGN neurons, an issue which also had not been addressed in the literature. Finally, this phase of the study afforded the opportunity for determining if abnormalities in somatic development of squint-reared animals are observed in layers innervated by both the squinted and normal eyes as is suggested by the cell body size profiles of animals reared with monocular paralysis (Garraghty et al., 1985) and by the termination patterns of retinal arbors in the dorsal laminae of the LGN of squinted animals (Roe, et al., 1990; Garraghty, et al., 1989).

Comparisons between the MD and squint phases of this study offered useful insights into the general utility of binocular competition as a way of explaining LGN cell development. The data provided by the experiments performed here have important implications with regard to the role of competitive mechanisms in the self-organization of sensory thalamic nuclei, and therefore it offers useful insights regarding the role of early visual experience in inducing modifications in the neural substrate which give rise to changes in the sensory capacities of young animals.

# CHAPTER II

# METHOD

## Subjects and Experimental Conditions.

Subjects used in this study were cats born and reared within the laboratory. Each animal was born in a whelping cage and socially reared in a 2.37 M x 2.42 M enclosure. All animals were cared for according to the guidelines established for the health and welfare of experimental animals.

Fifteen kittens were randomly assigned to one of five experimental or control conditions. A distribution of the cats used in each condition are listed in Table A-1 of the appendix and are described below. Cats were chosen for this study because the previous research (see introduction) on the role of competitive mechanisms in the LGN cell development has relied on this species. Hence, the extensive literature in this area supplies not only the intellectual justification for the study described below but also serves as a baseline against which to validate the results obtained here.

In order to study the role of cortically mediated binocular competition in the development of LGN cells, two experimental groups were used. The first group, illustrated in figure 2A, included animals (n=2) reared with monocular eyelid suture (i.e. monocularly deprived, MD). This condition results in

unbalanced competition for cortical space between cells in the LGN layers innervated from the deprived and non-deprived eyes. The second experimental condition (n=3), depicted in figure 2B, involved cats reared with MD and concomitant transection of their optic chiasm (MD-OX). This procedure results in the denervation of the two A layers, thereby leaving one LGN A1 layer innervated by the deprived eye and the other, contralateral A1 layer innervated by the non-deprived eye. Unlike conditions in which MD alone is performed, cells from the deprived layers of MD-OX reared animals are not forced to compete for cortical contact with cells innervated from the non-deprived eye. Transection of the optic chiasm releases deprived layer cells from the highly unfavorable competitive interactions established by MD. Hence MD-OX allows for deprived layer cells to compete unopposed by cells with retinal innervation originating from the non-deprived eye.

The second set of experiments, illustrated in figures 2C and 2D, was designed to address the same issues raised in the deprivation paradigm with animals reared with artificially induced strabismus. Hence these experiments also involved two groups, one with animals reared with squint alone (SQ, n=4) and the second group reared with squint plus concomitant section of their optic chiasm (SQ-OX, n=4).

**Figure 2.** Figure 2A shows a shematic of the cat visual system in animals reared with monocular deprivation. Under these conditions cells from the deprived layer (see dark arrows) compete with cells from the non-deprived layer to establish and maintain synaptic contacts in the visual cortex. As indicated by the thin dark arrow, the geniculocortical projection for the deprived eye is smaller than that for the non-deprived eye which is believed to have detrimental consequences for the further development of the cells in the deprived layer of the LGN. As depicted in figure 2B, transection of the optic chiasm creates a developmental scenario in which deprived layer cells do not compete with non-deprived layer cells for synaptic contacts in the visual cortex. Figures 2C and 2D offer a similar account of the differences in the binocularly mediated, competitive interactions in animals reared with artificially induced strabismus.

Figure 2.

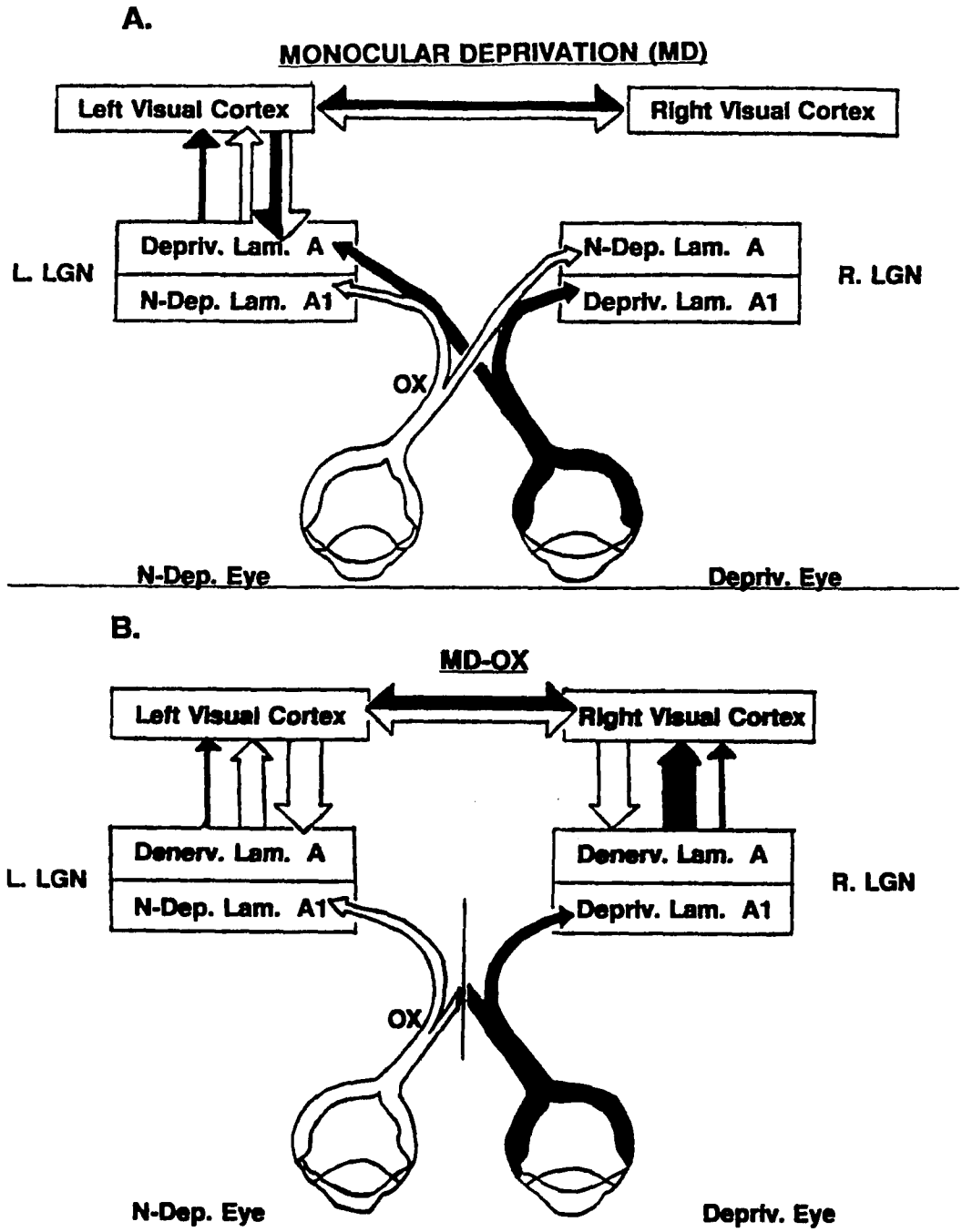
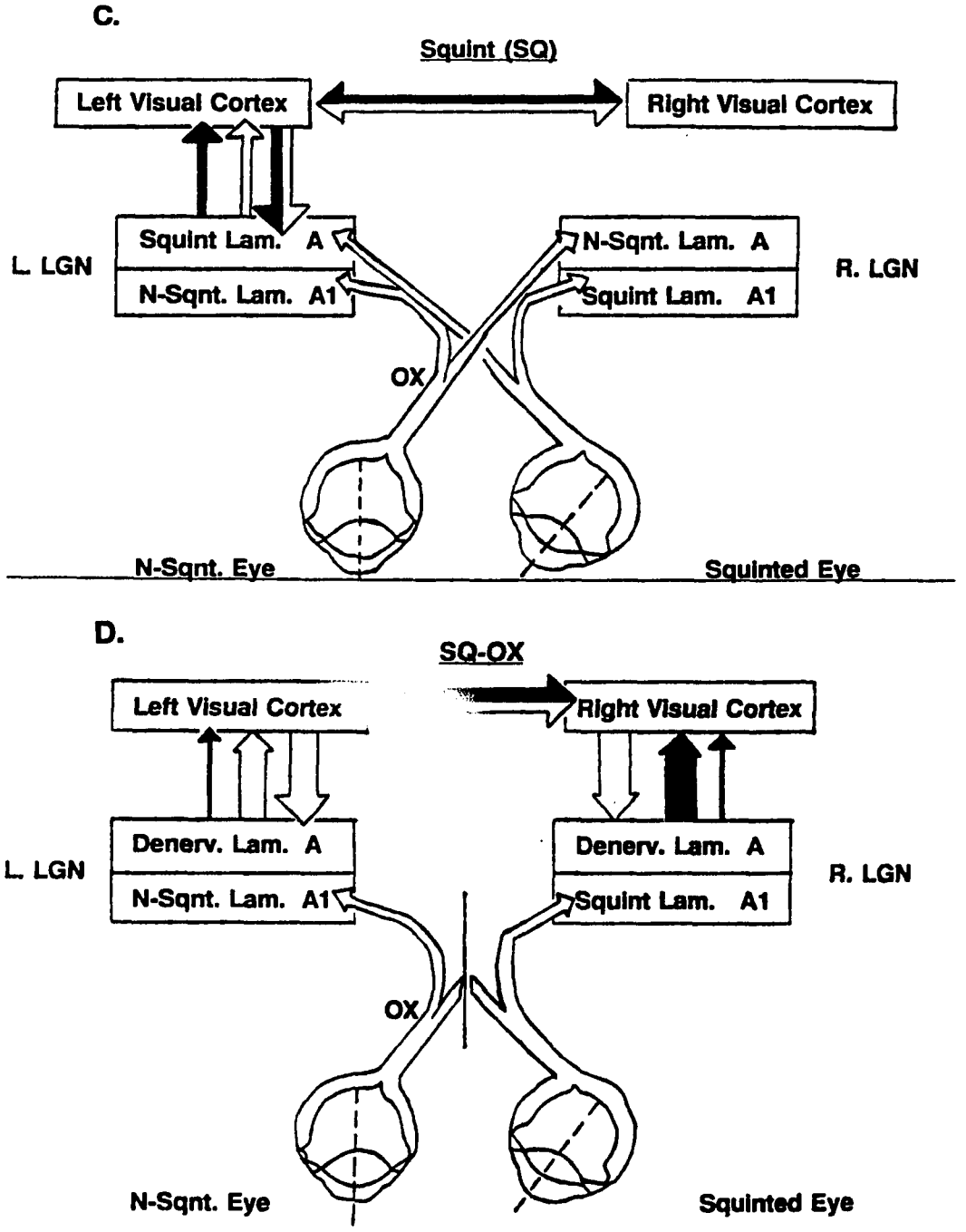

A.
MONOCULAR DEPRIVATION (MD)
Left Visual Cortex
Right Visual Cortex
Depriv. Lam. A
N-Dep. Lam. A1
N-Dep. Lam. A
Depriv. Lam. A1
L. LGN
R. LGN
OX
N-Dep. Eye
Depriv. Eye
B.
MD-OX
Left Visual Cortex
Right Visual Cortex
Denerv. Lam. A
N-Dep. Lam. A1
Denerv. Lam. A
Depriv. Lam. A1
L. LGN
R. LGN
OX
N-Dep. Eye
Depriv. Eye

**Figure 2.**


C.
Squint (SQ)
Left Visual Cortex
Right Visual Cortex
L. LGN
Squint Lam. A
N-Sqnt. Lam. A1
N-Sqnt. Lam. A
Squint Lam. A1
R. LGN
OX
N-Sqnt. Eye
Squinted Eye
D.
SQ-OX
Left Visual Cortex
Right Visual Cortex
L. LGN
Denerv. Lam. A
N-Sqnt. Lam. A1
Denerv. Lam. A
Squint Lam. A1
R. LGN
OX
N-Sqnt. Eye
Squinted Eye

Finally, the last condition served as a control for the effects of optic chiasm transection. Animals (n=2) reared in this condition underwent transection of their optic chiasm without either deprivation or squint.

Surgical Protocol

Two sets of surgical procedures were performed on these animals: the first surgeries, designed to alter the competitive interactions occurring during early development, were performed at either 27 or 28 days of age; and the second surgeries, used to prepare the animals for intracellular injections, were performed as adults. At the time of the initial surgical procedure, each kitten was removed from its mother during the morning of the surgery and replaced back with the litter approximately 8 to 10 hours later. During the period of absence from the care of its mother, each kitten was under constant care by trained graduate students who were in consultation with a licensed veterinarian. Daily checks were made on each kitten following the surgery to ensure that the appropriate temperature and weight gains (approximately 10 gr/day) were maintained and, if needed, their diet was supplemented by an orogastric administration of commercially manufactured kitten milk (KMR brand milk supplement; manufactured by Pet-Ag Inc.) until weaning. Penicillin-G and Tylosin (.5cc) were subcutaneously administered every 12 hours on the day of the surgery and for 7 days after the surgery to prevent post-operative infection.

***Safeguards Against Pain and Discomfort.*** For all surgical procedures, animals were deeply anesthetized with a combination of intraperitoneal

injections of a mixture of acepromazine maleate (1.45 mg/kg for kittens) and intramuscular injections of ketamine hydrochloride (10-15 mg/kg for kittens). This mixture combined with intermittent supplementation with halothane is sufficient to produce stage III, plane 2 anesthesia, as determined by the absence of nociceptive reflexes, the absence of a corneal blink and tendon reflexes, and abdominal instigation of the inspiratory phase of respiration (Cohen, 1975). During anesthesia and recovery, the animal's temperature and respiration were monitored and maintained within normal physiological limits. Furthermore, the long-acting effects of the acepromazine maleate protect the animal against post-operative discomfort by inducing pain tolerance.

***Surgical Procedures Performed on Kittens.***

*i. **Monocular Deprivation.*** Monocular deprivation of either the right or left eyes was performed by surgically removing the margins of the upper and lower eye lids. The wound edges were joined with sutures. A small pinhole in the nasal region was left unsutured to allow for drainage. Through this hole a single dose of ophthalmic antibiotic (containing bacitracin zinc, neomycin sulfate, and polymyxin B sulfate) was administered to protect the wound edges and eye from postoperative infection.

*ii. **Surgically Induced Esotropia.*** Artificially induced esotropia (i.e., a unilateral inward deviating squint) was accomplished on either the right or left eye by tenotomization of the lateral rectus muscle. The region was exposed to the surgeon by making small incisions at the external canthus of the eye. The

tendon attached to the lateral rectus muscle was lifted from the eyeball using a surgical hook and then excised at its point of insertion to the eyeball using ophthalmic scissors. The wound edges were sutured and the wound flooded with an ophthalmic antibiotic. Following recovery, it was clear that this procedure induced noticeable esotropias.

***iii. Transection of the Optic Chiasm.*** Using an intraoral approach through the soft palate and nasopharynx, the surgeon exposed the bone covering the ventral side of the optic chiasm. Using a fine dental burr, the ventral surface optic chiasm was exposed. This procedure allowed for the middle of the optic chiasm to be severed under visual control with the aid of surgical microscope. The wound was then packed with gelfoam and the soft palate was sutured closed.

***Surgical Procedures on Adult Animals.*** After reaching adulthood (> 8 months), the animals were prepared for a bilateral intracerebral injection of rhodamine beads. Retrograde transport of these beads to LGN cells allowed us to identify LGN relay cells. In preparation for this procedure, the animals were anesthetized using intramuscular injections of acepromazine maleate (2.9 mg/kg) and ketamine hydrochloride (25 mg/kg). They were then mounted into a stereotaxic apparatus to stabilize the head for surgery. The scalp was transected along the midline and retracted. A bilateral craniotomy was performed to expose the dorsal region of the occipital lobes beginning at tentorium and extending anterior for at least 2 cm. According to published visual topographic maps (Tusa, Palmer

and Rosenquist, 1979; Tusa, Rosenquist and Palmer, 1979) the region of the visual cortex exposed by this procedures includes the central representations of visual area 17 and 18 and portions of area 19.

A 10 $\mu$l syringe filled with the rhodamine microspheres was then inserted into the crown of the lateral gyrus where approximately 2 $\mu$l were injected. All injections were made perpendicularly to the cortical surface (approximately 2-6 mm under the dural surface) and each injection was performed at approximately 1-2 mm intervals, thus 10-15 injections were made in each cortical hemisphere. Rhodamine is a fluorescent dye which is taken up by axon terminals of LGN relay cells and then retrogradely fills the relay cell bodies. The labeling of LGN relay cell somata takes approximately two days before it is completed. Following these intracortical injections, the scalp was sutured together and the animal was allowed to recover from the anesthesia. The animals were then administered subcutaneous injections of penicillin and tylosin (1 cc) every 12 hours to protect against postoperative infection.

Two days after the intracranial injections the animals were given a lethal injection of sodium pentobarbital and then perfused intracardially using a saline rinse ($\approx$ 1 liter) followed by a paraformaldehyde solution (2% paraformaldehyde, .2% glutaraldehyde, in a .1 M phosphate buffer). The brain was removed from the skull and then the two LGNs were blocked and sectioned ($\approx$ 300 $\mu$m thick) using a vibratome (Series 1000). Sections containing the LGN were bathed in .1

M phosphate buffered saline solution and refrigerated until later used for the intracellular injections (described below).

For those animals whose optic chiasm had been sectioned as kittens, a block of the brain containing the optic chiasm was removed separately to ensure that its removal did not disrupt the status of the optic chiasm. Post mortem inspections of the optic chiasma were made and in each case revealed that a complete and successful transection of the crossed retinal fibers was obtained. In addition, independent confirmation of the completeness of the chiasma transections was demonstrated by microscopic analysis of the whole mounted retinae which had been stained with cresyl violet. These retinae revealed a clear line of decussation between nasal (i.e., cells whose fibers cross at the optic chiasm) and temporal (i.e., cells whose fibers project to the ipsilateral thalamus) retina. That is, ganglion cell perikarya in the nasal retina either were shriveled or non-existent, whereas ganglion cell perikarya in the temporal retina were large and appeared to be unaffected by early chiasm transection.

Slice Preparation

***Labeling.*** The intracellular labeling procedure for lightly fixed tissue has been described in detail elsewhere (Katz,1986, Ramoa, Campbell, and Shatz, 1987, Dann, Buhl, and Peichl, 1988). Briefly, two blocks containing the right or left LGN were trimmed, placed in a vibratome and sectioned into 300 $\mu$m thick sections. Sections were then stored in cold phosphate buffered saline and transferred to Petri dish for viewing with a Nikon epi-fluorescent microscope.

Fine-tipped, glass micropipettes filled with 10-20% lucifer yellow dissolved in .2 M phosphate buffer were attached to a deFonbrun micromanipulator and advanced into cells which contained the rhodamine microspheres. The rhodamine-containing relay cells were identified using epi-fluorescence at the appropriate wavelengths for both rhodamine and lucifer. The penetration of the lucifer-filled electrode into the cell can be performed with the aid of a 40x epi-fluorescent long working distance (ELWD) objective. Following penetration, the lucifer was iontophoresed through the tip of the electrode (2.5-10 nA at 3 Hz, 200 msec depolarizing current) into the cell for approximately 1 minute or until it became apparent that the lucifer was leaking from the cell. This technique yielded successful injections in as many as 15-20 cells in some slices.

***Processing.*** The tissue was then processed by recutting the slices into 100 $\mu$m thick sections using a cryostat and then placed on gel coated slides and coverslipped. At this thickness cells containing the double label (rhodamine and lucifer) could be easily visualized for light microscopy. Low power (4x) drawings were obtained for each section which show the position of each labeled cell in the slice.

Cells labeled with lucifer yellow are typically contained within the first 100 $\mu$m section entirely, thus serial reconstructions are not needed to obtain a cell's complete morphology. Slides of some of these neurons were made using a camera attachment and 20X (DPlanApo UV) lens. Camera lucida drawings of each well labeled neuron were made at a combined magnification of 1500X.

Each drawing was obtained by using an Olympus epi-fluorescent microscope in conjunction with a 100x oil immersion lens (Olympus, DPlanApo UV). The drawings for each cell were made using **blind coded** slides. Hence, the microscopist was unaware of the type of preparation (i.e. MD, squint etc..) from which the drawings were made in order to alleviate the potential for experimenter bias. A liberal judgment was applied in the determination of which cells from the sample could or should be drawn.

Extensive notes were made for each drawing for two principle reasons: 1) in order to characterize any damage of the dendritic tree; 2) in order to aid in the interpretation of the circuitous path of some dendrites. After all the cells had been drawn, cells in which more than two of the primary segments had been lost were rejected from the sample. Reasons for the loss of portions of a cell's dendrites include: (1) poor or inadequate filling of the cell's dendrites, (2) problems in the plane of section were discovered when attempting to fill the neuron (i.e., portions of a cell's dendrites were discovered to be missing during the initial *in vitro* preparation), and finally (3) damage produced to the cell's morphology due to tissue processing occurring after the cells had been injected with the lucifer.

Following completion of the camera lucida drawings, the coverslips were removed and the tissue was stained with cressyl violet (see appendix A-2 for details) in order to identify the layer in which the cells were located. This procedure washes the lucifer from the cells. Thus to elucidate the position of the

previously lucifer labeled cells 4x drawings, in conjunction with the drawing tube attachment, were used to determine the cell's laminar position. Tissue landmarks (e.g. blood vessels, tissue tears, and edges of section) in the sections that had been previously included in the 4x drawings were aligned with their position in the tissue as viewed through the objective overlaid with the image obtained from the drawing tube. In certain cases particularly when the cell was close to a tissue landmark, it was possible to actually identify the cell labeled with cresyl violet which had been previously drawn using the lucifer label.

Decoding the tissue with respect to the experimental history of the cell was not made until the laminar position of cells was determined using the cresyl violet procedures described above. Hence, at this point in the process cells had been drawn, measured, and their data entered into the computer. Classification of cell types (see qualitative analysis described below) was made with the experimenter aware of the visual rearing history of the LGN neuron.

Data Analysis

***Qualitative Anatomical Analysis.*** According to Guillery (1966), the dorsal layers of the LGN are composed of three morphologically distinct classes of LGN cells. He referred to them as class 1, 2, and 3 cells. Class 1 cells were noted for their large somata, radially symmetric dendritic tree, and the presence of tiny dendritic spines. Class 2 cells possessed medium sized somata, dendrites oriented perpendicular with respect to laminar borders, and grape-like clustered appendages found at or near dendritic branch points. Finally, class 3 neurons

were characterized by their small somata, thin, sinuous dendrites which run perpendicular to laminar borders and stalked dendritic appendages located between dendritic branch points.

Using physiological recordings combined with intracellular injections of horseradish peroxidase (HRP), Friedlander et al. (1981) were able to determine the functional classification (i.e., X or Y) of Guillery's (1966) three morphological classes of LGN neurons. Their results indicated that LGN Y cells typically displayed morphological features characteristic of class 1 neurons, but on occasion exhibited certain characteristics common to class 2 neurons (i.e., dendrites oriented perpendicular to laminar borders, occasional presence of grape-like clustered appendages). LGN X cells, on the other hand, typically maintained morphological features characteristic of either class 2 or class 3 cells. Despite the fact that some class 1 cells maintain dendritic features typical of class 2 cells, Friedlander et al. (1981) noted that class 2 Y cells were distinguishable from class 2 X cells based on their large somata.

In addition, both physiological and anatomical data support the notion that LGN Y cells are more sensitive than LGN X cells to perturbations of early visual experience. For this reason, it was imperative that cells in this study should be characterized according to a modified version of Guillery's classification scheme. In order to clearly discriminate LGN X and Y cells, I have modified Guillery's terminology so that our class 1 cells include Guillery's standard class 1 as well as his class 2 cells with large somata. Similarly, class 2

cells were defined as having medium-sized somata, an oriented dendritic tree and grape-like clustered appendages located at or near dendritic branch points. None of the cells in the sample met all of the Guillery criteria for class 3 cells, however, two cells (which were characterized as class 2 cells) did exhibit some features of class 3 morphology. These modifications of Guillery's classification scheme should meet with the experimental objective of identifying putative LGN X and Y cells based on morphological characteristics and is consistent with Friedlander et al.'s (1981) observations.

Friedlander et al. (1982) have demonstrated that approximately 80% of their sample of Y cells from the deprived laminae exhibit smaller somata than those of Y cells innervated by the non-deprived eye. Therefore, it was clear that for cells innervated by the deprived eye soma size should not be used as a criteria for classifying LGN cells according to their class 1/ class 2 morphological categories. However, Friedlander et al. (1981) report that only 5.5% of their sample of physiologically classified Y cells from normally reared cats displayed dendrites oriented perpendicular to laminar borders. Therefore, the degree of dendritic orientation was used as the primary method of classifying cells in deprived layers. In addition, cells characterized as class 1 cells from the deprived layers also revealed other pathological features (which have also been previously noted by Friedlander et al., 1982) such as a tangled and beaded dendrites which aided in their classification. Taken together, these findings suggest that the

ability to make cell classifications without soma size considerations was not significantly compromised by using dendritic orientation as our criteria.

***Quantitative Anatomical Analysis.*** The 1500X drawing of each well labeled neuron was subjected to a Sholl (1955) ring analysis. The Sholl ring procedure provides a method for quantitatively describing a cell's dendritic field relative to its distance from the soma. This method utilized a transparent acetate template centered over the cell body. The template includes six concentric rings plus vertically and horizontally oriented hatchings. Each ring increased in diameter by 50 $\mu$, thus affording the opportunity for a quantitative description of any morphological parameter with 50 $\mu$ resolution relative from the distance to the cell body. The vertical and horizontal hatchings were sectioned into quadrants which could be used to quantify the orientation of the cell's dendrites relative to the layer borders (See Appendix A-4 for picture of the Sholl ring procedure and thorough description of the calculation of the orientation index).

Quantitative measures of each cell's morphology were obtained using measures of soma size, number of primary dendrites, and number of branch points per cell. Morphometric software from Jandell (Java version 1.3) permitted extraction of the number and length for each dendritic segment. In addition an averaged width of each dendritic segment was obtained by measuring the diameter of each segment every 5 to 8 microns. All estimates of segment length and width were collected in a manner which maintained information regarding the segment's Sholl ring. From calculations of dendritic

length and width it was possible to derive estimates of the cell's surface area ***(Formula: $A=(2\pi r^2)+(2\pi rl)$)***. Collection of all parameters yielded a large data base whose dimensions were 2560 (number of measurements) x 76 (number of cells).

The quantitative measures listed above were selected in accordance with published descriptions (Guillery, 1966; Friedlander et al., 1981) of morphological cell types found in the LGN of normally reared adult cats. Moreover, the morphological parameters provided by this analysis were chosen with respect to previous *in vivo* reports on the anatomy of cells found in the deprived layers of MD animals and were complete enough to identify even the most subtle effects produced by any of the experimental rearing conditions. A detailed description of each quantitative measure is provided in Appendix A-3 and A-4.

***Statistical Analysis.*** Cells were characterized according to the type of developmental history to which they were exposed. Hence, the experiments described above resulted in cells that could be categorized into one of the following visual histories: 1) deprived layer cells in animals reared with an intact optic chiasm (i.e., cells found in LGN layers which received retinal input from the deprived eye); 2) squint layer cells in animals reared with an intact optic chiasm (i.e., cells found in LGN layers which received retinal input from the esotropic eye); 3) non-deprived layer cells from animals reared with an intact optic chiasm (i.e., cells in this condition were obtained from LGN layers innervated by the open eye of MD cats or from layers innervated by the

unsquinted eyes of animals reared with artificially induced strabismus); 4) deprived layer cells from animals reared with optic chiasm transection; 5) squint layer cells from animals reared with optic chiasm transection; 6) non-deprived layer cells from animals reared with optic chiasm transection; and finally, 7) cells from the denervated A layers of animals reared with optic chiasm transection. In addition to experimental rearing history, each cell was classified according to a modified version of Guillery's classification scheme (see description above).

The dependent measures subjected to statistical inquiry included: (1) soma size; (2) cumulative dendritic length; (3) average length per dendrite; (4) number of dendrites; (5) number of dendritic segments (6) number of dendritic branch points; (7) average width per dendritic segment; (8) cumulative dendritic surface area; (9) the ratio of dendritic length in the outer (i.e., rings 3-8) relative to the inner (rings 1 and 2) Sholl rings; (10) the ratio of dendritic surface area in the outer relative to the inner Sholl rings; and finally (11) Rall's power rule for dendritic branching. Analysis of the distributions for each of the dependent measures revealed skewed distributions, therefore, $\log_{10}$ transforms were performed on the distributions in order obtain normality. In each case, this transform removed the skew and yielded distributions in which parametric statistical procedures could be used.

Using the means for each LGN layer as the unit of measure, a series of analysis of variance (ANOVA) procedures was performed. The model for these statistical procedures was designed to assess the main effects of the various

visual rearing history, cell type, and the interaction between visual rearing history and cell type. Because of the imbalance in the number of layers contributing to the mean estimates (a result of the experimental design), type IV sums of squares was used to determine the main effects of these three factors (see Freund, Littell, and Spector, 1986). If the main effects of the statistical model yielded statistically significant ($\alpha$=.05) results, the F statistics associated with the type IV sums of squares for each of the independent variables was assessed. For those independent variables which yielded statistically significant type IV sums of squares, a series of planned comparisons (using partial F statistics) were performed to evaluate predictions of the experimental hypotheses.

As described above, laminae rather than individual neurons serve as the unit of measure for the analysis. This ensures statistical independence for the experimental units, thereby meeting one of the requirements of the ANOVA. In addition, $\log_{10}$ transforms of each dependent measure were used in order to produce normal distributions. Finally, the heterogeneity of variance for each level of the independent variables was tested using Hartley's maximum F test and in each case was found not to violate the assumptions of the ANOVA procedure (Byrkit, 1980).

# CHAPTER III

## RESULTS

Fifteen cats were included in this experiment. Data from one of these animals were lost due to a failure in the retrograde transport of rhodamine from the visual cortex to LGN cell somata. This same problem also occurred in one hemisphere of two other animals (cat numbers 1170 and 1191). Post mortem analysis of injection sites revealed no systematic differences in the loci and/or depth of the injections, nor was there any evidence of differences in regional trauma at the injection site between cortices in which the rhodamine transported successfully and cortices in which it did not.

A total of 110 LGN neurons was microscopically examined. Thirty four of these cells were deleted from the sample for one of three principle reasons: (1) fifteen cells were found to be in the C layers and therefore not included in the analysis; (2) the laminar position of 6 cells was lost to damaged tissue produced when the coverslips were lifted for Nissl staining; and (3) 13 cells were excluded from the sample because more than two of the primary segments had been cut or could not be clearly resolved due to poor lucifer labeling. Of the cells from this last group of excluded neurons, 8 cells were obtained from non-deprived laminae in animals reared with chiasm transection; 2 from non-deprived laminae in

animals with an intact chiasm; 1 from a squinted lamina; and 2 from squinted laminae in animals reared with chiasm transection.

Each of the 76 remaining neurons was identified as a LGN **relay** cell based on the observation of both rhodamine and lucifer label within the cell's soma. Moreover, each cell was labelled according one of seven different visual rearing histories. These histories were based on the type of retinal innervation to the LGN lamina. For example, cells found in the layers innervated by the deprived eye were designated as deprived cells. Two **groups** of cells designated as non-deprived were used in the present study (i.e., one group from animals with intact optic chiasms and the other group from animals reared with optic chiasm transection), and these non-deprived groups were compiled from one of several different sources. For animals reared with an **intact optic chiasm**, non-deprived cells were sampled from layers innervated by either the non-deprived eyes in monocularly deprived (MD) animals or from the non-squinted eyes of animals reared with artificially induced strabismus. For animals reared with **optic chiasm transection**, non-deprived cells were obtained from layers innervated by the non-deprived eye of animals reared with either MD, squint, or optic chiasm section alone. In animals reared with intact optic chiasms, statistical comparisons (t-tests) revealed no differences between the non-deprived layers of MD reared animals and the non-squinted layers in animals reared with artificially induced strabismus. In chiasm sectioned animals statistical comparisons (t-test) revealed no significant differences between the non-deprived layers of MD-OX animals,

non-squinted layers of animals reared with artificially induced strabismus, and the untreated (i.e., non-deprived) layers of animals reared with chiasm section alone. Hence, cells from these two groups of non-deprived layers were combined for subsequent statistical analysis.

Table 1 presents a precis of certain features of the seventy-six cells used for statistical analysis. As noted from the table, the majority of the sample was obtained from the non-deprived layers. The principle reason for this preponderance in the number of cells from non-deprived layers is the relative difference in the number of animals and/or laminae which contribute to this sample because of the experimental design. The mean number of cells per layer did not differ significantly between the various experimental rearing histories. The imbalance in experimental design which is attributable to differences in the numbers of layers contributing to the ANOVA was not a significant problem because type IV sums of squares were used for the test (Freund, Littel and Spector,1986). In addition, this sample of cells offers a good mixture of class 1 (n=30, 39.5%) and class 2 (n=46, 60.5%) neurons. With the exception of the denervated layer cells, there is also a relatively balanced proportion of class 1 and class 2 cells found among each of the experimental conditions.

Another feature of table 1 is information regarding the region of the LGN posterior (A/P) axis of the nucleus, data were accumulated from 42 coronal sections that ranged from A 7.5 to A 3.5 with a median of A 5.5 (Horsely Clarke coordinates). Variation in the A/P axis reflects changes in the elevation

Table 1

Summary Table of LGN Cell Characteristics as a Function of Visual Rearing History for the Present Sample.

| Visual Rearing History | Number of Cats | Number of Cells | Mean Cells per Lamina | Median A/P Location | M/L Location Med:ParCent:Lat | Class1:Class2 Ratio |
|---|---|---|---|---|---|---|
| 1. Non-Deprived OX[f] | 8 | 21 | 2.63 (±1.3) | 5.5 (3.5-6.0)[fff] | 9:6:0[ff] | 8:13 |
| 2. Deprived OX | 2 | 8 | 4.00 (±4.2) | 5.5 (5.5-6.0) | 6:2:0 | 4:4 |
| 3. Squint OX | 2 | 8 | 4.00 (±4.2) | 4.5 (4.0-7.0) | 2:4:2 | 4:4 |
| 4. Denervated OX | 4 | 6 | 1.50 (±0.6) | 6.0 (5.5-6.5) | 4:1:1 | 1:5 |
| 5. Non-Deprived | 6 | 18 | 2.57 (±2.4) | 5.5 (3.5-6.0) | 2:11:3 | 7:11 |
| 6. Deprived | 2 | 7 | 2.33 (±2.1) | 5.5 (5.0-6.0) | 3:3:0 | 3:4 |
| 7. Squint | 3 | 8 | 2.70 (±2.7) | 4.5 (3.5-5.0) | 6:1:1 | 3:5 |

[f] *OX refers to preparations in which the optic chiasm has been transected.*

[ff] *The number of LGN neurons located in the medial paracentral and peripheral locations of the LGN.*

[fff] *These numbers reflect the* ***range*** *of Anterior/Posterior locations from which the sample was obtained.*

of visual representations. Hence, posterior regions of the LGN represent higher elevations and the further anterior one travels in the nucleus the lower the visual fields will run (Sanderson, 1971). The majority (64%) of the sample was obtained from the middle third of the nucleus (A 6.3- A 4.6), whereas 22.7% and 13.3% of the sample were taken from the posterior and anterior thirds of the nucleus, respectively. Moreover, table 1 shows that the distribution of A/P coordinates for all experimental histories were overlapping and therefore it is unlikely that effects of the visual rearing histories could be attributed to the location of the LGN from which the cells were found.

Table 1 also provides information regarding cellular position in the mediolateral (M/L) axis. In the M/L axis, medial portions of the LGN represent central visual space and the further lateral one travels the more eccentric the visual representations become (Sanderson, 1971). For this study we segmented the LGN into 3 regions: (1) the medial portion (approximately 0° to 4°); (2) the paracentral portion (approximately 4°- 18°); and (3) the lateral portion (approximately 18° to 50°) of the visual field (Sanderson, 1971). In our sample, 42% (n=32) of the cells were obtained from the medial portion of the LGN, 37% (n=28) of the cells were obtained from the paracentral region, and 9% (n=7) of the cells were found in the lateral third of the nucleus. The M/L position for 12% (n=9) of the cells could not be determined due to damage sustained by the medial and lateral borders of the nucleus during tissue processing. With the exception of cells in the lateral portion of the LGN, table 1 demonstrates that

there is considerable overlap with respect to the M/L position of neurons within each of the visual rearing histories.

A series of analyses of variance (ANOVAs) were performed to determine if differences in any of the dependent measures (described below) could be accounted for based solely on a cell's position within the nucleus. These tests were performed on both laminar position in the A/P and M/L axes. Results of this test revealed no statistically significant differences for any of the dependent variables. These null results indicate that differences among cells could not be attributed solely to a cell's position within the body of the nucleus. Unfortunately, however, the range of locations in the A/P or M/L axes was not broad enough in this sample to support a test of the impact of visual rearing histories as a function of a cell's position within the nucleus.

In addition to the A/P and M/L locations of LGN neurons, another factor that might account for the variation in the dependent measures could be the layer of the LGN from which the cells were obtained. For example, it is possible that cells in the A layer yield different morphological characteristics than cells in the A1 layer. To address this possibility, Student t-tests were performed within three different rearing histories (i.e. deprived, non-deprived and squint layers) for animals reared with **intact** optic chiasms. These tests were performed using $\log_{10}$ transforms of the data in order to achieve normality in the sample. Differences between A and A1 layers in animals reared with optic chiasm transection could not be used in this analysis because all A layers in these animals were

denervated, hence, any difference between A and A1 layers could not be separated from differences in visual rearing history. Table 2, 3, and 4 show the results of these comparisons for the deprived, non-deprived, and squinted layers. As one can see from these tables, there were no apparent differences between the A and A1 laminae. Hence, it is not likely that the effects for the ANOVAs described below (see section on quantitative analysis) are attributable to differences between LGN layers.

Table 2.

Data From the Deprived Layers of Animals Reared With An Intact Optic Chiasm. Note that None of the A vs. A1 Comparisons Yield Statistically Significant Differences.

| Dependent Variable | Lamina | Num. of Cells | Num. of Lamina | Mean | Standard Deviation | $t_{(df=4)}$ test | Prob. |
|---|---|---|---|---|---|---|---|
| 1. Soma Size | A | 4 | 3 | 266.20 $\mu^2$ | 26.83 | 1.729 | .1589 |
| | A1 | 3 | 3 | 227.12 $\mu^2$ | 27.86 | | |
| 2. Cumulative Dend. | A | 4 | 3 | 524.69 $\mu$ | 262.89 | -0.726 | .5082 |
| Length | A1 | 3 | 3 | 765.05 $\mu$ | 253.51 | | |
| 3. Ave. Length per | A | 4 | 3 | 80.95 $\mu$ | 33.04 | -0.388 | .7180 |
| Dendrite | A1 | 3 | 3 | 96.34 $\mu$ | 43.48 | | |
| 4. Cumulative Dend. | A | 4 | 3 | 1217.53 $\mu^2$ | 453.77 | 0.756 | .4916 |
| Surface Area | A1 | 3 | 3 | 1019.95 $\mu^2$ | 1019.95 | | |
| 5. Number of Dend. | A | 4 | 3 | 27.67 | 15.04 | -1.359 | .2458 |
| Segments | A1 | 3 | 3 | 42.33 | 8.68 | | |
| 6. Dendritic Branch | A | 4 | 3 | 11.67 | 8.08 | -1.407 | .2323 |
| Points per Cell | A1 | 3 | 3 | 19.67 | 7.51 | | |
| 7. Ring Length Index | A | 4 | 3 | 51.43 | 79.01 | -0.847 | .4447 |
| (%In Rg : %Out Rg) | A1 | 3 | 3 | 452.53 | 9.39 | | |
| 8. Ring Sur.Area Id. | A | 4 | 3 | 72.49 | 104.94 | 0.576 | .5955 |
| (%In Rg : %Out Rg) | A1 | 3 | 3 | 22.46 | 25.04 | | |

Table 3.

Data From the Non-Deprived Layers of Animals Reared With an Intact Optic Chiasm. Note that None of the A vs. A1 Comparisons Yield Statistically Significant Differences.

| Dependent Variable | Lamina | Num. of Cells | Num. of Lamina | Mean | Standard Deviation | $t_{(df=6)}$ test | Prob. |
|---|---|---|---|---|---|---|---|
| 1. Soma Size | A | 6 | 2 | 378.08 $\mu^2$ | 78.65 | 0.541 | .6077 |
| | A1 | 11 | 6 | 345.41 $\mu^2$ | 9.88 | | |
| 2. Cumulative Dend. | A | 6 | 2 | 1194.71 $\mu$ | 210.31 | -0.163 | .8757 |
| Length | A1 | 11 | 6 | 1397.60 $\mu$ | 778.00 | | |
| 3. Ave. Length per | A | 6 | 2 | 198.47 $\mu$ | 60.13 | -0.603 | .5685 |
| Dendrite | A1 | 11 | 6 | 170.99 $\mu$ | 73.99 | | |
| 4. Cumulative Dend. | A | 6 | 2 | 2695.50 $\mu^2$ | 244.96 | 0.113 | .9135 |
| Surface Area | A1 | 11 | 6 | 2773.17 $\mu^2$ | 1114.09 | | |
| 5. Number of Dend. | A | 6 | 2 | 53.50 | 2.12 | -0.134 | .8977 |
| Segments | A1 | 11 | 6 | 61.83 | 33.43 | | |
| 6. Dendritic Branch | A | 6 | 2 | 23.00 | 1.41 | -0.380 | .7172 |
| Points per Cell | A1 | 11 | 6 | 38.33 | 15.44 | | |
| 7. Ring Length Index | A | 6 | 2 | 0.91 | 0.21 | -0.742 | .4859 |
| (%In Rg : %Out Rg) | A1 | 11 | 6 | 0.91 | 0.60 | | |
| 8. Ring Sur.Area Id. | A | 6 | 2 | 1.83 | 0.48 | -0.436 | .6779 |
| (%In Rg : %Out Rg) | A1 | 11 | 6 | 2.39 | 1.16 | | |

Table 4.

Data From the Squinted Layers of Animals With an Intact Optic Chiasm. Note that None of the A vs. A1 Comparisons Yield Statistically Significant Differences.

| Dependent Variable | Lamina | Num. of Cells | Num. of Lamina | Mean | Standard Deviation | $t_{(df=4)}$ test | Prob. |
|---|---|---|---|---|---|---|---|
| 1. Soma Size | A | 4 | 3 | 243.46 $\mu^2$ | 70.29 | -0.001 | .9992 |
| | A1 | 4 | 3 | 242.69 $\mu^2$ | 67.24 | | |
| 2. Cumulative Dend. | A | 4 | 3 | 458.55 $\mu$ | 123.96 | -0.528 | .6253 |
| Length | A1 | 4 | 3 | 613.59 $\mu$ | 430.01 | | |
| 3. Ave. Length per | A | 4 | 3 | 81.54 $\mu$ | 60.13 | -0.781 | .4785 |
| Dendrite | A1 | 4 | 3 | 113.20 $\mu$ | 62.54 | | |
| 4. Cumulative Dend. | A | 4 | 3 | 903.87 $\mu^2$ | 362.45 | -0.616 | .5711 |
| Surface Area | A1 | 4 | 3 | 1330.04 $\mu^2$ | 973.84 | | |
| 5. Number of Dend. | A | 4 | 3 | 32.00 | 9.54 | 0.092 | .9307 |
| Segments | A1 | 4 | 3 | 34.67 | 23.03 | | |
| 6. Dendritic Branch | A | 4 | 3 | 12.67 | 4.04 | -0.218 | .8379 |
| Points per Cell | A1 | 4 | 3 | 16.33 | 11.06 | | |
| 7. Ring Length Index | A | 4 | 3 | 3.74 | 3.74 | 1.096 | .3344 |
| (%In Rg : %Out Rg) | A1 | 4 | 3 | 7.70 | 8.43 | | |
| 8. Ring Sur.Area Id. | A | 4 | 3 | 19.19 | 11.14 | 0.680 | .5338 |
| (%In Rg : %Out Rg) | A1 | 4 | 3 | 18.49 | 23.02 | | |

Qualitative Analysis of LGN Cells.

*LGN Class 1 and Class 2 Cells.* As described in both the introduction and methods, the dorsal layers of the LGN are composed of three morphologically distinct classes of neurons which Guillery (1966) termed class 1, class 2, and class 3 cells. Using physiological recordings combined with intracellular injections of horse radish peroxidase (HRP), Friedlander et al. (1981) were able to identify the structure/function relationship of LGN X and Y cells. They demonstrated that Y cells typically exhibit morphologies consistent with class 1 and class 2 cells, whereas X cells typically reflect morphological features of class 2 and class 3 cells. Moreover, these researchers report that class 2, Y cells were rare and distinguishable from their class 2, X cell counterparts. This difference is based on the observation that class 2, Y cells had large cell bodies which were consistent with those of class 1 cells, whereas class 2, X cells had significantly smaller somata.

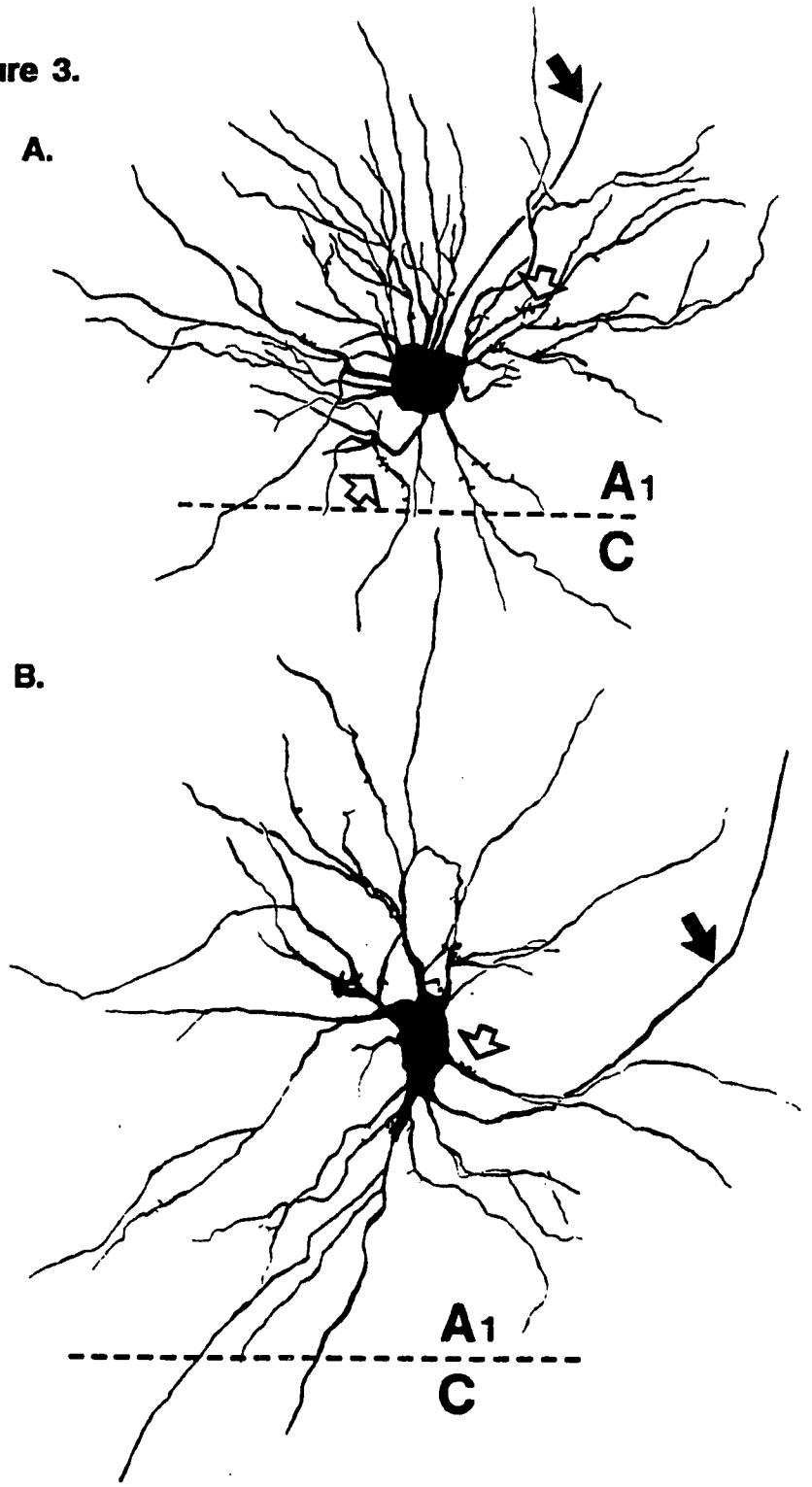
According to Guillery, class 1 cells are characterized by their large somata, radially symmetric dendritic arbors, tendency to cross layer borders, and smooth dendrites accompanied by intermittent dendritic spines. One of the goals of the present study was to use morphological criteria to discriminate LGN X and Y cells because previous reports have indicated that the effects of deprivation are for the most part selective for the Y class (see introduction for details). Since Y cells are morphologically a heterogeneous class (i.e. either class 1 or class 2), Guillery's terminology was modified so that the class 1 cells in this sample included

Guillery's standard class 1 as well as class 2 cells with large somata. This change is consistent with Friedlander et al.'s (1981) observations, and therefore should meet with the objective of the present study for identifying LGN Y cells. Moreover, it should be noted that the present sample of modified class 1 cells (i.e. large cells with oriented dendritic arbors) constitutes only 4% of the entire sample and if their classification were changed there would be no alterations in the pattern of significant results obtained in the quantitative analyses (described below).

Figure 3 shows two examples of class 1 cells. Both cells are confirmed relay cells as determined from the double (i.e. lucifer and rhodamine) label, and exhibit an axon (marked by straight black arrows) which courses dorsally toward the A lamina. Unfortunately, it was rarely possible to follow axons outside of their respective layer because they tended to run out of the plane of section. Thus, it was not possible to use axon terminal morphology as a way of characterizing the sample according to class 1/ class 2 criteria (Guillery, 1966). The cell in figure 3A was obtained from the non-deprived, A1 layer of an animal reared with MD and intact optic chiasm. This cell was located at the base of the A1 layer and displays all of the features of class 1 cells mentioned above. The cell in figure 3B was taken from the non-deprived, A1 layer of an animal reared with MD combined with sagittal transection of the optic chiasm. This A1 layer cell was not located near the A/A1 boundary and maintains an extensive dendritic radius within the A1 layer. This cell exhibits many class 1 features

**Figure 3.** Two cells characterized as exhibiting type 1 morphologies. Figure 3A shows an image of a type 1 cell located near the bottom region of the A1 lamina. Note the radially symmetric pattern of the cell's dendritic arbors combined with its large cell body (490 $\mu^2$), and dendritic spines. This cell possesses all of the features characteristic of Guillery's class 1 cells. Figure 3B demonstrates a class 1 cell obtained from the non-deprived layer of an animal reared with optic chiasm transection. This cell possesses a large soma (565 $\mu^2$), radially symmetrical dendrites and proximally located dendritic spines (open arrow) all common features of class 1 cells. In both cells the solid arrow represent the cell's axon.

**Figure 3.**



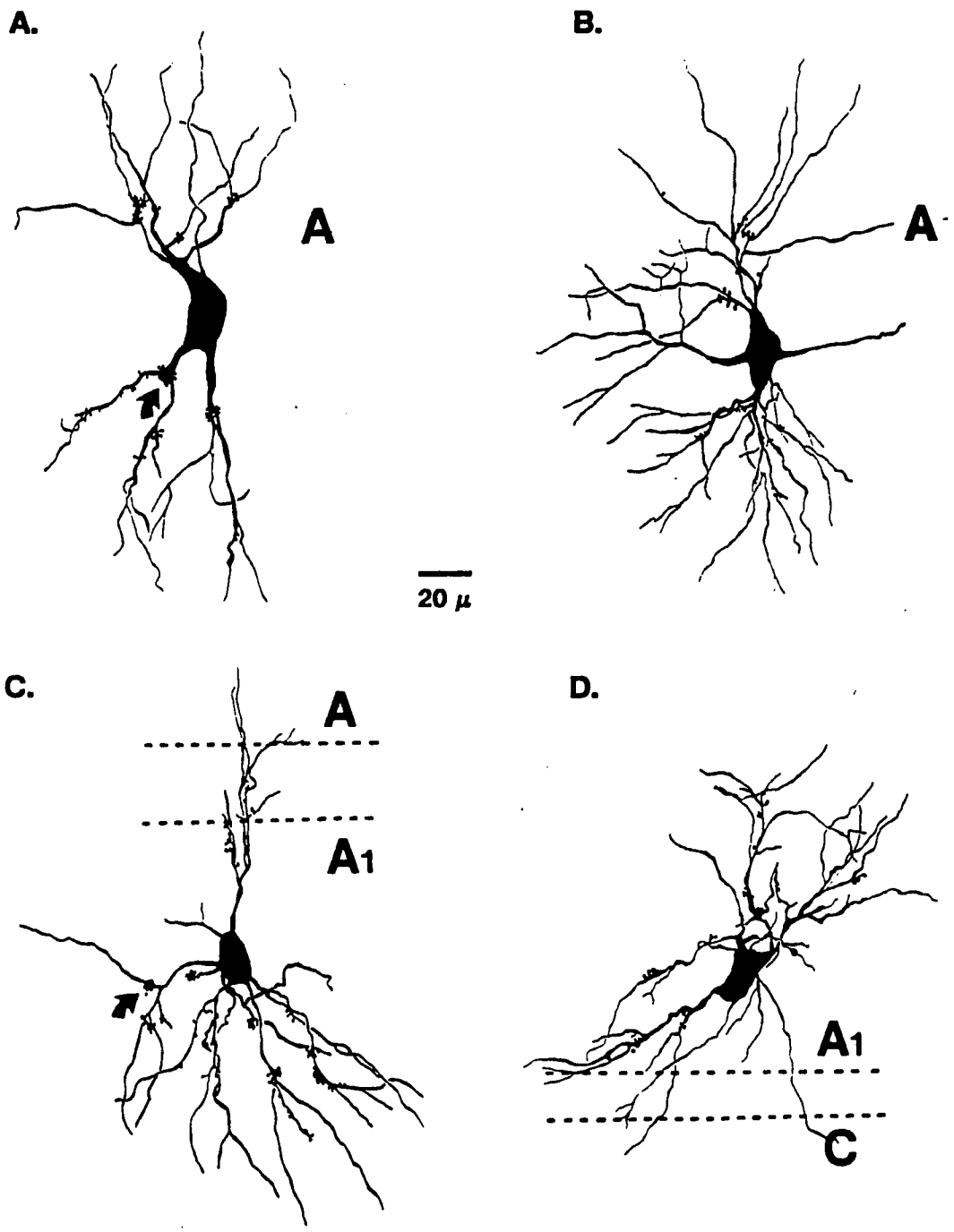
such as: (1) a large somata (565 $\mu^2$); (2) radially symmetric dendritic arbors; and (3) tiny dendritic spines.

One final feature of figure 3 (see open arrows) is the presence of dendritic spines located along the proximal (Fig. 3B) and distal (Fig. 3A) portions of the cells' dendrites. Visualization of these spines demonstrate that our injection procedures were successful in labeling even the most tiny features of the cell's morphology.

Class 2 cells, on the other hand, are noted for their relatively small somata, dendrites oriented perpendicular to the laminar borders, and presence of grape-like clustered dendritic appendages (see Figure 4, solid, curved arrows) usually located at or near dendritic branch points. Figure 4 presents four examples of class 2 cells, two of which (Fig. 4A; 4B) are from lamina A and two (Fig. 4C; 4D) from lamina A1. These cells were also confirmed relay cells as demonstrated by the double labeling procedure used in this study (see methods). Cells in figures 4A and 4B are from non-deprived laminae and are located in the middle of their respective layers. Although these cells were oriented perpendicular to the layer borders, they failed to cross into adjacent layers. By contrast, the cells in figures 4C and 4D which are also from non-deprived laminae are located near the A/A1 (Fig. 4C) and A1/C borders, respectively, and do possess dendrites which cross the interlaminar zone (i.e., region marked between the hatchings), and also maintain a few processes which reach into the adjacent layers. These findings are inconsistent with initial claims of Friedlander

**Figure 4.** Demonstration of 4 cells possessing features characteristic of class 2 morphologies. For example, all 4 cells are vertically oriented with respect to their borders, they all possess soma profiles consistent with class 2 cells (range 228-352 $\mu^2$), and they all exhibit grape-like clusters (see solid curved arrows) at some point along their dendritic tree. Cells in figures A and B are two class 2 cells located in the middle (i.e. with respect to laminar borders) of the A lamina. These cells were taken from the non-deprived layers of animals reared with either MD (Fig. 4A) or squint (Fig. 4B). Figures 4C and 4D are reconstructions of class 4 cells from the non-deprived layers of animals reared with either optic chiasm transection combined with squint (Fig. 4D) or optic chiasm section alone (Fig. 4C). Note that both cells (Fig. 4C and 4D) cross their respective laminar borders, a finding which is consistent with reports from Humphrey and Weller (1988). The dashed lines in figures 4C and 4D indicate the region of the interlaminar zone between the A1 layer and the adjacent layer.

**Figure 4.**



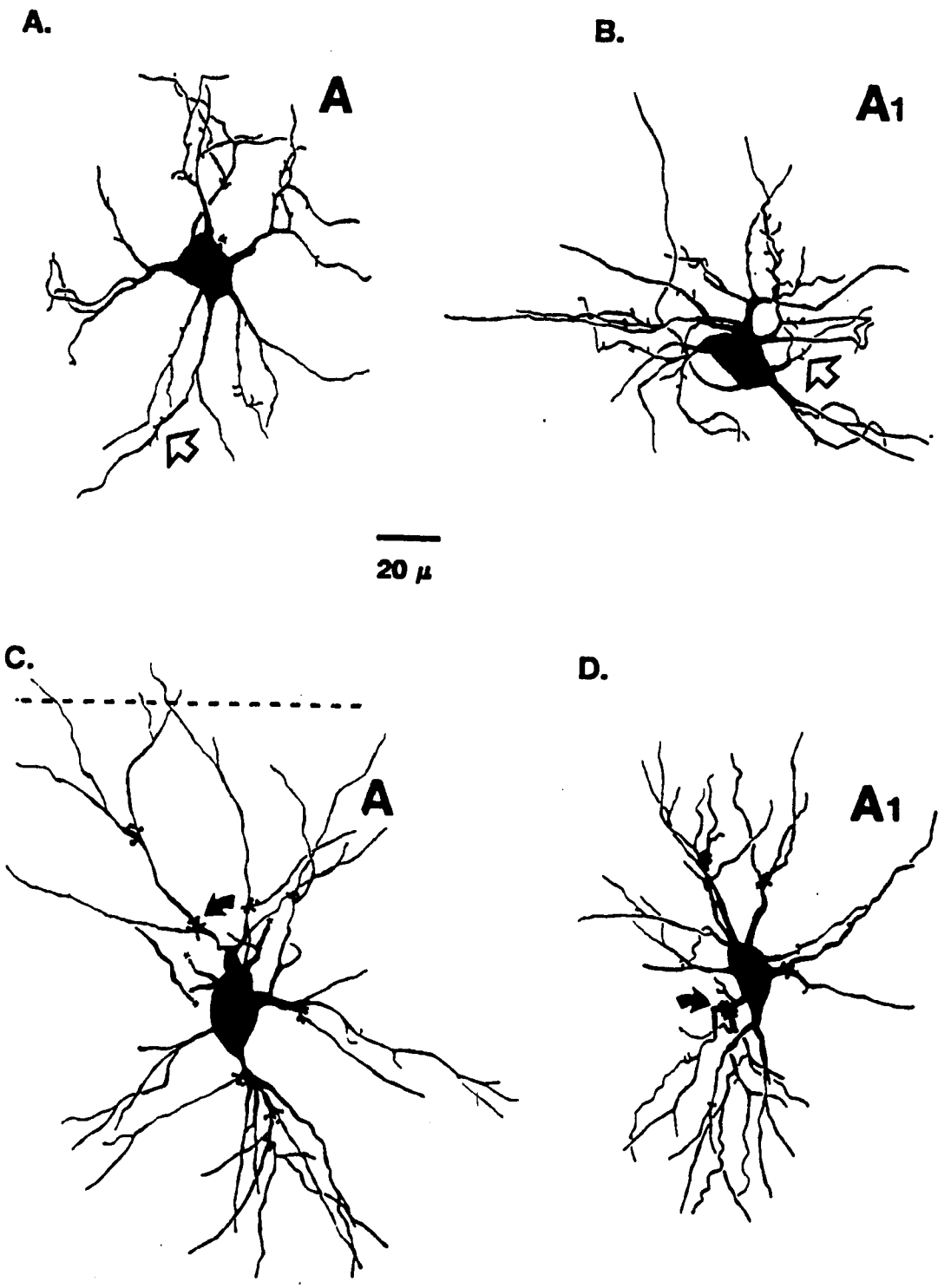
et al. (1981) which suggested that class 2 cells terminate their dendrites within a layer, and agrees with Humphrey and Weller's (1988) claim that LGN neurons (irrespective of class) located near laminar borders tend to have dendrites which are not restricted to their respective cell layer.

***Determination of Cell Typology within Deprived Layers.*** As described in the introduction, monocular deprivation is known to reduce the soma size profiles of cells in the LGN. Moreover, Friedlander et al. (1982) have shown that the somata profiles of LGN Y cells (i.e., those cells which typically possess class 1 morphologies) are more sensitive to the developmental consequences of MD than are LGN X cells, which maintain either class 2 or 3 morphologies. This finding raises a problem since a major criterion for classifying cells according to Guillery's class 1/class 2 typology is based on the cell's soma size. When characterizing cells from the deprived layers, therefore, soma size could not be used as one of the criteria for classification. Both in the present sample of non-deprived class 1 cells and in published reports of physiologically classified Y cells (Friedlander et al., 1981), the occurrence of class 1 cells with oriented dendritic trees is quite low. Thus, cells from deprived layers were judged class 1 if their indices of orientation approached (i.e. $>.5$ & $<1.5$) a value of 1 (see appendix A-4).

Figure 5 presents the reconstructed morphology of four neurons which were obtained from the deprived layers of the LGN. Cells from figures 5A and 5B were classified as class 1 cells. The soma size of these neurons (243 $\mu^2$ and

**Figure 5.** Four LGN neurons from the deprived layer of animals reared with MD and an intact optic chiasm. Neurons depicted in figures 5A and 5B were classified as class 1 cells based on orientation indices and the presence of dendritic spines (see open arrows). The image of cell 5B reflects the tangled nature of dendrites typical of some class 1 cells from the deprived layers of the LGN (Friedlander et al., 1982). Our sample of class 1 neurons, unlike Friedlander et al.'s (1982), have relatively impoverished dendritic structure. Figures 5C and 5D present reconstructions of two class 2 cells. These neurons possess features typical of class 2 such as vertically oriented arbors, grape-like dendritic appendages (curved arrows), and somata within the class 2 range. Note that the scale of this figure is greater than that of figures 1 and 2, and lastly, they obtain soma profiles compatible with their respective cell class.

**Figure 5.**



252 $\mu^2$, respectively) is not characteristic of type 1 cells in normally reared animals. However, both cells exhibit orientation indices (5A: OI=1.07 and 5B: OI= 1.25) consistent with type 1 cells, and they also possess dendrites which maintain simple, spine-like appendages (see clear arrows). The neurons from figures 5C and 5D are examples of class 2 cells obtained from the deprived layers. Note that both cells exhibit morphologies typical of Guillery's criteria for class 2 cells. For example, they are both oriented perpendicular (5C: OI= 2.25 and to laminar borders and they display grape-like clustered appendages located at or near dendritic branch points (see curved arrows).

The cell in figure 5B exhibits a phenomenon which Friedlander et al. (1982) have previously reported for abnormally responsive Y cells from the deprived laminae. Note the sinuous and tangled (i.e., dendrites wrapping their processes around themselves) nature of the distal segments of the dendritic tree. As was the case for Friedlander et al.'s (1982) sample, this tangling does not appear to be a characteristic of the proximal dendritic segments. Although the distal segments of the class 2 cells (Figs. 5C and 5D) do appear complex in their branching patterns, their outer segments appear to be relatively straighter than their counterparts found on deprived class 1 cells.

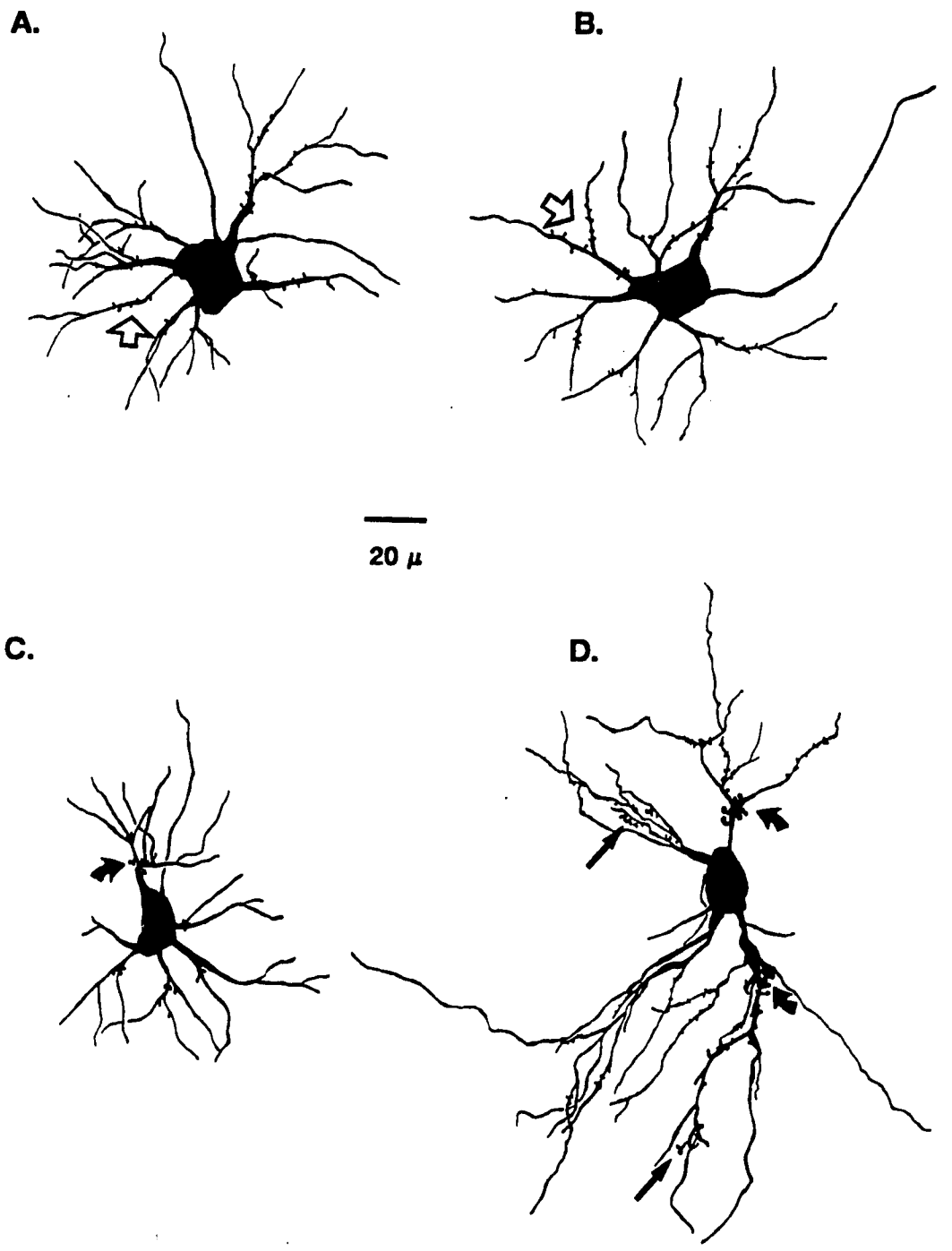
***Determination of Cell Typology within Squinted Layers.*** To date there are no existing reports of the developmental impact of squint on the dendritic morphology of geniculate neurons. Previous work (Tremain and Ikeda, 1982; Garraghty et al., 1985) in cats reared with artificially induced strabismus has

demonstrated that cell shrinkage is present for cells located in the medial regions of the LGN. It was not clear whether soma size would be a useful criterion for distinguishing between class 1 and class 2 cells, orientation indices (see appendix A-4) and types of dendritic appendages were used to determine if the class 1 somata were larger than those of class 2 cells within layers innervated by the squinted eye. As it turns out, however, the soma size distribution of type 1 cells (classified on the basis of their dendritic characteristics) from the squinted layers (300-340 $\mu^2$) was larger than the distribution obtained from class 2 cells (182-227 $\mu^2$). Unlike the soma size profiles from deprived layers, the observations from layers innervated by the squinted eye appear to permit the use of soma size as a method for classifying cells as class 1 or class 2.

Figure 6 presents examples of two class 1 (Figs 6A and 6B) cells and two class 2 cells obtained from layers innervated by the squinted eye. As one can see from figures 6A and 6B, the dendrites of the class 1 cells exhibit radial symmetry in their dendritic arbors and also reveal tiny spine-like appendages (see open arrows in figure 6A & 6B), both of which are typical of class 1 cells. Cells depicted in figures 6C and 6D have characteristics common to class 2 cells. For example, they both have small somata (227 and 209 $\mu^2$), dendritic arbors oriented perpendicular to layer borders and grape-like appendages (see curved arrows) at dendritic branch points. The cell depicted in figure 4D, however, also displays features intermediate between classes 2 and 3. Note the complex, stalked appendages which are distant from the dendritic branch points (see long

**Figure 6.** Figure 6 presents the four LGN relay cells innervated by the squinted eye. Figures 6A and 6B represent cells which maintain a class 1 profile. The cell depicted in figure 6C represents an example of a class 2 cell and figure 6D offers an example of a cell with features intermediate between classes 2 and 3. Note that the cell shown in figure 6D has grape-like clusters located near dendritic branch points, in addition to the complex, stalked appendages located along portions of dendrites distal to dendritic branch points.

**Figure 6.**



solid arrow). Using physiological recordings combined with intracellular injections of HRP, Friedlander et al. (1981) have described LGN relay cells with these morphological features. Moreover, they report that all of their cells of this morphological type exhibited physiological response characteristics typical of LGN X cells.

Quantitative Analysis of LGN Cells.

The quantitative portion of the analysis is divided into three sections. The first two sections deal with the effects of deprivation and squint on somatic and dendritic development. The principle measure for somatic development is that of soma size. There are, however, many features of dendritic morphology considered here ranging from variation in cumulative dendritic length to differences in the geometric configuration of a neuron's dendritic arbors. The last portion of the quantitative analysis explores the possible role of experience-dependent competitive mechanisms on the morphological substrates which determine a cell's electrotonic properties in the LGN.

All statistical procedures were performed on the means for LGN layers. Thus, laminae rather than individual neurons serve as the unit of measure for the analyses described below. This ensures statistical independence for the experimental units, thereby meeting one of the requirements of the ANOVA. In addition, log transforms of each dependent measure were used in order to meet the assumption of normality for the ANOVA. Also, the heterogeneity of variance for each level of the independent variables was tested using Hartley's maximum F

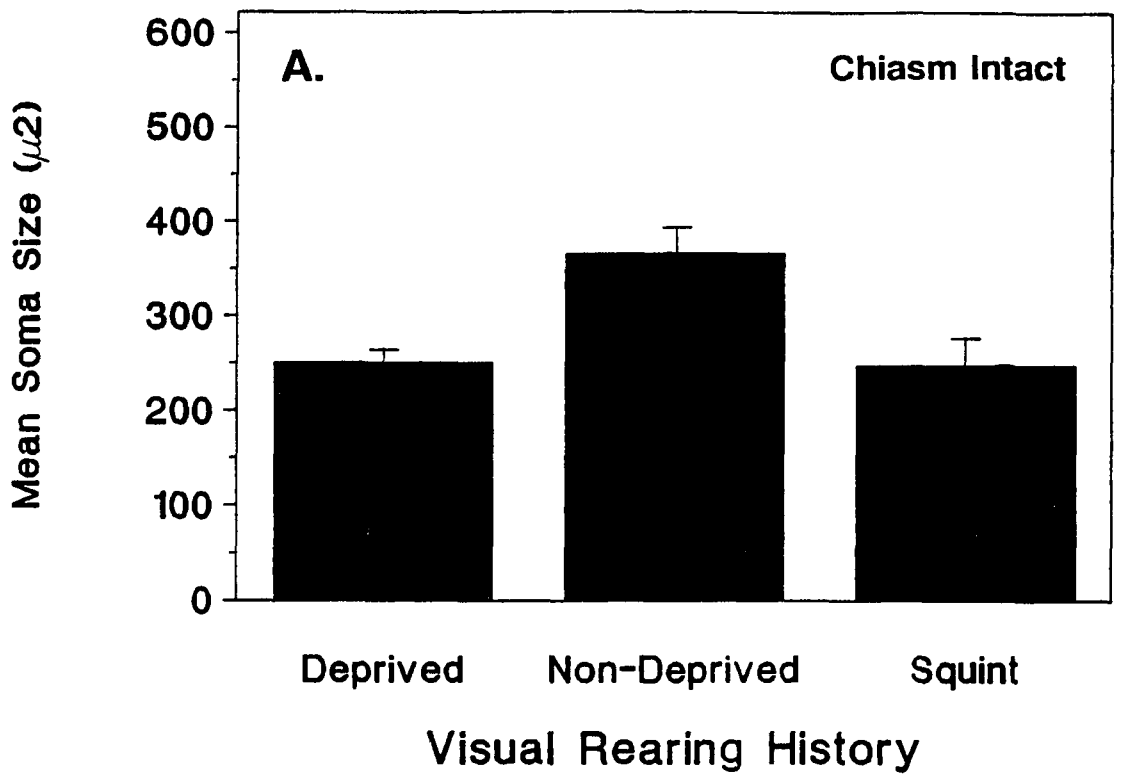
test and in each case was found not to violate the assumptions of the ANOVA procedure (Byrkit, 1980).

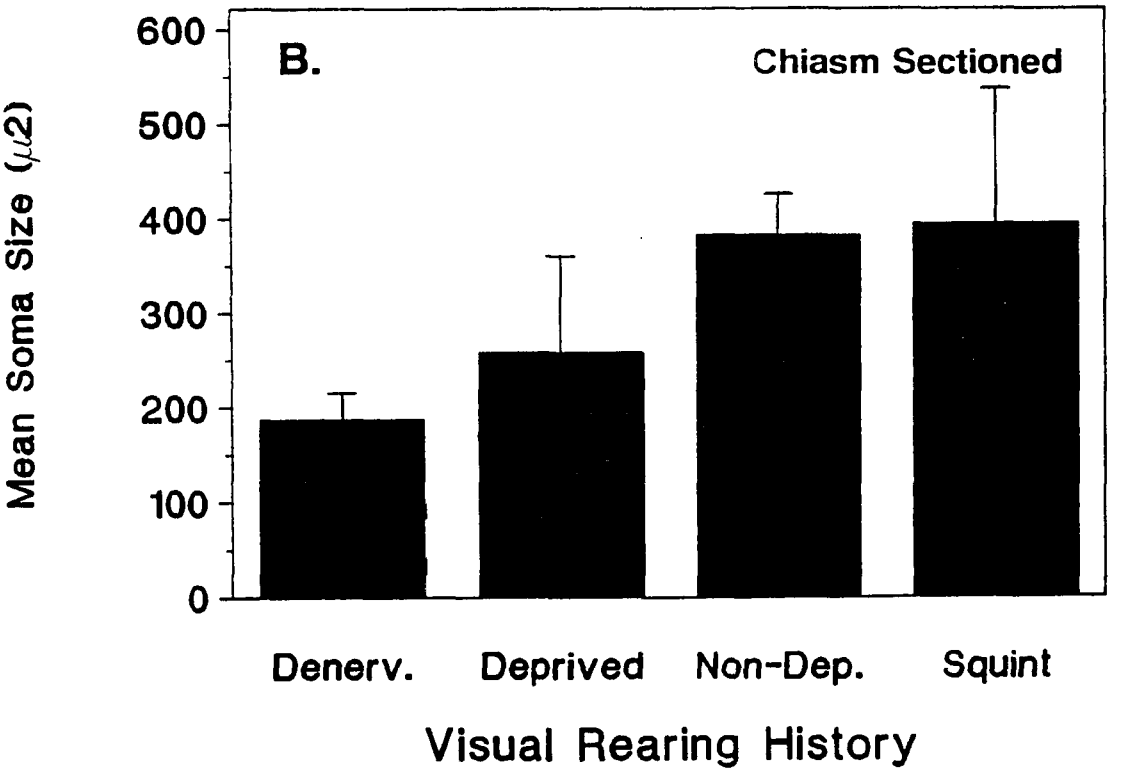
Finally, frequency distributions for each dependent variable that yielded significant main effects are presented in appendix C. These figures are based on individual cells and demonstrate changes in each dependent measure as a function of modifications in early visual experience. In addition, each histogram presents the data with respect to cell class affiliation.

***Somatic Development.*** Figure 7 presents data on the effects of either deprivation or squint on the soma size in animals reared with or without an intact optic chiasm. The experimental groups are labeled according to the type of retinal innervation for a given layer. For example, laminae categorized as non-deprived received their retinal input from either the opened eye of MD animals or from the unsquinted eye in animals reared with artificially induced strabismus. The group means were derived using the averaged soma size per layer as the unit of measure. Standard error bars, therefore, reflect variation of the averaged soma size per layer rather than the variation among individual cells. Averaging the data from individual cells by layer is necessary to provide independent samples for statistical tests.

An analysis of variance (ANOVA) for soma size was performed using visual rearing history (e.g., deprived, squint, non-deprived), and cell type (class 1 or class 2), and their respective interaction as independent variables. This analysis revealed significant main effects of this statistical model

**Figure 7.** Figure 7 presents data on the effects of monocular deprivation (MD), squint and denervation on the soma size in animals reared with (7A) or without (7B) an intact optic chiasm. Soma size estimates were obtained from a quantitative analysis (using morphometric software, JAVA 1.3, from Jandel) of the camera lucida drawings of lucifer labeled LGN neurons. Only cells which revealed a nucleolus (as determined under high powered 1000x microscopy using UV illumination) were permitted in the sample. This condition ensured that the estimates of the cross sectional, somatic surface area were obtained from the middle of the cell. The mean estimates (see ordinate) were based on the average somatic cross sectional area of cells within a layer. Thus, error bars reflect the standard error estimates based on variation in somatic cross-sectional area across laminae rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their visual input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 7A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 7B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.


A.
Chiasm Intact
Mean Soma Size (μ2)
600
500
400
300
200
100
0
Deprived
Non-Deprived
Squint
Visual Rearing History


B.
Chiasm Sectioned
Mean Soma Size (μ2)
600
500
400
300
200
100
0
Denerv.
Deprived
Non-Dep.
Squint
Visual Rearing History

($F_{(df=13,27)}=13.80$, p=.0001). In addition, this statistical procedure allowed for an assessment of the contribution of each independent variable separately (using type IV sums of squares). The result of this analysis demonstrated that visual rearing history (p=.0009), cell type (p=.0001), and the visual rearing history by cell type interaction (p=.005) each contributed to a significant amount of the variation in soma size.

Table 5

Results of Planned Comparisons for the Effects of Deprivation, Squint or Denervation on Soma Size for LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.06467 | 9.17 | 0.0054 *[f1] |
| Deprived OX vs Non-Dep OX[f2] | 0.01593 | 2.26 | 0.1446 |
| Squint vs Non-Deprived | 0.55439 | 14.04 | 0.0009 * |
| Squint OX vs Non-Deprived OX | 0.00493 | 0.70 | 0.4089 |
| Denervated vs Non-Deprived OX | 0.10155 | 14.41 | 0.0008 * |

[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

Five planned comparisons (using partial F statistics) were used to parse out specific differences between the various rearing histories (see table 5 above). In animals reared with **intact optic chiasm**, it appears that deprivation significantly reduces the soma size of LGN neurons relative to non-deprived controls. Comparison between the deprived and non-deprived laminae of **chiasm sectioned** animals, however, did **not** yield statistically significant differences. Thus, transection of the optic chiasm abolished the effects of deprivation on somatic development noted in animals reared with MD alone. As with

deprivation, cells from laminae innervated by the squinted eye yielded significantly smaller somata than cells from the non-deprived layers. Chiasm section also appears to protect cells in the squinted layer from the effects of squint as revealed by the non-significant difference between squinted and non-deprived layers in animals reared with squint and concomitant transection of the optic chiasm. Finally, a small sample of denervated neurons was also obtained from the A layers of chiasm sectioned animals. Cells from the denervated layers exhibited significantly smaller somata than cells from the non-deprived layers of chiasm sectioned animals.

As expected from the existing literature, class 1 cells were, on average, significantly larger than class 2 cells in the LGN. This difference between the two classes, however, was expected given that soma size was an important variable for determining the cell's classification. Since a statistically significant interaction was obtained, a probe of differences among the various groups with respect to both visual rearing history and cell type was required. Planned comparisons using partial F statistics were performed. Because there were six degrees of freedom associated with the type IV sums of squares only six experimental comparisons were supported by the statistical procedures that were used (see Freund, Littel, and Spector, 1986). The most important experimental comparisons involved assessing differences among class 1 and 2 cells between layers innervated by the deprived or squinted eye and layers innervated by the non-deprived eye. If significant differences between these two groups were

revealed then the experimental hypotheses compelled an assessment of the effects of chiasm section in order to evaluate possible contributions of interocular competition on somatic development.

**Table 6**

Results of Planned Comparisons for the Effects of Deprivation Squint or Denervation on the Average Soma Size Profiles of LGN Class 1 and Class 2 Cells from Animals Reared With or Without Optic Chiasm Transection.

| Experimental Comparisons | Cell Type | Mean Square | F Value | Prob. | |
|---|---|---|---|---|---|
| Deprived vs Non-Deprived | Class 1 | 0.07846 | 7.23 | 0.0197 | *[f1] |
| Deprived OX vs Non-Dep OX[f2] | Class 1 | 0.00096 | 0.09 | 0.7704 | |
| Squint vs Non-Deprived | Class 1 | 0.02396 | 2.17 | 0.1662 | |
| Deprived vs Non-Deprived | Class 2 | 0.00632 | 1.58 | 0.2280 | |
| Squint vs Non-Deprived | Class 2 | 0.04829 | 12.06 | 0.0034 | * |
| Squint OX vs Non-Deprived OX | Class 2 | 0.09377 | 13.22 | 0.0024 | * |

[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

Table 6 presents data from the planned comparisons. Note that class 1 cells from the deprived layers yielded significantly smaller somata than class 1 cells in the non-deprived layers. This is an important finding in that it corresponds to Friedlander et al.'s. (1982) results which demonstrate that deprivation selectively alters the morphological development of class 1 cells in the LGN. Moreover, these results support and extend previous work (Garraghty et al., 1984) which indicated that transection of the optic chiasm, a manipulation which alters the competitive imbalance imposed by MD, reduced the difference between soma size profiles for cells found in deprived and non-deprived laminae.

Table 6 also reveals that squint induced a reduction in the cell body size

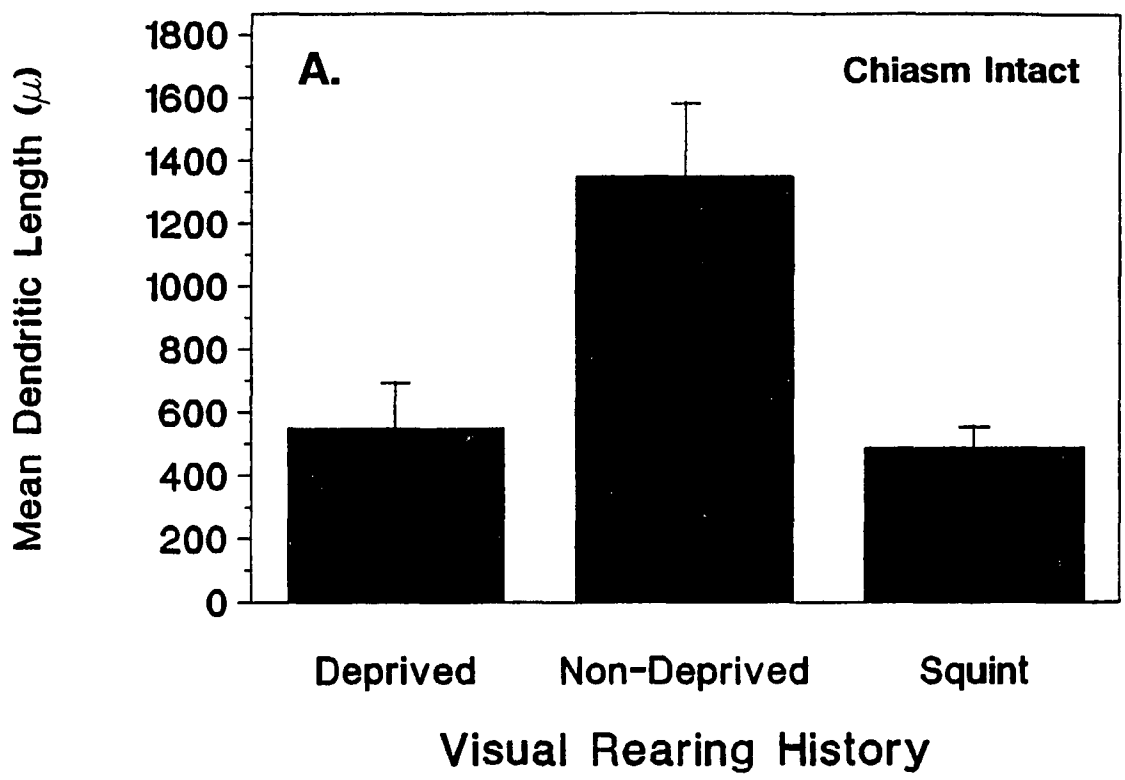
of class 2 cells. This reduction in the soma size of squinted class 2 neurons appears to be **unaffected** by optic chiasm transection. These findings are interesting in that they suggest that squint selectively influences the somatic development of class 2 cells in a manner that is **not** based on competitive interactions among geniculo-cortical axons. The squint induced effects on soma size are different from those obtained from MD animals in two ways: (1) **squint appears to impose its reduction in soma size primarily on class 2 rather than class 1 cells**; and (2) **these effects do not appear to be blocked by optic chiasm transection.** It should be noted, however, these results were not predicted from previous work (see discussion below). Given the small sample size it is possible that such results may have arisen from chance. Clearly, more data will be needed to confirm these results.

One final result from this analysis involves the effects of denervation on the somatic development of LGN neurons. Because the degrees of freedom for the planned comparisons would not allow for further tests without modifying experimentwise error rate, Scheffe's multiple comparison procedure was used to assess the effects of denervation on soma size. The effects of denervation on class 1 cells, however, could not be addressed here because only one cell of this class was obtained from the denervated layers. Results from the statistical analysis of class 2 cells revealed that the somata of cells in the denervated layers were significantly smaller ($p<.05$) than those of cells found in the non-deprived layers of chiasm sectioned animals.

***Dendritic Development.*** Assessment of variation in 11 different dendritic attributes was made. Variation among these attributes, unless otherwise mentioned, was assessed using the same statistical model as implemented in the soma size ANOVA (i.e. the independent variables include: visual rearing history, cell type, and the visual rearing history by cell type interaction). Also, as was the case in the soma size analysis, the mean estimates for each LGN layer were used as the unit of measure in order to obtain statistically independent observations for population estimates.

***i. Cumulative Dendritic Length.*** Variation in dendritic length was assessed with respect to differences in visual rearing history, cell classification, or in the interaction between rearing history and cell classification. Estimates of cumulative dendritic length were obtained by summing the length of each dendritic segment across each neuron's entire dendritic tree. Figure 8 presents the mean cumulative dendritic length for each of the experimental rearing histories for animals reared with or without an intact optic chiasm. The ANOVA performed on cumulative dendritic length provided significant main effects for the full statistical model ($F_{(13,27)}=3.71$, $p=.0019$), of which only visual rearing history ($p=.0015$) yielded statistically significant type IV sum of squares.

**Figure 8.** Figure 8 presents data on the effects of monocular deprivation (MD), squint and denervation on the cumulative dendritic length in animals reared with (8A) or without (8B) an intact optic chiasm. Estimates of the cumulative dendritic length were obtained by a quantitative analysis (using morphometric software) of the camera lucida drawings of lucifer labeled LGN neurons. The length of individual dendritic segments were calculated and stored in a data base. To obtain the estimates of a neuron's cumulative dendritic length a computer program was used to total the length of each dendritic segment. Mean estimates of cumulative dendritic length (see ordinate) were based on the cumulative dendritic length of cells within a layer. Thus, the error bars reflect the standard error estimates based on the variation in cumulative dendritic length across LGN lamina rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their retinal input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 8A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 8B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.


A.
Chiasm Intact
Mean Dendritic Length (μ)
1800
1600
1400
1200
1000
800
600
400
200
0
Deprived
Non-Deprived
Squint
Visual Rearing History

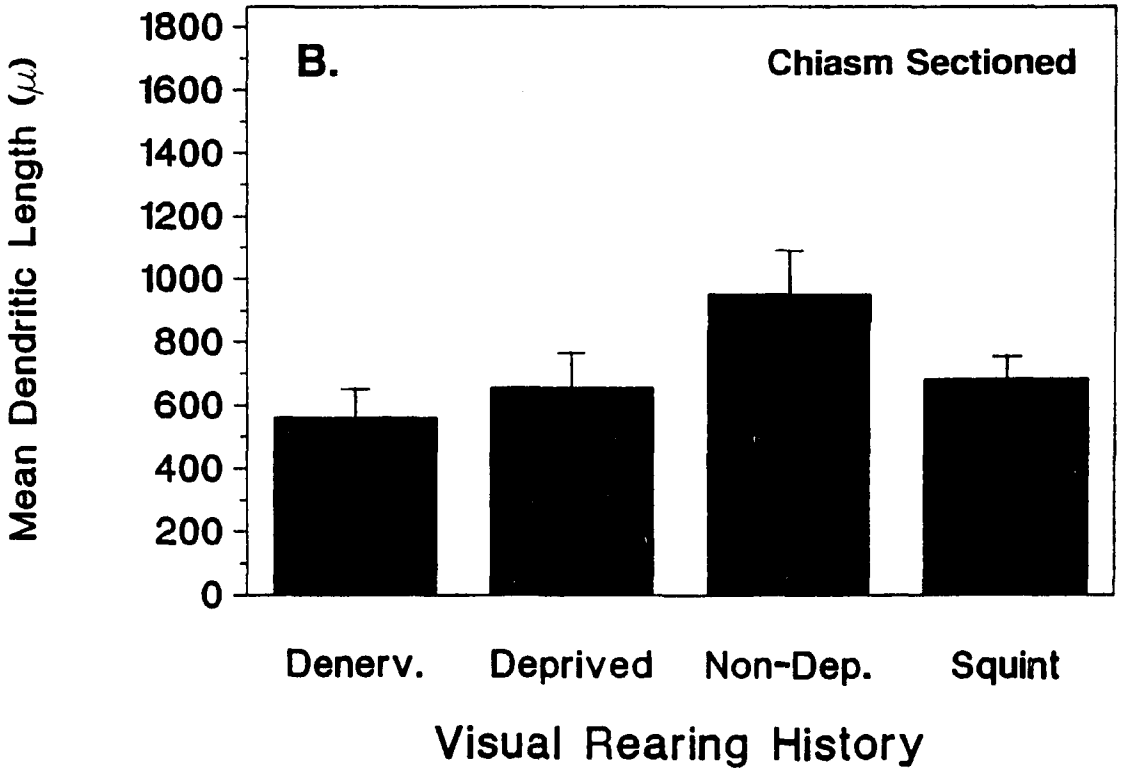

B.
Chiasm Sectioned
Mean Dendritic Length (μ)
1800
1600
1400
1200
1000
800
600
400
200
0
Denerv.
Deprived
Non-Dep.
Squint
Visual Rearing History

Table 7

Results of Planned Comparisons for the Effects of Deprivation, Squint or Denervation on Cumulative Dendritic Length for LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.43771 | 11.09 | 0.0025 *[f1] |
| Deprived OX vs Non-Dep OX[f2] | 0.08913 | 2.26 | 0.1446 |
| Squint vs Non-Deprived | 0.55439 | 14.04 | 0.0009 * |
| Squint OX vs Non-Deprived OX | 0.02778 | 0.70 | 0.4089 |
| Denervated vs Non-Deprived OX | 0.37481 | 9.49 | 0.0047 * |

[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

Five planned comparisons (using partial F statistics) were used to parse out specific differences between the various rearing histories (see table 7 above). It appears from this analysis that deprivation significantly reduced the cumulative dendritic length of LGN neurons. Moreover, the comparison between the deprived and non-deprived laminae of animals reared with optic chiasm transection did not yield a statistically significant difference. Thus, transection of the optic chiasm diminishes the effects of deprivation on cumulative dendritic length. As with deprivation, the sample of cells from laminae innervated by the squinted eye yielded significantly shorter estimates of cumulative dendritic length than did cells from the non-deprived layers. Chiasm section, however, reduced the effects of squint as revealed by the non-significant difference between squinted and non-deprived layers within animals reared with optic chiasm transection. Finally, cells from the denervated layers exhibited significantly

shorter estimates of cumulative dendritic length than cells from the non-deprived layers of chiasm sectioned animals. In fact, the mean (318.80 $\mu$) cumulative dendritic length of denervated laminae was shorter than estimates obtained from any of the other visual rearing histories assessed.

Given these differences in cumulative dendritic length across the experimental rearing conditions, an attempt was made to characterize which morphological factors could lead to such modifications in cumulative dendritic length. Two **possible** factors could account for these differences: (1) the number of dendrites could be changing across the experimental conditions; and (2) the length of each dendritic arbor could be modified by the developmental manipulations. Either or both of these factors could account for the systematic differences in cumulative dendritic length and therefore are analyzed below.

***ii. Number of Dendrites.*** To assess the first of these alternatives, an ANOVA was performed on the number of dendrites per cell within each of the experimental conditions. The results of this procedure yielded **no** statistically significant main effects for the independent variables assessed in this study ($F_{(13,27)}=1.38$, $p=.23$). Therefore, it appears that the differences in cumulative dendritic length **may not** be accounted for by modifications in the number of dendrites.

***iii. Average Length per Dendrite.*** Modifications in dendrite number could not account for the results obtained in cumulative dendritic length, thus analysis of the average length per dendrite varied was assessed with respect to the

independent variables. Figure 9 presents the average length per dendrite for each of the experimental rearing histories in animals reared with or without an intact optic chiasm. The ANOVA performed on average length per dendrite provided significant main effects for the full statistical model ($F_{(13,27)}=2.90$, $p=.0004$), of which visual rearing history ($p=.003$), but not cell type nor the cell type by visual rearing history interaction, yielded statistically significant type IV sums of squares.

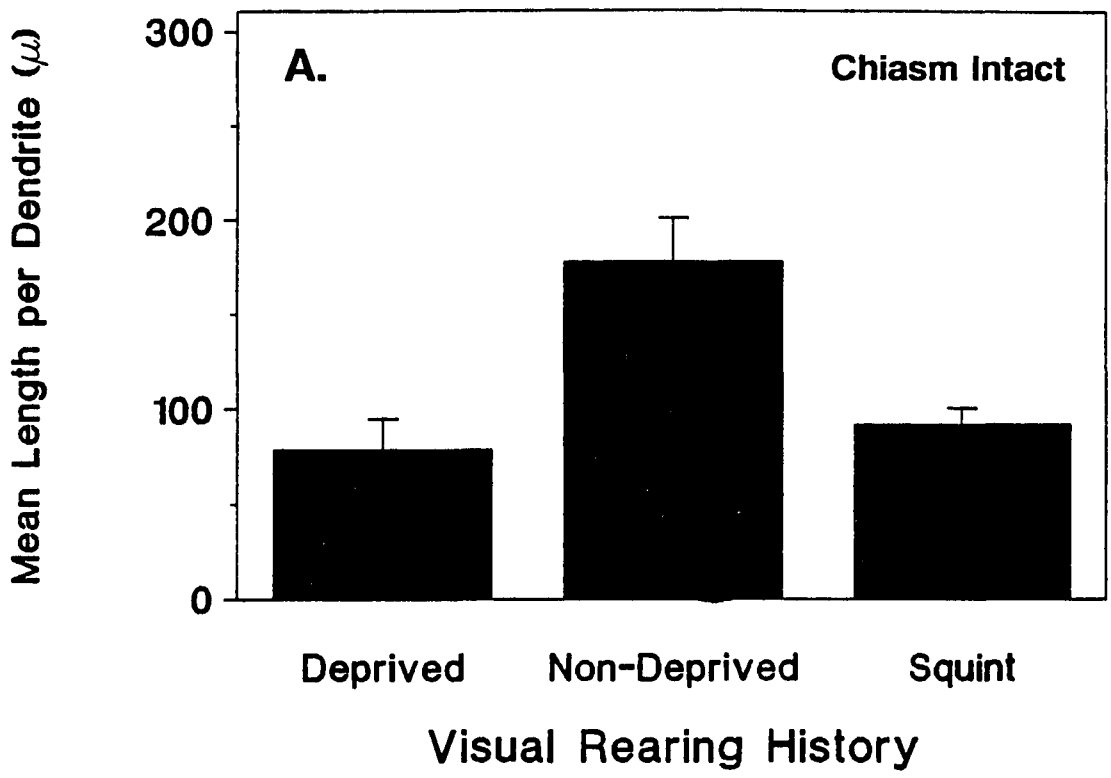
Table 8

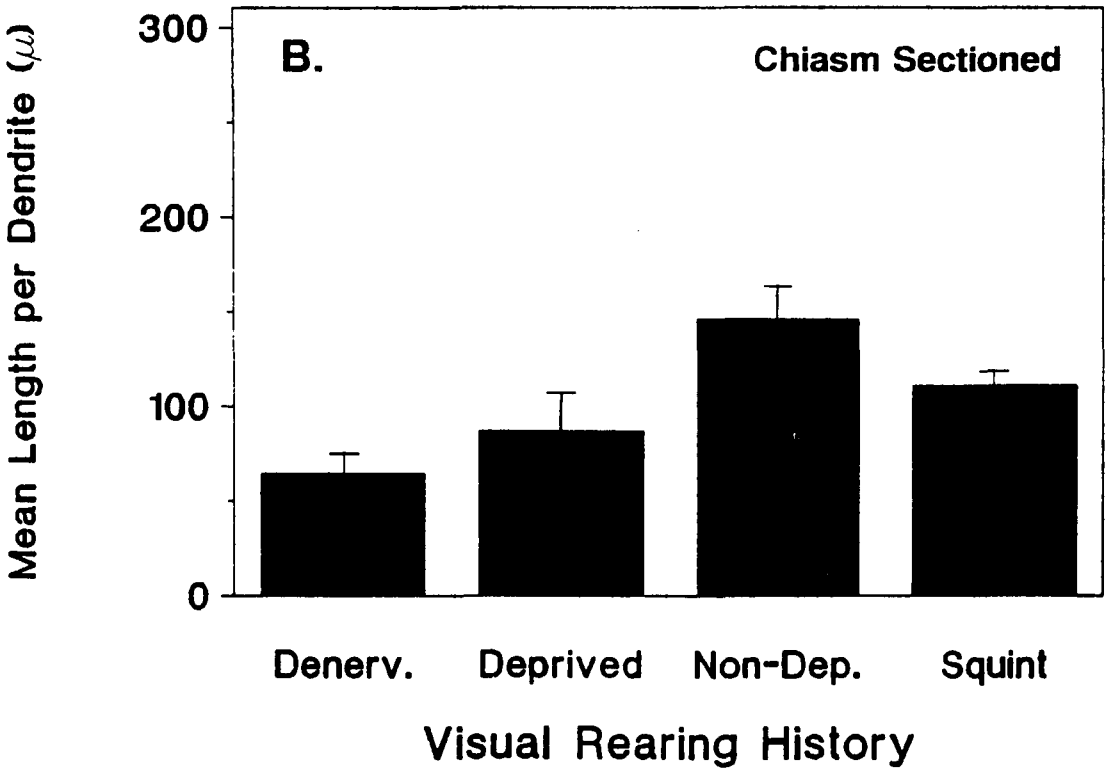
Results of Planned Comparisons for the Effects of Deprivation, Squint or Denervation on Average Length per Dendrite for LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.35166 | 11.76 | 0.002 *[f1] |
| Deprived OX vs Non-Dep OX[f2] | 0.09796 | 3.28 | 0.081 |
| Squint vs Non-Deprived | 0.22613 | 7.56 | 0.003 * |
| Squint OX vs Non-Deprived OX | 0.01223 | 0.41 | 0.528 |
| Denervated vs Non-Deprived OX | 0.31331 | 10.48 | .003 * |

[f1] *Values with asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

**Figure 9.** Figure 9 presents data on the effects of monocular deprivation (MD), squint and denervation on the average length per dendrite in animals reared with (9A) or without (9B) an intact optic chiasm. Estimates of the average length per dendrite were obtained by a quantitative analysis (using morphometric software) of the camera lucida drawings of lucifer labeled LGN neurons. The length of individual dendritic segments were calculated and stored in a data base. To obtain the estimates of a neuron's average length per dendrite a computer program was used to total the length of each dendritic segment and this number was then divided by the number of dendrites for that cell. The mean estimates (see ordinate) were based on the average length per dendrite of cells within a layer. Thus, the error bars reflect the standard error estimates based on the variation in average length per dendrite across LGN laminae rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their retinal input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 9A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 9B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.


A.
Chiasm Intact
300
200
100
0
Mean Length per Dendrite (μ)
Deprived
Non-Deprived
Squint
Visual Rearing History


B.
Chiasm Sectioned
300
200
100
0
Mean Length per Dendrite (μ)
Denerv.
Deprived
Non-Dep.
Squint
Visual Rearing History

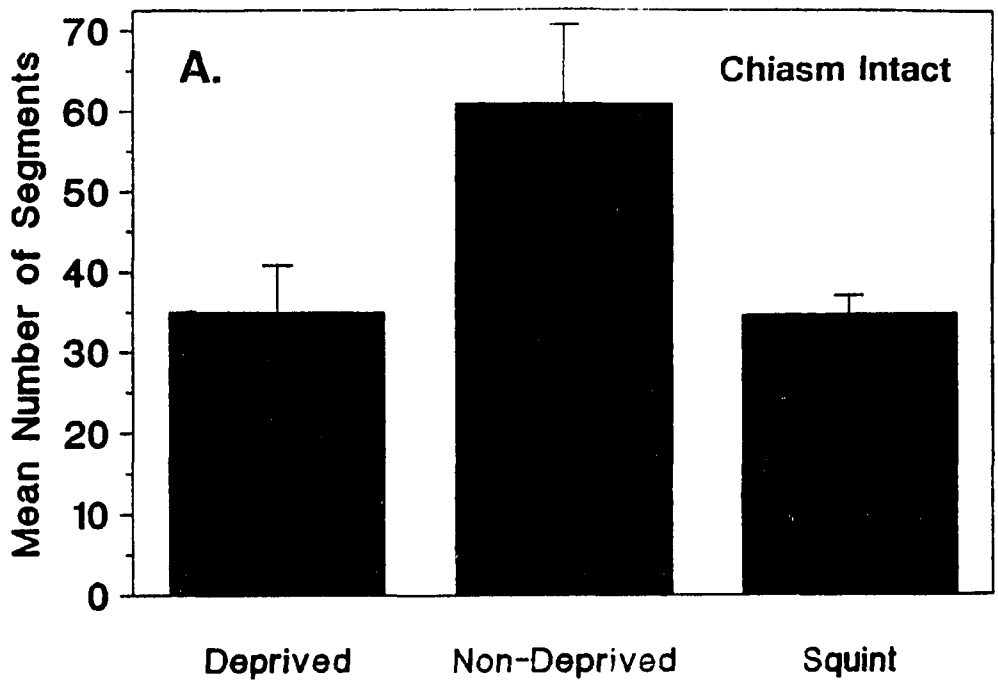
As demonstrated in table 8 (see above), planned comparisons for average length per dendrite revealed the same pattern of significant results as obtained for the analysis of cumulative dendritic length. Animals with **intact optic chiasms** showed a significant reduction in the arboreal length of dendrites innervated by the deprived or squinted eye when compared to cells in laminae innervated by the non-deprived eye. By contrast, animals reared with **optic chiasm transection** showed no significant differences in average length per dendrite for layers innervated by the deprived and squinted eyes when compared to layers innervated by the non-deprived eye. In addition, cells from the denervated laminae yielded significantly shorter estimates in their average length per dendrite. Taken together, these results show that variation in the cumulative dendritic length (described above) observed across these different visual rearing histories is largely due to changes in the average length of the dendritic arbors. Two possible factors could separately or together account for modifications in the length of dendritic arbors: (1) the individual dendritic segments could be changing in length and therefore resulting in the observed modifications of dendritic length; or (2) there could be differences in the average number of dendritic segments in dendrites across the visual rearing histories. Both of these potential explanations are evaluated below.

***iv. Dendritic Segment Length.*** An ANOVA performed on the average length per dendritic segment failed to demonstrate significant main effects for the independent variables assessed in this study ($F_{(13,27)}=1.79$, $p=.09$). This finding

implies that the segment lengths were roughly uniform across the visual rearing conditions.

*v. **Number of Dendritic Segments.*** The null result for average segment length suggests that systematic changes in the overall number of segments per cell play an important role in determining the cumulative dendritic length effects described above. Figure 10 presents the mean number of dendritic segments for each of the experimental rearing histories in animals reared with or without an intact optic chiasms. As for all dependent measures described above, the group means depicted in this figure were derived using the total number of dendritic segments per cell averaged across LGN laminae. Thus, LGN layers rather than individual neurons were used as the unit of measure for this analysis. The ANOVA performed on the mean number of dendritic segments per cell provided significant main effects for the full statistical model ($F_{(13,27)}=2.51$, $p=.02$), of which only visual rearing history ($p=.008$) yielded a statistically significant type IV sum of squares ($p=.008$).

**Figure 10.** Figure 10 presents data on the effects of monocular deprivation (MD), squint and denervation on the number of dendritic segments in animals reared with (10A) or without (10B) an intact optic chiasm. The number of dendritic segments was obtained by a quantitative analysis of the camera lucida drawings of lucifer labeled LGN neurons. The number of dendritic segments was derived from the matrix of data on dendritic length and was determined by simply counting the number of dendritic segments for which a dendritic length had been entered. Means (see ordinate) for the total number of dendritic segments per cell were averaged by layer. Thus, the error bars reflect the standard error estimates based on the variation in average number of segments across LGN laminae rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their retinal input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 10A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 10B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.


A.
Chiasm Intact
Mean Number of Segments
70
60
50
40
30
20
10
0
Deprived
Non-Deprived
Squint
Visual Rearing History

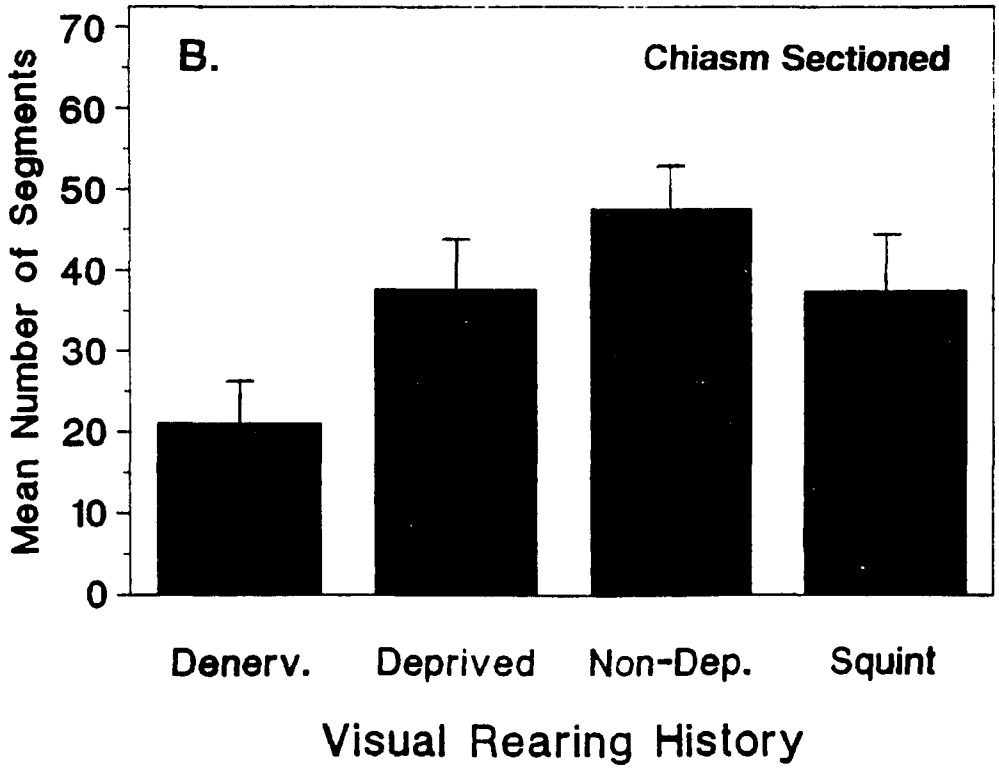

B.
Chiasm Sectioned
Mean Number of Segments
70
60
50
40
30
20
10
0
Denerv.
Deprived
Non-Dep.
Squint
Visual Rearing History

Table 9

Results of Planned Comparisons for the Effects of Deprivation, Squint or Denervation on the Number of Dendritic Segments for LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.14891 | 4.65 | 0.040 *[f1] |
| Deprived OX vs Non-Dep. OX[f2] | 0.02970 | 0.93 | 0.344 |
| Squint vs Non-Deprived | 0.16695 | 5.21 | 0.030 * |
| Squint OX vs Non-Dep. OX | 0.01774 | 0.55 | 0.463 |
| Denervated vs Non-Dep. OX | 0.41187 | 12.86 | 0.001 * |

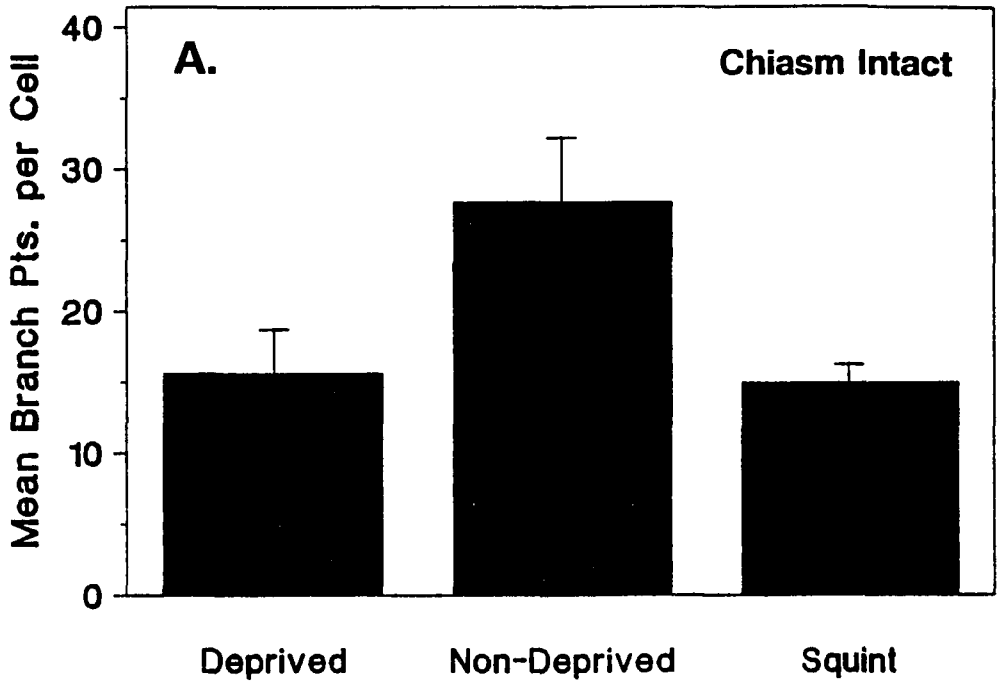
[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

Table 9 (see above) presents the results of the planned group comparisons on the number of dendritic segments. The pattern of significant differences revealed by this table mirrors those discovered for both cumulative dendritic length and average length per dendrite. Among animals reared with an intact optic chiasm, cells within layers innervated by the deprived or squinted eye generate about the same number of dendrites but with fewer dendritic segments than cells innervated by the non-deprived eye. By contrast, animals reared with optic chiasm transection showed no significant differences in number of dendritic segments for layers innervated by either the deprived or the squinted eyes as compared to layers innervated by the non-deprived eye. Also, significant differences were revealed between cells found in the denervated layers and those obtained from the non-deprived layers of chiasm sectioned animals. These systematic differences in the number of dendritic segments per cell help to

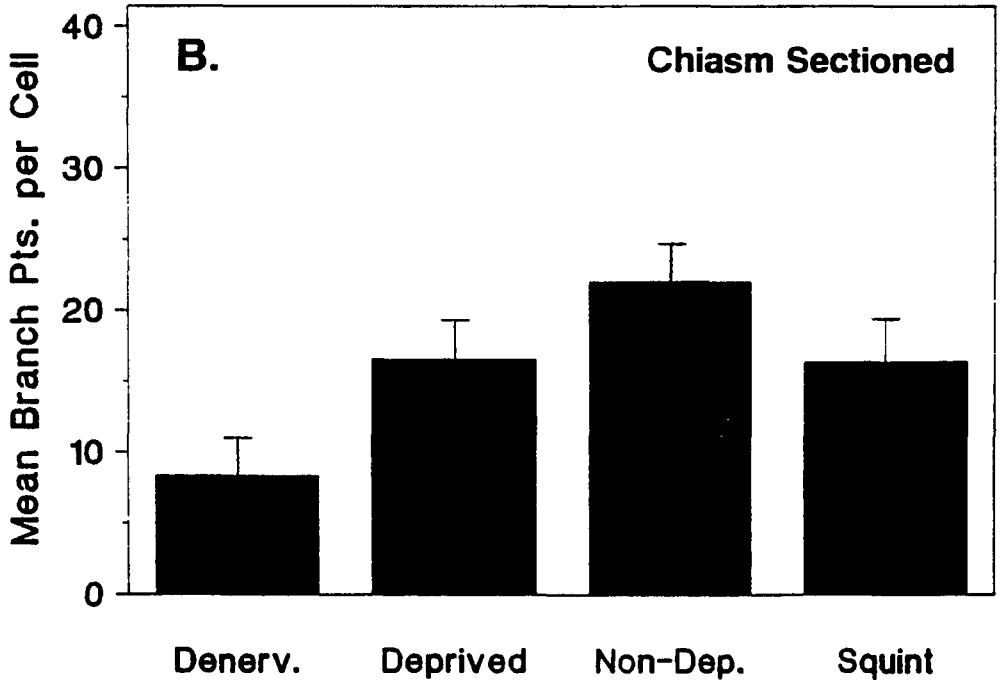
explain why the same pattern of results was obtained for cumulative dendritic length. Thus, developmental conditions which produce cells with dendrites that have more dendritic segments will correspondingly produce cells with higher estimates of dendritic length.

***vi. Dendritic Branching.*** There are 2 ways to produce a change in the total number of dendritic segments per cell. One way to increase the number of dendritic segments is to increase the number of dendrites per cell, and the other way is to increase the amount of dendritic branching. The null result for dendrite number (described above) ruled out the first possibility. Therefore, the following analysis addressed the possibility that modifications in the amount of dendritic branching could account for the differences obtained in the number of dendritic segments. Figure 11 presents the mean number of dendritic branch points for each of the experimental rearing histories in animals reared with or without an intact optic chiasm. The ANOVA performed on the number of dendritic branch points provided significant main effects for the full statistical model ($F_{(13,27)}=2.79$, $p=.01$), of which visual rearing history ($p=.002$), but not cell type nor the cell type by visual rearing history interaction, yielded statistically significant type IV sums of squares.

**Figure 11.** Figure 11 presents data on the effects of monocular deprivation (MD), squint and denervation on the number of dendritic branch points per cell in animals reared with (11A) or without (11B) an intact optic chiasm. The number of dendritic branch points was obtained from a quantitative analysis of the camera lucida drawings of lucifer labeled LGN neurons. The number of branch points for each cell was recorded and stored in a data file. Means (see ordinate) for the total number of dendritic branch points per cell were obtained by averaging our estimates of branch points by layer. Thus, the error bars reflect the standard error estimates based on the variation in average number of branch points across LGN laminae rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their retinal input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 11A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 11B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.




Visual History of the LGN Cell




Visual History of the LGN Cell

**Table 10**

Results of Planned Comparisons for the Effects of Deprivation, Squint or Denervation on the Number of Branch Points for LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.17461 | 4.00 | 0.0456 *[f1] |
| Deprived OX vs Non-Dep OX[f2] | 0.04619 | 1.06 | 0.3125 |
| Squint vs Non-Deprived | 0.02657 | 4.93 | 0.0349 * |
| Squint OX vs Non-Deprived OX | 0.79149 | 0.61 | 0.4419 |
| Denervated vs Non-Deprived OX | 0.77028 | 18.15 | 0.0002 * |

[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

Table 10 (see above) presents the results of planned group comparisons on the number of dendritic branch points per cell. The pattern of significant differences which emerged for this dependent measure was the same as those observed for the total number of dendritic segments. Among animals reared with an intact optic chiasm, cells within layers innervated by the deprived and squinted eye generated fewer dendritic branches than did cells innervated by the non-deprived eyes. By contrast, animals reared with optic chiasm transection showed no significant differences in dendritic branching for layers innervated by the deprived or squinted eyes as compared to layers innervated by the non-deprived eye. In addition, significant differences in dendritic branching were observed between cells found in the denervated layers and cells obtained from the non-deprived layers of chiasm sectioned animals.

Taken together, these findings indicate that differences in dendritic branching, rather than differences in the number of dendrites, are the key factor

responsible for the pattern of variation in the number of dendritic segments. Also, these findings support the emerging hypothesis that measures of cumulative dendritic length are, in many respects, tied to differences in the branching patterns among cells in these various experimental conditions. The data demonstrate that rearing histories conducive for dendritic branching (e.g., non-deprived conditions) also produce greater cumulative dendritic length.

***vii. Dendritic Width.*** Variation in the width of dendritic segments was also assessed. An ANOVA performed on the average width per dendritic segment revealed no significant main effects for the independent variables assessed in the present study ($F_{(13,27)}=1.16$, $p=.36$).

***viii. Dendritic Surface Area.*** Another feature of dendritic morphology addressed by this study was that differences in cumulative dendritic surface area could arise as a result of modifications in early visual experience. Dendritic surface area is an important variable because it offers a measure of the potential for synaptic space afforded by the dendrites of a neuron and because it has consequences for the electrotonic cable properties of the cell (Rall, 1959; Rall, 1970; Rall, 1977). In order to estimate dendritic surface area, dendritic segments were assumed to be cylinders. With this assumption, the measures of width and length for each dendritic segment were used to calculate the surface area of a cylinder [formula: $A=(2\pi r^2)+(2\pi rl)$]. Estimates of surface area for each dendritic segment were then summed across the entire dendritic tree of a neuron. The resulting number provided an estimate of the cumulative dendritic

surface area. Next, means were derived for these estimates by averaging across LGN lamina.

Figure 12 presents the mean cumulative dendritic surface area for each of the experimental rearing histories in animals reared with or without an intact optic chiasm. The ANOVA performed on dendritic surface area provided significant main effects for the full statistical model ($F_{(13,27)}=4.61$, $p=.0004$), of which visual rearing history ($p=.0004$) yielded a statistically significant type IV sum of squares.

Table 11

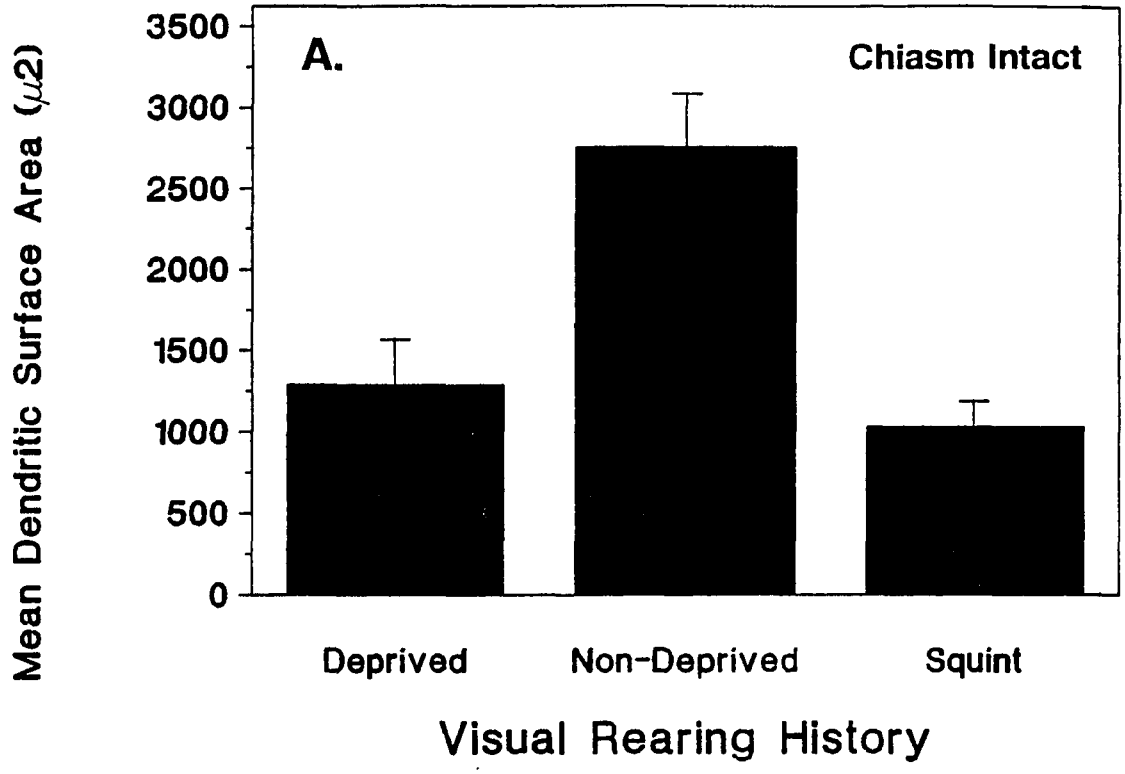
Results of Planned Comparisons for the Effects of Deprivation and Squint on the Cumulative Dendritic Surface Area for LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

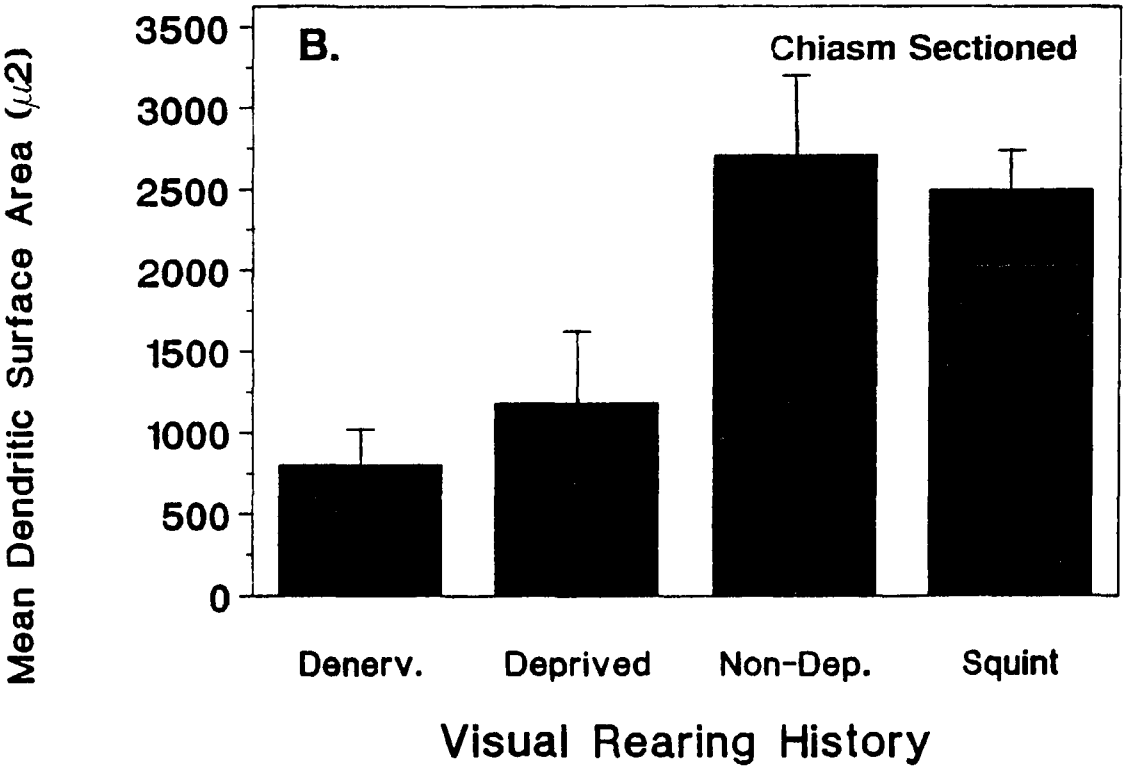
| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.31716 | 8.09 | 0.0084 *[f1] |
| Deprived OX vs Non-Dep. OX[f2] | 0.23189 | 5.91 | 0.0220 * |
| Squint vs Non-Deprived | 0.57711 | 14.71 | 0.0007 * |
| Squint OX vs Non-Deprived OX | 0.04369 | 1.03 | 0.3194 |
| Denervated vs Non-Deprived OX | 0.62765 | 16.00 | 0.0004 * |

[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

As displayed in table 11 (see above), the planned comparisons for cumulative dendritic surface area reveal a slightly different pattern of results from those observed for the other dependent variables. On this measure LGN layers innervated by the deprived eye revealed a significant reduction in estimates of their cumulative dendritic surface area. Moreover, this reduction

**Figure 12.** Figure 12 presents data on the effects of either monocular deprivation (MD), squint or denervation on the cumulative dendritic surface area in animals reared with (12A) or without (12B) an intact optic chiasm. Estimates of the cumulative dendritic surface area were obtained by a quantitative analysis (using morphometric software) of the camera lucida drawings of lucifer labeled LGN relay neurons. Dendritic segments were assumed to be cylindrical and the surface area for each dendritic segment was based on the estimated length and average width of each dendritic segment [formula: $A=(2\pi r^2)+(2\pi rl)$]. Estimates of the cumulative dendritic surface were then calculated with a computer by summing surface area of each dendritic segment. Mean estimates of cumulative dendritic surface area (see ordinate) were based on the cumulative dendritic surface area of cells within a layer. Thus, the error bars reflect the standard error estimates based on the variation in cumulative dendritic surface area across LGN lamina rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their retinal input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 12A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 12B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.


A.
Chiasm Intact
Mean Dendritic Surface Area (μ2)
3500
3000
2500
2000
1500
1000
500
0
Deprived
Non-Deprived
Squint
Visual Rearing History


B.
Chiasm Sectioned
Mean Dendritic Surface Area (μ2)
3500
3000
2500
2000
1500
1000
500
0
Denerv.
Deprived
Non-Dep.
Squint
Visual Rearing History

among deprived laminae appeared unaffected by optic chiasm transection. Hence, it appears that **transection of the optic chiasm does not protect LGN dendrites from deprivation induced reductions in dendritic surface** as it did for the other dependent measures described above. These results suggest, therefore, that the developmental mechanisms responsible for decreasing the dendritic surface area of cells within deprived layers are not based on competitive interactions occurring at the geniculocortical synapse (see discussion).

In contrast, squint elicits its effects on dendritic surface area in a way that **depends** on competitive interactions at the level of geniculocortical synapse. This idea is supported by two observations: (1), squint induced a significant decrease in the cumulative dendritic surface area in animals reared with intact optic chiasms; and (2) squint **failed** to produce a detectable decrease in estimates of dendritic surface area for animals who had been reared with optic chiasm transection. Hence, the effects of squint on dendritic surface area for animals reared with intact optic chiasms is likely to be a consequence of binocularly mediated competition geniculocortical axon terminal which occurs during early postnatal development in the cat (see Discussion). Taken together, these findings from deprived and squinted animals suggest that the effects of deprivation or squint reflect differences in the developmental processes which underlie the reductions in dendritic surface area described above.

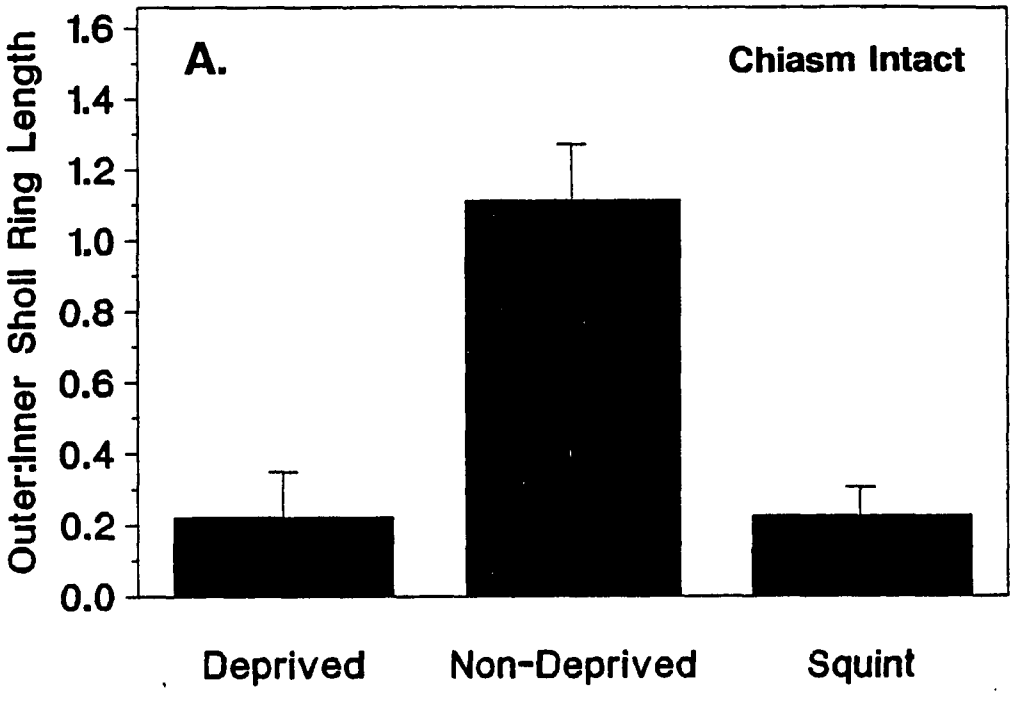
***ix. Sholl Ring Analysis.*** Simple measures of dendritic length cannot provide estimates of the radial expansion in LGN cell dendrites because dendrites do not

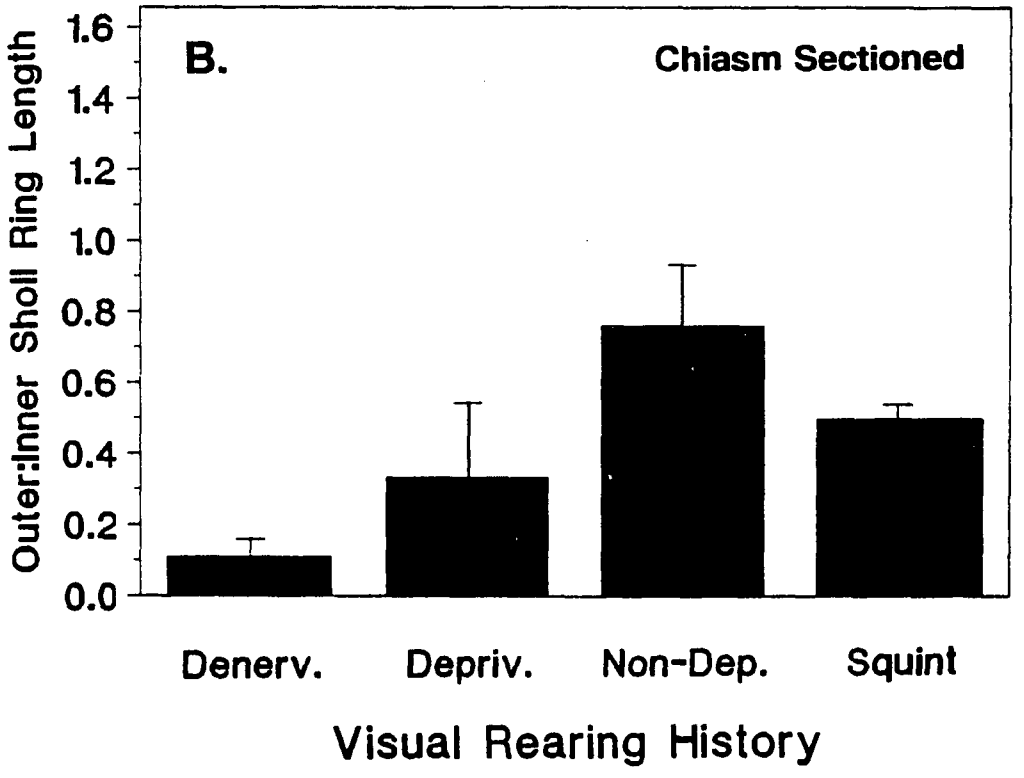
always depart from the soma in a radial fashion. Therefore, Sholl ring procedures were used in order to determine the radial expansion of geniculate cell dendrites. Sholl rings are concentric circles with diameters that increase in 50 $\mu$ increments. They offer a useful way to estimate the radial expansion of a neuron's dendritic tree (see Sholl, 1955; Friedlander et al., 1981).

As described in the Methods, all dendritic measurements were collected and stored in a manner which preserved information regarding the Sholl ring position of each dendritic segment. It was possible then to determine what percentage of the dendritic tree was found in each ring of the Sholl ring template. Using this information, two Sholl ring indices were generated: the first index estimated the percentage of **dendritic length** obtained from the inner rings (rings 1-2) relative to the outer (rings 3-8) Sholl rings; the second index employed the same procedures except that **dendritic surface area** rather than dendritic length was used as the dependent measure.

Figure 13 presents the mean Sholl ring index of dendritic length for each of the experimental rearing histories in animals reared with or without an intact optic chiasm. This analysis is important because Wilson et al. (1984) have shown that in normal animals retinal input to LGN dendrites is typically restricted within the first two Sholl rings, whereas extraretinal inputs (particularly from the visual cortex) are located in the outer Sholl rings. The ANOVA performed on Sholl ring length provided significant main effects for the full statistical model test ($F_{(13,27)}=3.69$, $p=.002$), of which visual rearing history

**Figure 13.** Figure 13 presents data on the effects of monocular deprivation (MD), squint and denervation on the ratio between the dendritic length in the outer relative to the inner Sholl rings in animals reared with (13A) or without (13B) an intact optic chiasm. Estimates of the outer : inner Sholl ring length were obtained by a quantitative analysis of the camera lucida drawings of lucifer labeled LGN relay neurons. Data regarding length of dendritic segments were obtained and stored in the computer in a manner that preserved information regarding a segment's Sholl ring affiliation. Cumulative estimates of dendritic length for each Sholl ring were calculated and then normalized with respect to the percentage of length obtained from the inner (i.e., rings 1 & 2) and outer (rings 3-8) rings. Ratios at or near 1 on this Sholl ring index reflect a high degree of balance between the proportion of dendritic structure found in the inner and outer regions of the dendritic tree. Mean estimates of outer : inner sholl ring length (see ordinate) are based on LGN layers. Thus, the error bars reflect the standard error estimates based on the variation in outer:inner Sholl ring length across LGN lamina rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their retinal input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 13A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 13B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.


A.
Chiasm Intact
Outer:Inner Sholl Ring Length
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Deprived
Non-Deprived
Squint
Visual Rearing History


B.
Chiasm Sectioned
Outer:Inner Sholl Ring Length
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Denerv.
Depriv.
Non-Dep.
Squint
Visual Rearing History

(p=.002), but not cell type nor the cell type by visual rearing history interaction, yielded statistically significant type IV sums of squares.

Table 12
Results of Planned Comparisons for the Effects of Deprivation, Squint or Denervation on the Sholl Ring Index for Dendritic Length in LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.00344 | 12.83 | 0.0013 *[f1] |
| Deprived OX vs Non-Dep. OX[f2] | 0.00044 | 1.67 | 0.2074 |
| Squint vs Non-Deprived | 0.00391 | 14.60 | 0.0007 * |
| Squint OX vs Non-Deprived OX | 0.00048 | 1.79 | 0.1925 |
| Denervated vs Non-Deprived OX | 0.00157 | 5.87 | 0.0223 * |

[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

Table 12 (see above) presents the results of planned group comparisons on Sholl ring dendritic length. Significantly less dendritic length was found in the outer Sholl rings for cells innervated by either the deprived or squinted eye relative to cells innervated by the non-deprived eye. By contrast, animals reared with optic chiasm transection showed no significant differences in Sholl ring dendritic length among layers innervated by the deprived or squinted eyes as compared to layers innervated by the non-deprived eye. Significant differences in this index were also observed between cells found in the denervated layers as compared to cells obtained from the non-deprived layers of chiasm sectioned animals. Taken together these results revealed that cells found in the deprived, squinted, and denervated layers are likely to have a high proportion of their dendritic arbors located near the soma where retinal synapses are prominent

(Wilson et al., 1984). Moreover, the effects of deprivation and squint can be diminished by optic chiasm transection.

Figure 14 displays the mean Sholl ring index of dendritic surface area for each of the experimental rearing histories in animals reared with or without an intact optic chiasm. This measure is an index of the relative percentage of dendritic surface area in the inner and outer rings. The ANOVA performed on Sholl ring surface area provided significant main effects for the full statistical model ($F_{(13,27)}$=3.12, p=.006), of which visual rearing history, but not cell type nor the cell type by visual rearing history interaction, yielded statistically significant type IV sums of squares (p=.003).

Table 13

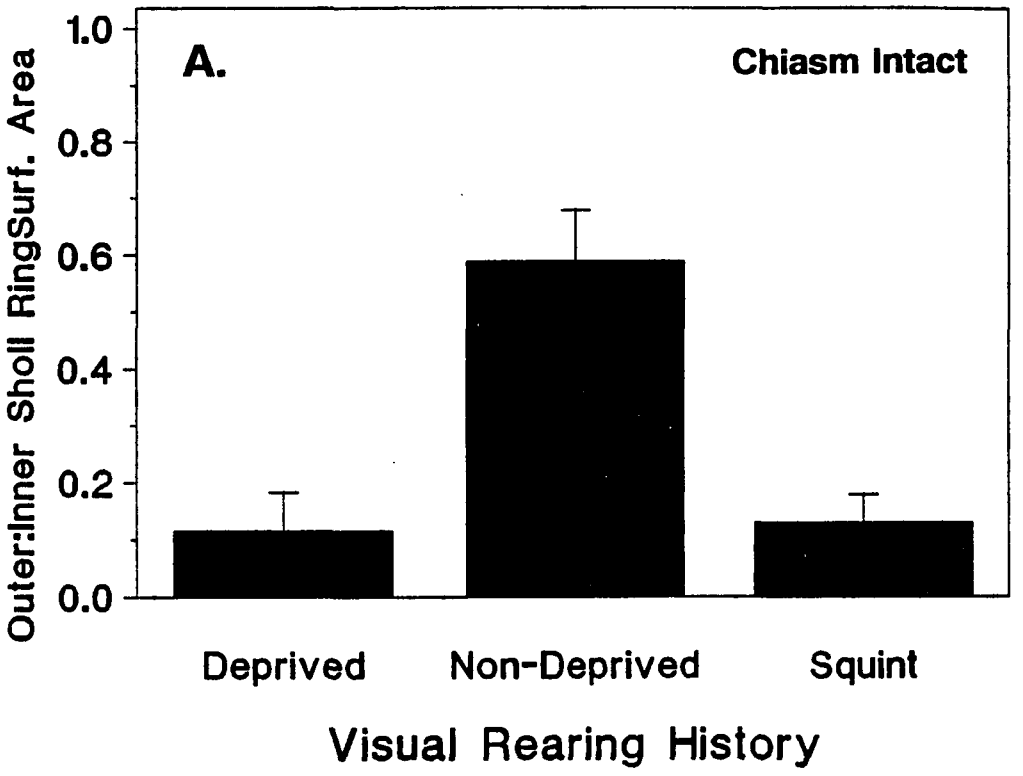
Results of Planned Comparisons for the Effects of Deprivation, Squint on the Sholl Ring Index for Dendritic Surface Area in LGN Cells from Animals Reared With or Without Optic Chiasm Transection.

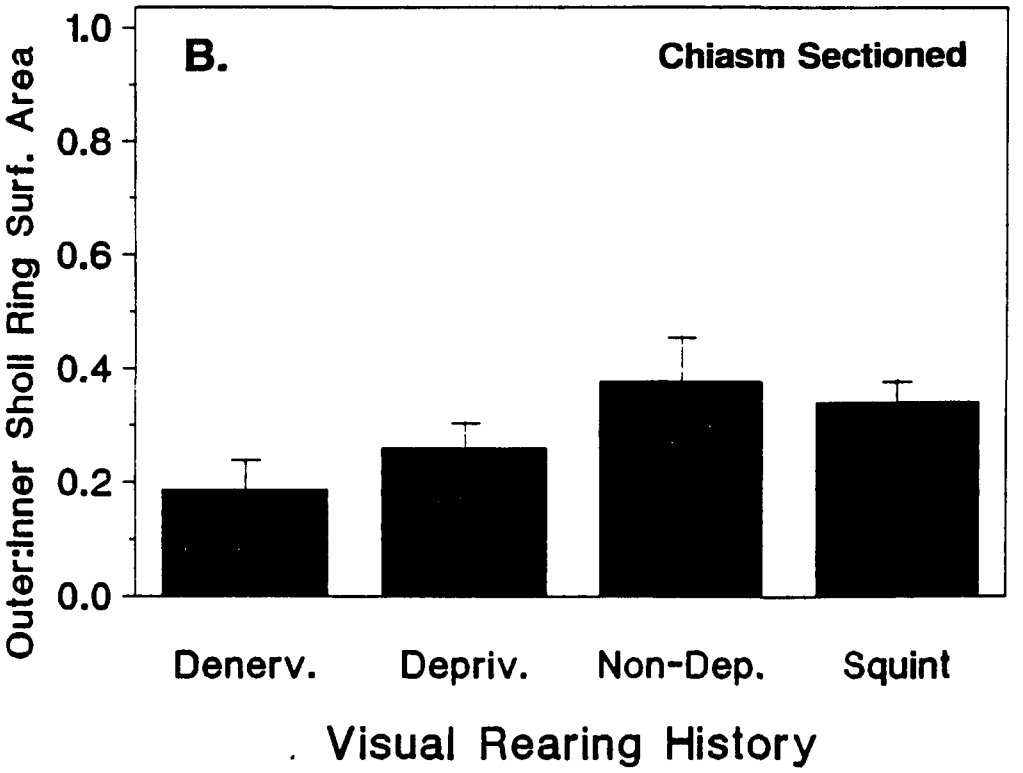
| Experimental Comparisons | Mean Square | F Value | Prob. |
|---|---|---|---|
| Deprived vs Non-Deprived | 0.00104 | 12.13 | 0.002 *[f1] |
| Deprived OX vs Non-Dep OX[f2] | 0.00018 | 2.12 | 0.156 |
| Squint vs Non-Deprived | 0.00114 | 13.27 | 0.001 * |
| Squint OX vs Non-Deprived OX | 0.00016 | 1.97 | 0.171 |
| Denervated vs Non-Deprived OX | 0.00045 | 5.27 | 0.029 * |

[f1] *Values marked by asterisks represent statistically significant differences.*
[f2] *OX refers to preparations in which the optic chiasm has been transected.*

Table 13 (see above) presents the results of the planned group comparisons on the Sholl ring index for dendritic surface area. These comparisons reveal the same pattern of significant differences for this dependent

**Figure 14.** Figure 14 presents data on the effects of monocular deprivation (MD), squint and denervation on the ratio between the dendritic surface area (SA) in the outer relative to the inner Sholl rings in animals reared with (14A) or without (14B) an intact optic chiasm. Estimates of the outer : inner Sholl ring SA were obtained by an analysis of the camera lucida drawings of lucifer labeled LGN relay neurons. Data regarding length and width of dendritic segments were obtained and stored in the computer in a manner that preserved information regarding a segment's Sholl ring affiliation. These measure were then used to calculated estimates for the SA of each dendritic segment. Cumulative estimates of dendritic SA for each Sholl ring were calculated and then normalized with respect to the percentage of SA obtained from the inner and outer rings. Ratios at or near 1 on this Sholl ring index reflect a high degree of balance between the proportion of dendritic SA found in the inner and outer regions of the dendritic tree. Mean estimates of outer : inner Sholl ring SA (see ordinate) are based on LGN layers. Thus, the error bars reflect the standard error estimates based on the variation in outer:inner Sholl ring SA across LGN lamina rather than across cells. LGN layers were classified according to one of seven different visual rearing histories (see abscissa). These classifications were based on the type of the visual input to the layer. For example, deprived layer cells received their retinal input from the sutured eye and squinted layers received their retinal innervation from the strabismic eye. Comparisons presented in figure 14A were obtained from animals with intact optic chiasms, whereas the comparisons revealed in figure 14B were taken from adult animals whose optic chiasms were transected on either the $27^{th}$ or $28^{th}$ day of life.


A.
Chiasm Intact
Outer:Inner Sholl RingSurf. Area
1.0
0.8
0.6
0.4
0.2
0.0
Deprived
Non-Deprived
Squint
Visual Rearing History

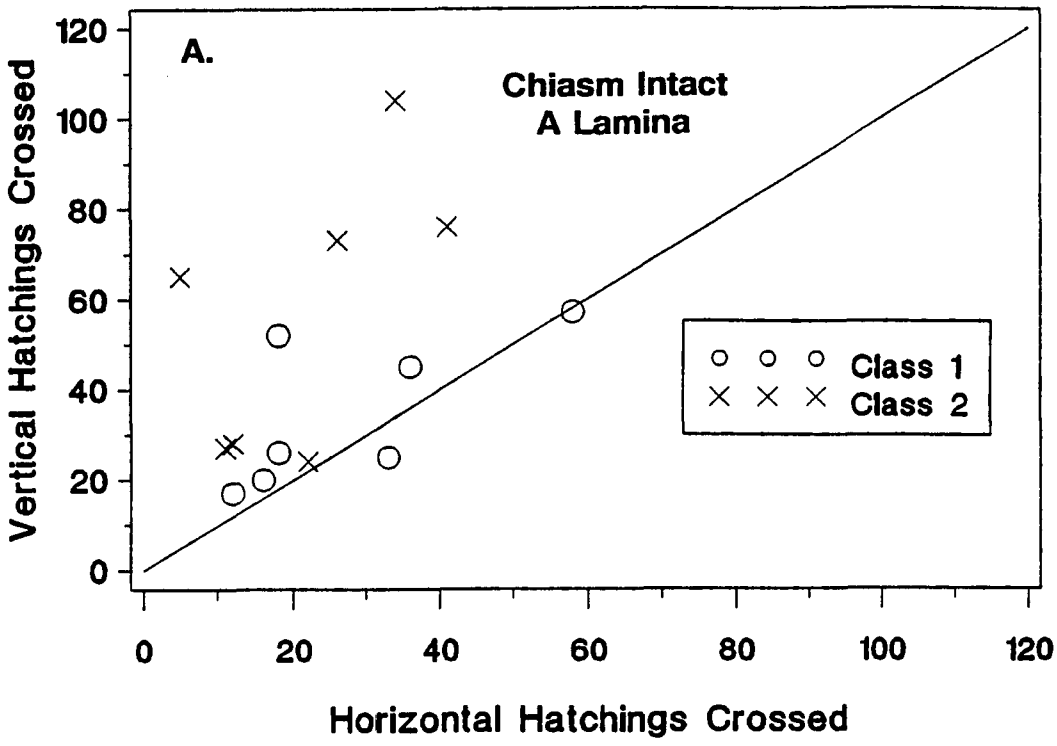

B.
Chiasm Sectioned
Outer:Inner Sholl Ring Surf. Area
1.0
0.8
0.6
0.4
0.2
0.0
Denerv.
Depriv.
Non-Dep.
Squint
Visual Rearing History

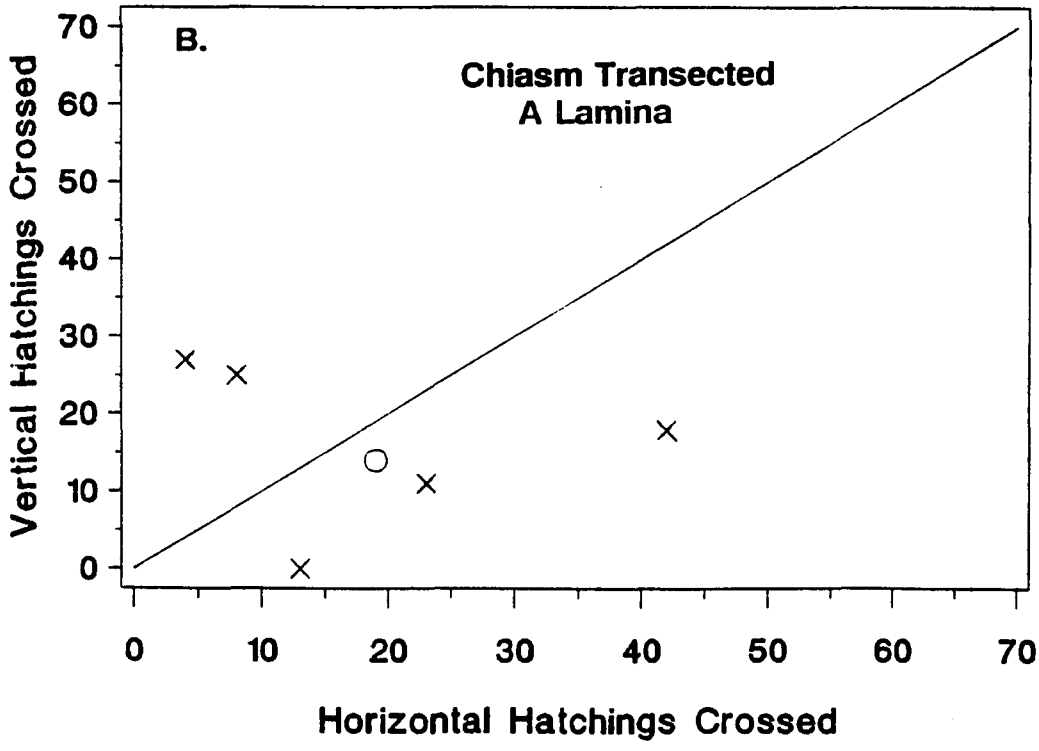
measure as obtained for the Sholl ring index in dendritic length. Among animals reared with an intact optic chiasm, cells from layers innervated by the deprived and squinted eye **do not** exhibit a balanced Sholl ring index (i.e., index does not approach one) like those indices which are prevalent among cells innervated by the non-deprived eyes. In essence, these results demonstrate that significantly less dendritic surface area is found in the outer rings of the Sholl ring plots for cells innervated by either the deprived or squinted eye. By contrast, animals reared with optic chiasm transection showed no significant differences in the Sholl ring index for dendritic length among layers innervated by the deprived or squinted eyes as compared to layers innervated by the non-deprived eye. Also, significant differences in this index were observed between cells found in the denervated layers and non-deprived layers of chiasm sectioned animals.

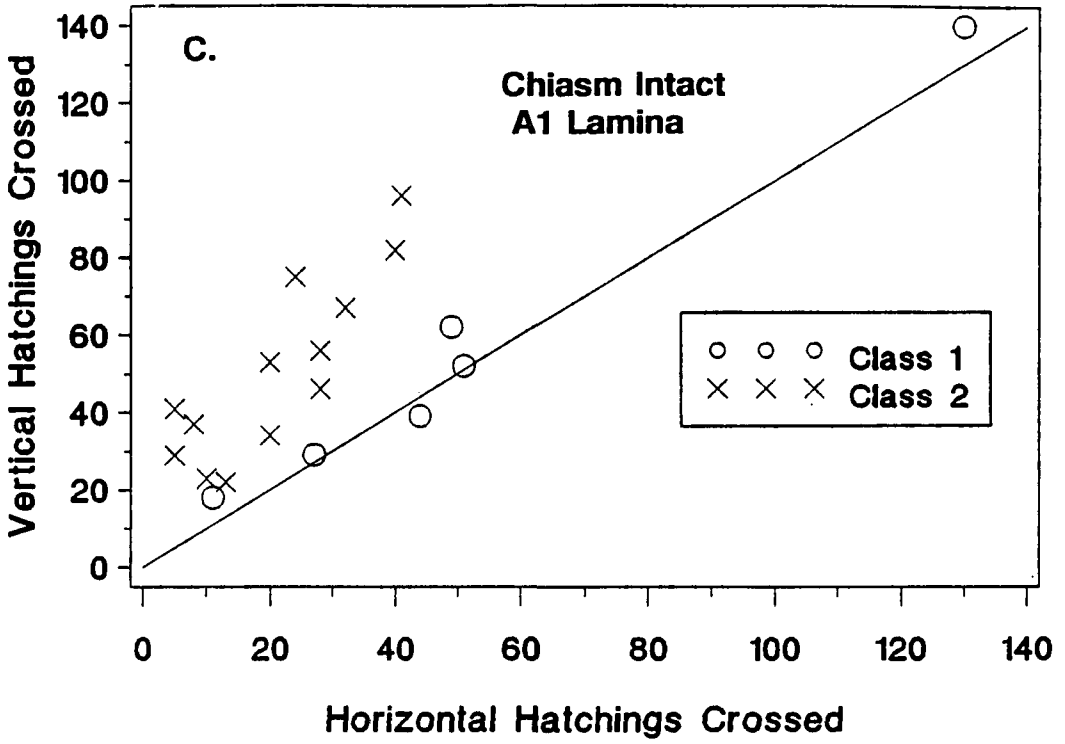
***x. Dendritic Geometry.*** In addition to the afore-mentioned effects of early visual perturbations on dendritic morphology, cells were also evaluated with respect to the geometry of their dendritic arbors. A cell's index of orientation is based on the number of vertical relative to horizontal hatchings crossed by that cell's dendrites within the four Sholl ring quadrants (see appendix A-4). Vertical and horizontal in this analysis are terms referenced with respect to axes perpendicular and parallel to the LGN laminae, respectively. Figure 15 presents an orientation scatter plot of class 1 and class 2 neurons for the A (Figs. 15 A & B) and A1 (Figs. 15 C & D) layers of animals reared with (Figs. 15 A and C) or without (Figs. 15 B and D) intact optic chiasms.

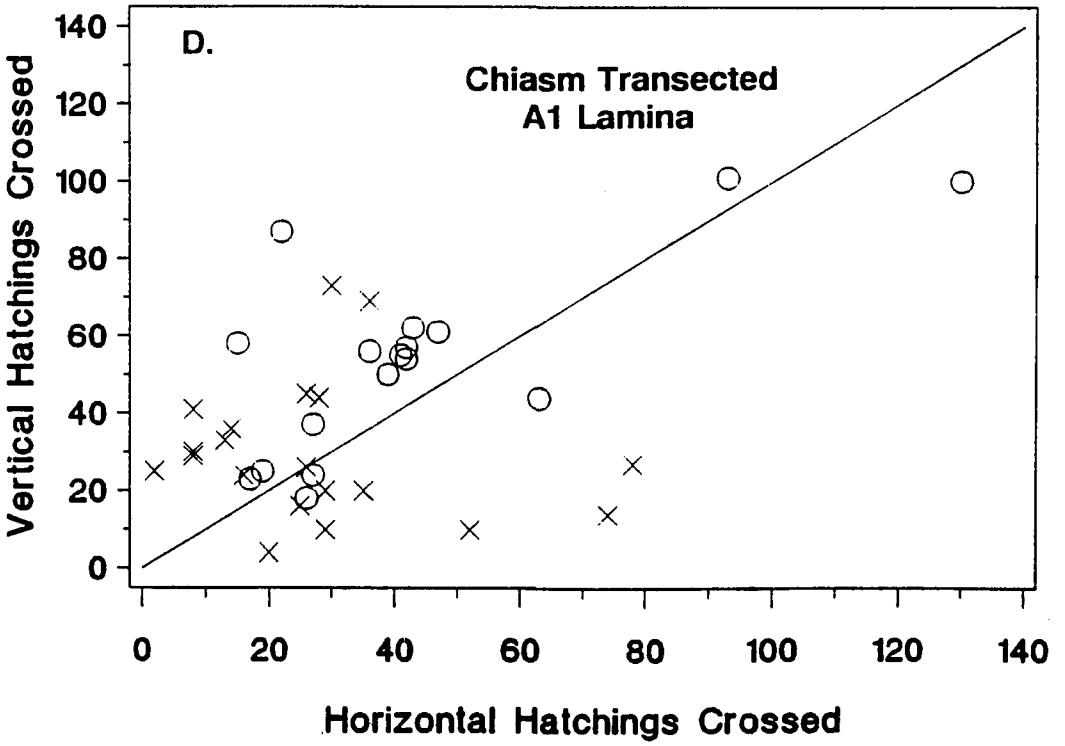
As one can see from these figures, class 1 neurons (denoted by circles) **typically** maintain orientation indices at or near 1 (values of 1 run along the oblique lines in the figures). This trend is consistent with Guillery's classification scheme for class 1 neurons and holds true throughout the sample with the

**Figure 15.** Scatter plot of the dendritic orientation for class 1 (denoted by the circles) and class 2 (denoted by Xs) LGN cells. Using the Sholl ring quadrants (see appendix A-4), the values are plotted as a function of the number of vertical (see ordinate) and horizontal hatchings (see abscissa) crossed by LGN cell dendrites. The orientation of the vertical or horizontal hatchings in each of the Sholl ring quadrants reflect axes which run either perpendicular or parallel to the lamina borders, respectively. Figures 15 A and B present orientation plots obtained from cells in the A laminae of cats reared with (fig. 15A) and without (fig. 15B) an intact optic chiasm. Figures 15 C and D present orientation plots obtained from cells in the A1 laminae of cats reared with (fig. 15C) and without (fig. 15D) an intact optic chiasm. The diagonal line in this figure represents values in which the ratios of horizontal to vertical hatchings crossed are equal to a value of one.


A.
Chiasm Intact
A Lamina
Class 1
Class 2
Vertical Hatchings Crossed
Horizontal Hatchings Crossed
0
20
40
60
80
100
120


B.
Chiasm Transected
A Lamina
Vertical Hatchings Crossed
Horizontal Hatchings Crossed
0
10
20
30
40
50
60
70


C.
Chiasm Intact
A1 Lamina
Class 1
Class 2
Vertical Hatchings Crossed
Horizontal Hatchings Crossed
0
20
40
60
80
100
120
140


D.
Chiasm Transected
A1 Lamina
Vertical Hatchings Crossed
Horizontal Hatchings Crossed
0
20
40
60
80
100
120
140

exception of 3 cells. These 3 LGN cells maintained dendritic arbors oriented perpendicular to the LGN laminae (features typical of class 2 cells) but also possessed exceptionally large somata (> 400 $\mu^2$ a feature typical of class 1 cells). Based on previous observations by Friedlander et al (1981) (see discussion of qualitative analysis above) these 3 neurons are likely to be physiologically characterized as LGN Y cells. Therefore, because the objective in classifying LGN cells according to Guillery's criteria was to characterize them with respect to their physiological properties, the class 1 criteria used in this study have been modified to include cells with oriented dendrites and large somata. This modification in the class 1 criteria is in accordance with Friedlander's structure/function analysis of LGN neurons.

Alternatively, the orientation indices for class 2 geniculate neurons (denoted by X's) are typically greater than 1 which places them above the oblique lines in figure 15. This simply means that dendrites from class 2 cells cross hatchings in the vertical axis (i.e., perpendicular to laminar borders) more frequently than they do in the horizontal axis (i.e., parallel to laminar borders). This phenomena is a defining feature of class 2 cells and can be seen in every class 2 cell in animals reared with intact optic chiasms (see figures 15 A & C)

Among the present sample of class 2 cells, however, there was a curious result noted in dendritic orientation which appeared to be related to the status of the optic chiasm. Among animals whose optic chiasm had been transected early in development (see figures 15 C & D), a preponderance of cells were discovered with class 2 features (e.g. these cells exhibited small to medium sized somata and grape-like appendages located at or near dendritic branch points) and which demonstrated dendritic orientation biases which ran either parallel or diagonal to the laminar borders as opposed to the normal pattern of vertically oriented

dendrites. These cells were not typically located near laminar borders and were clearly within either the A or A1 layers. Therefore, it is unlikely that these cells were either located in the interlaminar zone or that they were class 4, relay cells from the C laminae, both of which have been shown to possess dendritic arbors elongated parallel to laminar boundaries (Friedlander et al., 1981; Guillery, 1966; Stanford, Friedlander and Sherman, 1983). Hence, these cells were classified as class 2 relay neurons with abnormally oriented dendritic trees.

Figures 15 A and B demonstrate the effects of optic transection on the dendritic orientation of class 2 cells in the A layer. Note that class 2 cells obtained from A laminae of animals reared with intact optic chiasms exhibit dendritic arbors which yield more crossings in the vertical than in the horizontal axis. In the A laminae of chiasm sectioned animals, however, 60 % (n=3) of the class 2 cells maintain dendritic arbors in which there are more hatchings crossed in the horizontal than in the vertical axis. Cells from the A layers of chiasm sectioned animals have had their retinal input eliminated. Thus, these findings suggest the potential role of retinal axons in the organization of the orientation for dendritic arbors of class 2 cells (see discussion for details).

Figures 15 C and D provide an even more surprising result with respect to the dendritic orientation of class 2 cells from chiasm sectioned animals. Note that all class 2 cells from the A1 layers of animals reared with intact optic chiasms exhibited dendrites which were more oriented in the vertical axis. The orientation plot of LGN cells from the A1 layers of chiasm sectioned animals, however, reveals that 40 % (n=8) of the cells were more oriented in the axis parallel to laminar borders. In addition, one cell yielded an orientation index of 1 (as demonstrated by the X on the diagonal line of figure 15D), which is typical of class 1 cells. This cell, however, did exhibit an orientated dendritic arbor

which ran diagonal relative to the laminar borders, thus making it indistinguishable from class 1 cells on this measure of dendrite orientation. These observations from the A1 layers of chiasm sectioned animals are even more surprising than those noted in the denervated layers because they suggest that retinal axons destined for the A layers can influence the organization of dendritic arbors in class 2 cells found in the A1 layer. See discussion below for a more detailed analysis.

***The Morphological Substrates of Dendritic Electrotonus and the Role of Experience Dependent Competitive Mechanisms in its Development.***

***Rall's Power Rule.*** To understand the integrative performance of neurons, one must provide an adequate description of the passive electrical cable properties of a cell's dendritic field (Bloomfield et al., 1987). These properties are important because they, in conjunction with synaptic location, play a crucial role in determining amplitude, direction, and duration of post synaptic potentials recorded at the soma and are capable of influencing the axon hillock (for review, see Jack, Noble and Tsien, 1975; Rall, 1959, 1970, 1977). Rall's model (1959) is widely used for predicting the passive spread of electrical current within a neuron's dendritic arbor in many areas of nervous system.

Rall's equivalent cylinder model is based on several assumptions regarding the physiological and morphological attributes of a cell (for review see Rall, 1959; 1977). One of the morphological assumptions of this model is that the diameter (D) of the mother (m) branch raised to the 3/2 power is equal to the sum of the daughter (d) branches raised to the 3/2 power (i.e., $D_m^{3/2} = \Sigma(D_d)^{3/2}$). Using computer simulations, C. Koch (personal communication referenced in Bloomfield et al., 1987) has demonstrated that branching patterns which maintain this 3/2 power rule not only permit efficient electrotonic

conduction of post-synaptic potentials, but also allow for a symmetrical passage of electrical current both from the dendrites to the soma and from the soma to the dendrites. Such a relationship has important implications with respect to integration of electrical events occurring over the dendritic tree of the LGN cell (see Discussion for a more detailed treatment).

Bloomfield et al. (1987) have shown that the dendrites of geniculate cells from normally reared animals obey the 3/2 power rule. The validity of Rall's 3/2 power rule for the present data is assessed below. In addition, the question of whether this relationship is affected by cell class or visual rearing history was also assessed.

Using an electron microscope for calibration, Bloomfield et al. (1987) estimated a measurement error of approximately 0.5 $\mu$ in determining the diameter of dendritic segments with light microscope techniques (100x oil emersion lens, similar to optics used in this study). In addition, their measurements of segment diameter were obtained using a graticule eyepiece, a method which is somewhat more direct than estimates based on measures taken from the camera lucida drawings as used in this study. Therefore, it was expected that the estimates of segment diameter obtained for this study, particularly for the thin caliber dendrites, would be less accurate than those obtained by Bloomfield et al. (1987). In order to compensate for some of this measurement error, branch points having a mother segment diameter of less than .7 $\mu$ were eliminated from the analysis.

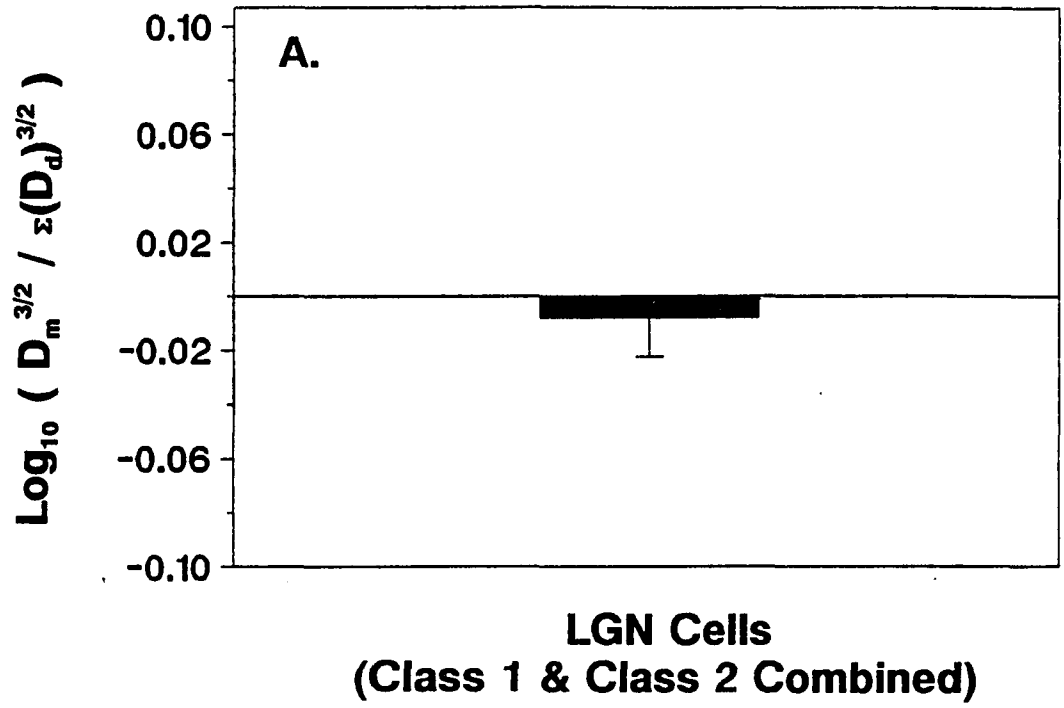
In order to provide independence between the experimental units, the mean ratio (i.e. $D_m^{3/2} / \Sigma(D_d)^{3/2}$) of dendritic branch points for each cell was obtained and then these values were averaged for each layer, thereby making LGN laminae the experimental unit of analysis. Because the distribution of

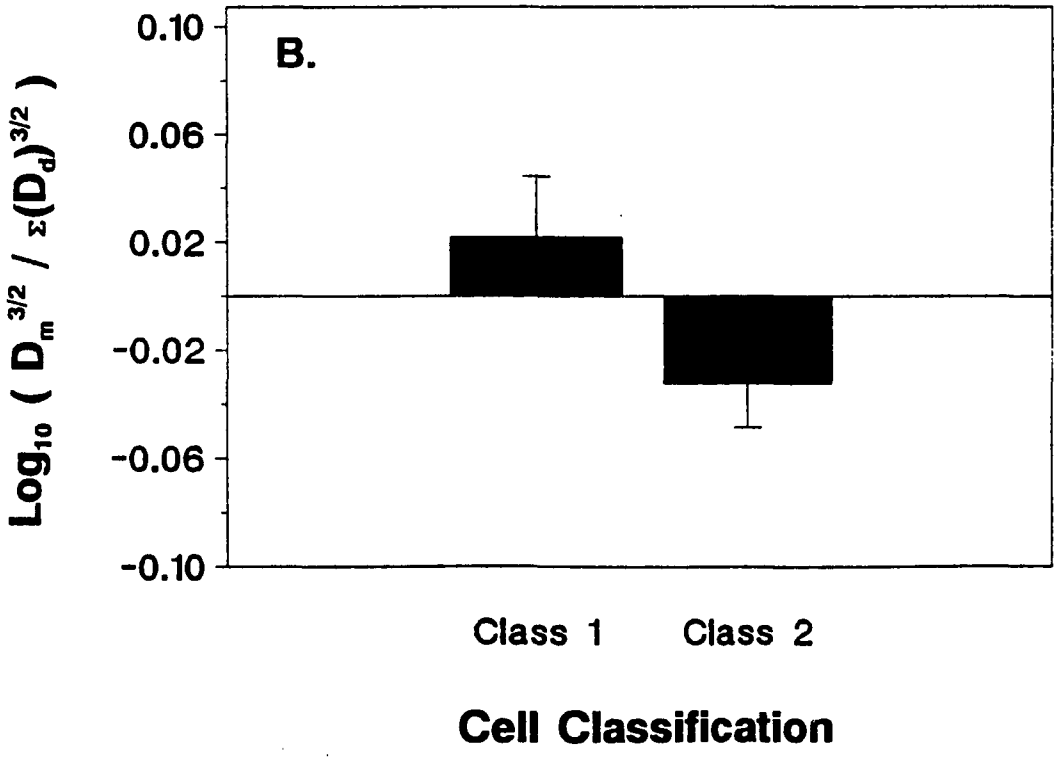
values for this ratio were not normally distributed, a $\log_{10}$ transform of the data was performed so that means would provide a valid measure of central tendency among the different experimental conditions. Hence, since Rall's power rule predicts that the $D_m^{3/2} / \Sigma(D_d)^{3/2} = 1$, our $\log_{10}$ values for this ratio should reveal a number close to 0 (i.e., the $\log_{10}$ of 1 is equal to 0).

Figure 16A shows the mean value for Rall's power rule averaged across LGN cells for both classes of geniculate neurons. A t-test performed on these data demonstrate that this value was **not** statistically different from zero ($t_{(39)}$, $p>.75$). This result confirms Bloomfield et al.'s (1987) in that geniculate neurons tend to obey Rall's 3/2 power rule. An ANOVA was used to address the possibility that significant differences in this ratio might arise across the experimental conditions. This statistical procedure detected statistically significant main effects of the model ($F_{(13,27)}= 2.07$, $p=.05$). Analysis of the type IV sum of squares revealed statistically significant differences between the two cell classes ($p=.007$) but not between the different visual rearing histories ($p=.14$) and not between the rearing history by cell class interaction ($p=.11$).

Figure 16B graphically presents the means for the ratio ($\log_{10}(D_m^{3/2} / \Sigma(D_d)^{3/2})$) in class 1 and class 2 cells. Student's t-tests reveal that **neither** class 1 ($t_{(17)}=.924$, $p>.85$) **nor** class 2 ($t_{(21)}=-2.01$, $p>.05$) LGN cells produced mean estimates on this ratio which were significantly different than 0. This result indicates that both classes appear, for the most part, to follow Rall's power ruleassumption. Because the direction of departure for the mean ratio of class 1 andclass 2 ratios were in opposite directions from 0, they did demonstrate statistically significant differences from each other. Personal communications with Koch suggest, however, that these differences in class 1 and class 2 cells are too small to cause functional differences. It seems quite remarkable that this

**Figure 16.** This figure presents the mean value of Rall's 3/2 power rule for LGN neurons. The ordinate reflects to $\log_{10}$ ratio of the mother branch raised to the 3/2 power divided by the sum of the daughter branches raised to the 3/2 power. According to Rall's model, the optimal branching relationship between the diameters of the mother and daughter branches in this equation should yield a $\log_{10}$ value of 0. The ratios for 935 branch points (class 1: n=412; class 2: n=523) were averaged for each cell (n=76) and then averaged for each LGN layer (n=40) in order to provide independent observations for our statistical analysis. Hence, error bars reflect variation on this ratio as a function of LGN layer rather than either cells or branch points. Figure 16A, presents the average 3/2 power ratio for the entire sample of LGN neurons. Note that the mean for the entire sample are not significantly different than Rall's predicted optimal branching relationship. Figure 16B, presents the average 3/2 power ratio for class 1 and class 2 cells separately. Neither of these two values are significantly (at $\alpha$=.05) different from 0, however, they are significantly (p=.007) different from each other.


A.
0.10
0.06
0.02
-0.02
-0.06
-0.10
$Log_{10}$ ( $D_m^{3/2}$ / $\Sigma(D_d)^{3/2}$ )
LGN Cells
(Class 1 & Class 2 Combined)


B.
0.10
0.06
0.02
-0.02
-0.06
-0.10
$Log_{10}$ ( $D_m^{3/2}$ / $\Sigma(D_d)^{3/2}$ )
Class 1
Class 2
Cell Classification

relationship between the diameters of mother and daughter branches would be maintained in the present study, given the large number of morphological changes which appear to accompany perturbations of early visual experience noted above. See discussion below for a more detailed treatment of this measure.

# CHAPTER IV

# DISCUSSION

Competitive mechanisms contribute to the development of early neural organization. The importance of competition is particularly well documented in the formation of central visual pathways in mammals (for review, see Guillery, 1988; Sherman and Spear, 1982; Sherman, 1985). Binocular competition, which involves competitive interactions between geniculocortical axons for establishing and maintaining ocular representation in the visual cortex, is thought to be especially important for controlling many aspects of cell development in the lateral geniculate nucleus (LGN) (Garraghty et al., 1984; Guillery, 1972; Lin and Sherman, 1978; Sherman et al., 1974; Sherman, Wilson, and Guillery, 1975; Sherman and Spear, 1982).

The present study was designed to extend the analysis of the influence of binocular competition beyond the context of animals reared with monocular deprivation (MD) to that provided by animals reared with artificially induced strabismus (squint). In addition, the present experiment assessed the possibility that somatic and dendritic development are controlled by different developmental mechanisms in addition to binocular competition.

## The Role of Binocular Competition in Cats Reared With MD: *Somatic Development.*

***Soma Size: Main Effect of Visual Rearing History.*** Consistent with previous reports (Guillery and Stelzner, 1970; Hickey et al., 1977; Kalil, 1980; LeVay and Ferster, 1977; Sherman et al., 1974; Wiesel and Hubel, 1963), the present data from animals reared with **intact optic chiasms** indicate that MD produces a significant reduction in the soma size of cells from the deprived layer. By contrast, cells in the deprived layer of animals reared with **chiasm transection** (MD-OX) exhibited somatic profiles which were not significantly different from cells in the non-deprived layers. Because MD-OX blocks the effects of MD by eliminating the opportunity for binocularly mediated competitive interactions at the geniculocortical synapse, the deprivation-induced deficits in somatic growth of LGN cells in animals reared with MD alone is likely to result from binocular competition. This conclusion regarding the role of binocular competition is consistent with those reached by earlier work (see also, Guillery, 1972; Sherman et al., 1974; but see Garraghty et al., 1984 which report only a partial block of the effects of deprivation after chiasm section).

***Soma Size: Cell Type by Visual Rearing History Interactions.*** In addition to the main effects of deprivation described above, the significant interaction between cell type and visual rearing history permitted further analysis. In animals reared with **intact optic chiasms**, monocular deprivation caused a significant reduction in the somatic development of class 1, but not class 2, LGN

relay cells in the deprived layers. The effects of MD on soma size were so powerful that they could have prevented accurate cell class assignments if soma size had been included as the criterion for determining cell class distinction in the deprived layers. However, besause cell class distinction in the deprived layers was based solely on dendritic morphology, it is unlikely that errors in cell classification could account for this reduction in soma size of class 1 cells.

By contrast, in animals reared with MD-OX, the soma size profiles of class 1 relay cells from the deprived layer were different in two ways from those from animals reared with MD alone. **First, the somata of LGN class 1 cells from the deprived layer of MD-OX reared animals were not smaller than their non-deprived counterparts** (i.e., optic chiasm section blocked the effects of MD on deprived class 1 cells). This observation from MD-OX reared animals suggests, therefore, that the decrease in soma size of class 1 cells in animals reared with MD (alone) is dependent on mechanisms which require binocularly mediated competitive interactions between geniculate axons in cortex. **Second, LGN class 1 cells in MD-OX reared animals were larger than deprived class 2 cells (see appendix C pg. 231).** This difference in soma size between deprived class 1 and class 2 LGN cells is **unlike** that observed in animals reared with MD alone and indicates that soma size in MD-OX reared animals, although not relied on in this study, would have been a useful criterion for distinguishing class 1 from class 2 cells in the LGN.

These results from MD and MD-OX reared animals are consistent with previous findings in the literature in several ways. First, the soma size reduction in MD reared animals is consistent with an extensive body of research (Guillery and Stelzner, 1970; Hickey, 1977; Kalil, 1980; LeVay and Ferster, 1977; Sherman et al., 1974; Wiesel and Hubel, 1963). Second, the selective nature (i.e., class 1 relay cells) and degree of reduced soma size confirms previous findings from Friedlander et al. (1982) which demonstrate that the soma size of LGN Y (i.e., class 1) cells are so severely affected by deprivation that they are indistinguishable from those of deprived X (i.e., class 2) cells in cats reared with MD. This result underscores the fact that soma size for deprived layer cells is not an adequate criterion for distinguishing between class 1 and class 2 relay cells in MD reared animals. Third, the observation that chiasm transection protects LGN relay cells from the deleterious effects of MD has also been demonstrated by previous studies (Garraghty et al., 1984) using the MD-OX preparation and by other studies which involve similar preparations (Guillery, 1972; Sherman et al., 1974). Hence, experiments designed to eliminate binocularly mediated competition at the geniculocortical synapse have consistently demonstrated that the decrease in soma size, particularly for class 1 LGN relay cells, in MD reared animals is dependent on the effects of binocular competition imposed by monocular eyelid suture.

The correspondence between results obtained in this study and those of previous studies is important because it validates the methods employed here and

offers confidence for examination of other phenomena (e.g., morphological analysis of LGN cells in animals reared with squint) for which a consistent literature does not exist (see discussion of squint below). Moreover, it is also important to note that even though the staining techniques used to label cells in this study (i.e., intracellular injections of lucifer yellow) were different from those used in the previous MD and MD-OX studies (i.e., cresyl violet or intracellular injections of HRP) similar findings were obtained, which suggests that differences in experimental procedure were unimportant for detecting deprivation-induced modifications in soma size.

Finally, given the difference in sampling procedures between this study and those described above, it is interesting that the lucifer injection procedures employed here and the cresyl violet labeling techniques used by others (see Garraghty et al., 1984; Hickey et al., 1977; Kalil, 1980) produced similar findings in MD animals. Cresyl violet procedures label both relay cells and interneurons in the LGN, whereas the methods employed by the present study selectively labeled LGN relay cells. Therefore, because the present results indicate that the effect of MD on LGN relay cells correspond to previous studies which have sampled both relay cells and interneurons, these findings suggest that deprivation yields similar effects on the somatic development of both LGN relay cells and interneurons.

## The Role of Binocular Competition in Cats Reared With MD: *Dendritic Development.*

***Qualitative Observations.*** In addition to the effects of deprivation on somatic development noted above, deprivation also induces several different effects on dendritic development, most of which appear to result from binocular competition. Confirming Friedlander et al.'s (1982) observations in animals reared with MD, the dendrites of some deprived class 1 LGN cells appeared pathological. These dendrites (see figure 3A) were thin and sinuous, and even though the primary segments departed the soma in an orderly, radial fashion, the second and third order dendritic segments appeared tangled (i.e. wrapped around each other, and exhibiting a highly torturous course). Although the physiological correlates of this morphological effect were not assessed in the present study, Friedlander et al. (1982) report that cells which possess these features typically display either sluggish responses or are unresponsive to visual and/or electrical stimulation. Qualitatively, the dendritic pathologies noted in the present study were present in 33% of the class 1 cells from the deprived layers of animals reared with MD alone. However, none of these pathological features were noted in the dendrites of cells from the deprived layers of chiasm sectioned animals (MD-OX). These observations would suggest, therefore, that binocular competition is involved in the production of these morphological anomalies.

These morphological differences between LGN cells in animals reared with MD (alone) and MD-OX raise the question of whether or not the physiological

correlates of MD (i.e., loss of recordable Y cells [Sherman et al., 1972], sluggish response properties [Friedlander et al., 1982], reductions in visual acuity [Lehmkuhle et al., 1980]) exist in animals reared with early transection of the optic chiasm. Because the dendritic morphology of deprived LGN class 1 cells in MD-OX reared animals looks like that of non-deprived cells, one might predict that the physiological properties of these deprived MD-OX class 1 cells would appear essentially normal. Recent behavioral data (Maffei et al., 1990) from animals reared with MD-OX have demonstrated that transection of the optic chiasm eliminates the visual amblyopia normally observed for the deprived eye of animals reared with MD (Dews and Wiesel, 1970; van Hof-van and Duin, 1976). Hence, the present data offer corroborating anatomical evidence which support the idea that the detrimental effects of MD on class 1 cell dendrites are diminished by removal of imbalanced binocularly competitive interactions between geniculocortical afferents, and that removal of these interactions has important consequences for the functional integrity of the LGN cells.

The present study reveals that binocular competition is an important mechanism for dendrite development among LGN cells. In normal development, cortically mediated competitive interactions are balanced. Thus, the resulting effects of these competitive interactions on LGN cell dendrites are not demonstrated in ways like that noted among deprived cells from MD reared animals. However, the present complement of experimental condition involving

animals reared with MD or MD-OX has shown that binocular competition is a normal feature regulating certain features LGN morphological development.

***Quantitative Effects of MD on Cumulative Dendritic Length.*** In animals reared with intact optic chiasms, quantitative analysis of cumulative dendritic length for LGN cells revealed that dendrites of cells in the deprived layers of MD reared animals were shorter than those of cells in the non-deprived layers. Unlike results from the soma size analysis, however, the ANOVA failed to detect a significant cell type by visual rearing history interaction, thus suggesting that the effects of deprivation on cumulative dendritic length may be present in both class 1 and class 2 LGN relay cells. Because a significant cell type by visual rearing history interaction was obtained for soma size but not for cumulative dendritic length, this suggests that different factors guide somatic and dendritic development in the LGN (see discussion of developmental mechanisms below for details). It is clear from these results in animals reared with MD that deprivation causes a significant reduction in the estimates of cumulative dendritic length.

By contrast, observations from MD-OX reared animals demonstrate that optic chiasm transection protects the dendrites of the deprived cells in the LGN from the detrimental effects of unbalanced binocular competition induced by monocular eyelid suture. This conclusion follows from the observation that dendrites of cells from the deprived layers of chiasm sectioned animals were not significantly shorter than cells in the non-deprived layer, whereas in animals reared with MD alone, cells from the deprived layers have significantly shorter

dendrites than cells in the non-deprived layer. Therefore, because chiasm transection eliminates the possibility for binocularly mediated competition between geniculocortical afferents in cortex, and because deprived layer cells in MD-OX (but not in MD alone) reared animals were not statistically different from cells in the non-deprived layer, it appears likely that cumulative dendritic length is controlled *via* mechanisms which involve binocular competition. In this respect, MD affects dendrites like it does on somata in the LGN.

***Factors Involved in the MD Induced Modifications of Cumulative Dendritic Length.*** Further analysis revealed that the effects of MD on cumulative dendritic length were due to a decrease in the length of individual dendritic arbors among deprived layer cells (see results on average length per dendrite above), rather than changes in the number of dendrites. This decrease in dendrite length for deprived layer cells is due to reductions in the amount of dendritic branching, and does **not** appear to be related to changes in the length of individual dendritic segments.

In animals reared with MD-OX, deprivation does **not** significantly decrease either the length of dendritic arbors or the number of dendritic branch points. Because chiasm section eliminates competitive interactions among deprived and non-deprived LGN arbors in cortex, these observations regarding dendrite length and the amount of dendrite branching indicate that chiasm section protects LGN dendrites from MD induced reductions in these two features of LGN cell morphology. Taken together, these data from MD and MD-OX reared animals

indicate that binocular competition is an important mechanism for controlling dendritic arbor length and dendritic branching in LGN cells.

The relationship between dendritic length and the amount of dendritic branching has important functional implications. A dendrite's length, in part, determines the electrotonic distance which electrical signals must travel to reach the soma and axon hillock where they are summated to produce action potentials. Because cells from the **non-deprived** layer exhibit longer dendritic arbors than cells in the deprived layers, it would seem that synaptic events (i.e., EPSPs and IPSPs) occurring on the terminal segments of **non-deprived** dendrites would be less likely to affect the cell's activity state than would synaptic events occurring on the terminal segments of deprived layer cells. However, variation in cumulative dendritic length does not neccessarily imply a change in dendritic electrotonic length, particularly if cumulative dendritic length is introduced by dendritic branching. Therefore, the large estimates of cumulative dendritic length produced by dendritic branching observed in cells from the non-deprived layer provides a means by which synaptic events occurring in distal segments may reach the soma with only minimal degradation. Moreover, because more dendritic branching results in more dendritic segments, and because the number of segments increase the opportunity for synaptic contacts, the morphological substrate exhibited by cells in the **non-deprived** layers provides an opportunity for an increase in the number synaptic contacts relative to cells in the deprived layers, thereby possibly permitting a greater sensitivity of these cells to incoming

synaptic events compared to deprived cells. By contrast, the terminal segments of the dendrites of cells in the **deprived** layer are not far from the soma. However, their reduced dendritic length and smaller number of dendritic segments suggests fewer opportunities for synaptic contacts (see dendritic surface area discussion below). This, in turn, may result in a relative decrease in their sensitivity to incoming synaptic events.

Finally, it is interesting to note that the average length per dendritic segment of cells in deprived and non-deprived layers did **not** differ. This observation suggests that the length of individual dendritic segments, as opposed to the length of LGN dendrites, are relatively impervious to the competitive imbalance imposed by monocular eye lid suture. This finding seems particularly interesting in light of the various morphological changes which were noted among cells in the deprived LGN layers. These results indicate that the mechanisms controlling segment length are mediated by epigenetic mechanisms that are unrelated to factors which control dendritic length and dendritic branching.

***Effects of MD on Dendritic Surface Area.*** For animals reared with intact optic chiasms, deprivation results in a decrease in cumulative dendritic surface area. Like the results from cumulative dendritic length, the ANOVA failed to detect a significant cell type by visual rearing history interaction. This suggests that the effects of deprivation on cumulative dendritic length are present in both class 1 and class 2 LGN relay cells. Hence, it is clear from the results in animals

reared with MD that deprivation causes a significant reduction in cumulative dendritic surface area.

Binocular competition does **not** appear to be the factor responsible for modifying dendritic surface area as revealed by the non-significant difference between estimates of dendritic surface area for cells in the deprived and non-deprived layers of animals reared with MD-OX. Surface area is the only dimension in which transection of the optic chiasm failed to protect LGN relay cells from the deleterious effects of monocular lid suture.

In general, differences in dendritic surface area are important because they reveal possible differences in the potential for synaptic space as well as potential differences in the cable properties of LGN neurons (Bloomfield et al., 1987). In the present study, differences in cumulative dendritic surface area suggest that cells in the deprived layers could have fewer synaptic contacts than cells in the non-deprived layers. Two sources of experimental evidence support this possibility. First, deprivation is known to diminish the physiological responsiveness of cells in the deprived layers of the LGN (Derrington and Hawkin, 1981; Eysel et al., 1979; Friedlander et al., 1982; Geisert, Spear, Zetlan and Langsetmo, 1982; Kratz, Webb and Sherman, 1978; Sherman et al., 1972; Wiesel and Hubel, 1963) but appears to have **no** detectable effects on the physiological response of retinal ganglion cells (Cleland et al., 1980; Sherman and Stone, 1973; Wiesel and Hubel, 1963). These findings indicate that the disruption in signal transfer between deprived retinae and LGN is likely to result

from problems arising in either the function or number of retinogeniculate synapses. Second, anatomical (Garraghty et al., 1986; Sur et al., 1982) evidence suggests that deprivation severely disrupts the size and bouton density of retinogeniculate arbors, particularly for retinal Y cells. These complementary sets of findings suggest that the observed reduction of dendritic surface area for deprived cells may have important implications for synaptic transmission in the LGN of MD reared animals.

These observations suggest that a detailed account of the deprived neuropil is required to explore the possibility that deprivation decreases synaptic contacts by way of a general decrease in dendritic surface area. Alternative explanations are possible; for example, increases in synaptic density for cells in the deprived layer could compensate for a loss of dendritic surface area. This possibility seems unlikely, however, since Sur et al.'s (1982) study of deprived retinal axon terminals revealed a decrease in the number of boutons, particularly for retinal Y cells (Sur et al., 1982). Examination of the neuropil could also address the possibility of changes in the ratio of retinal to non-retinal innervation of LGN cells. Existing data from normal animals (see below) suggest a highly specified topography for the innervation of retinal and extraretinal sources onto the dendritic tree of LGN neurons. Sholl ring analysis was used to address the possibility that deprivation may selectively result in a systematic change in the opportunity for synaptic innervation by either of these two sources.

***Effects of MD on Sholl Ring Length and Surface Area.*** The final two effects of deprivation on dendritic development assessed in the present study were detected with a Sholl ring analysis (Sholl, 1955) performed on both dendritic length and dendritic surface area. Simple measures of dendritic length or dendritic surface area do not provide estimates of the radial expansion of LGN dendrites because dendrites do not always expand in a radial fashion from the soma. Therefore, a Sholl ring procedure was used to determine an index of the radial expansion of geniculate cell dendrites. The index was calculated from the percentage of dendritic length or dendritic surface area in the outer (i.e., rings 3-8) relative to the inner (i.e., rings 1-2) Sholl rings. Hence, indices of 1 reflect a relatively balanced proportion of dendritic tree near (< 100 $\mu$) and far (> 100 $\mu$) from the center of the soma; indices less than 1 indicate that the majority of the dendritic tree is located near the soma; and indices greater than one indicate that the majority of the dendritic tree is located far from the soma. "Near" in this context (Sholl rings 1-2) was based on published reports from normally reared animals which indicate that the majority of the retinal terminals are located within a 100 $\mu$ radius of the center of the soma, whereas "far" represents the distal portion (> 100 $\mu$) of dendritic tree where afferent terminals from the visual cortex are more prominent (Sherman and Koch, 1986; Sherman, 1988; Weber and Kalil, 1987; Wilson, Friedlander, and Sherman, 1984). Since Sholl ring indices of dendritic length and dendritic surface area yielded comparable effects of deprivation, the discussion of both variables is presented together.

In animals reared with an intact optic chiasm, MD results in a reduction in the relative proportion of dendritic length and dendritic surface area located at distances > 100 $\mu$ from the center of the cell. Two morphological differences between cells in the deprived and non-deprived layers could contribute to the observed differences in the Sholl ring indices. First, since cells in the deprived layer yield smaller estimates of cumulative dendritic length and surface area, it is possible that the reduction in radial dendritic extension for deprived layer cells is due to the fact that the morphology of these cells is simply impoverished relative to cells in the non-deprived layers. Second, deprivation results in tangled, sinuous dendrites among class 1 cells as described above. This pattern differs from that of the radially projecting dendrites of cells in the non-deprived layers. Hence, these Sholl ring indices, in part, could reflect the concentration of deprived LGN cell dendrites near the soma. The mean dendritic length and mean dendritic surface area for the first two Sholl rings (< 100 $\mu$) indicate, however, that cells in the deprived layers have less dendritic length and surface area than do cells in the non-deprived layers. These results suggest that deprivation produces its effects on radial dendritic extension by merely decreasing the total length of the dendritic tree rather than by concentrating the dendritic length within the first 2 Sholl rings.

As described above, the location of synaptic innervation of retinal and extraretinal sources is topographically specified in normally reared animals. Excitatory retinal inputs and inhibitory extraretinal inputs both tend to be

localized within the first 100 $\mu$ from the soma, whereas excitatory extraretinal inputs are more prominent at intermediate (100-150 $\mu$) distances from the soma (Sherman and Koch, 1986; Weber and Kalil, 1971; Wilson et al., 1984). Because afferent input to the dendritic tree of LGN cells in normally reared animals appears to be regionally defined according to distances from the somata, the results from the Sholl ring indices of cells in the deprived layers suggest either significant modifications in synaptic topography and/or a loss of synaptic contacts (possibly from corticofugal fibers) are likely to result from rearing animals with MD. The functional implications of this possible synaptic remodeling and the relative strength of retinal and non-retinal influences in deprived layer cells require further physiological and anatomical study.

In animals reared with MD-OX, Sholl ring indices for LGN cells in the deprived layers **were not** different from those obtained from cells in the non-deprived layers. This finding from animals reared with MD-OX suggests that it is binocular competition which is likely responsible for the deprivation induced reduction in radial dendritic extension noted among animals reared with MD alone.

***Possible Residual Effects of Deprivation in Chiasm Sectioned Animals.*** It should be noted in chiasm sectioned animals (i.e., MD-OX) that, even though not statistically different, deprived LGN layers reliably yielded mean estimates on the dendritic measures described above which were lower than those obtained from the non-deprived layer. This suggests the possibility that deprivation might have

an effect on these morphological parameters (i.e., cumulative dendritic length, average length per dendrite, and number of branch points) even in the absence of binocular competition. The effect that is produced, however, is too small to be detected by the ANOVAs with the current sample size. In order to address this possibility more observations are needed. If residual effects of deprivation persist following optic chiasm section in addition to the more robust effects of binocular competition discussed above, this would suggest the possible contribution of one of four alternative mechanisms: (1) abnormal **binocular** interactions introduced by **callosal** projections in the visual cortex (i.e., callosal projections from the non-deprived cortex may induce abnormal binocularly mediated competitive interactions with deprived geniculocortical afferents, thereby causing modifications in LGN cell morphology); (2) **non-binocular** competitive interactions between **X and Y retinal axon terminals** (Garraghty et al., 1986; Sherman, 1985); (3) the contribution of **non-competitive** processes (i.e. **deprivation *per se***); and (4) the contribution of non-visual competitive interactions arising from mismatches in binocular proprioception. For a detailed discussion of these mechanisms see section entitled "*Possible developmental mechanisms underlying LGN cell development*" below.

Issues to be Considered Regarding Experimental Controls

The size of the effects of deprivation, particularly on soma size, reported here are possibly exaggerated because cells from the non-deprived layers were used as within animals experimental controls. Although the use of cells from

the non-deprived layers has the advantage of offering within animal comparisons, it has been suggested (see Sherman and Spear, 1982) that cells from non-deprived layers may reflect enlarged morphological features resulting from hypertrophy. This argument has been advanced particularly for somatic development (Guillery, 1973a; Hickey et al., 1977) and is based on the possibility that geniculocortical afferents from the non-deprived eye are placed at a competitive advantage over cells innervated by the deprived eye due to biased competition imposed by monocular lid suture. Recent support for this hypothesis comes from Friedlander, Martin and Wassenhove-McCarthy (1991) who have demonstrated that non-deprived geniculocortical Y afferents from MD cats possess larger than normal arbors in area 18 of the visual cortex. Despite this support for an enlarged non-deprived geniculocortical projection among LGN Y-cells, data from Kalil (1980) suggests that hypertrophy is not reflected in the somatic development of LGN cells from non-deprived layers of MD reared cats. Nonetheless, the possibility that hypertrophy might exist must be accepted and cannot be directly addressed by the present experiment because data from normal animals were not provided. If hypertrophy does exist among our sample of geniculate neurons then this would tend to amplify the morphological differences noted between deprived and non-deprived layer cells, thereby increasing the chances of detecting deprivation effects. Regardless of whether atrophy (in deprived layers) or hypertrophy (in the non-deprived layers) or both contribute to morphological differences in the deprived and non-deprived layers

of MD animals, this does not detract from the thesis of the present study, namely that chiasm transection protects the LGN from most of the detrimental effects of biased competition imposed by monocular lid suture.

Another issue regarding the experimental controls used in the present study has to do to the possibility that optic chiasm transection itself produces effects on LGN cells which are unrelated to the animal's deprivation status. Consistent with the possibility, comparisons between the non-deprived layers of animals reared with or without optic chiasm transection reveal some differences on many of the dependent measures. Although not definitive, statistical comparisons between the non-deprived layers of chiasm sectioned and chiasm intact animals revealed no significant differences on any of the quantitative measures except for dendritic orientation (see discussion below), thus suggesting that chiasm transection by itself does not induce significant changes in LGN cell morphology. More importantly it should be noted that even if effects due to chiasm transection should arise, the present studies used within-animal experimental comparisons from non-deprived layers which maintain optic chiasm status constant (i.e., either OX intact or OX transected). Thus, the experimental comparisons used in the present study did control for the possibility that chiasm section itself may have induced changes in the morphology of LGN neurons. At the same time, however, they preclude definitive analysis of the possible effects of chiasm section itself on LGN cell morphology.

One final issue regarding the experimental controls used in this study deals with the composition of cells included in the non-deprived group. Cells designated as non-deprived were compiled from several different sources. For animals reared **with an intact optic chiasm,** non-deprived cells were taken from layers innervated by both from the non-deprived eyes in monocularly deprived (MD) animals and from the unsquinted eyes of animals reared with artificially induced strabismus. These groups of cells were combined for statistical analysis since they exhibited **no significant differences.** For animals reared **with optic chiasm transection,** non-deprived cells were obtained from layers innervated by non-deprived eyes of animals reared with MD-OX, SQ-OX, and optic chiasm section alone. In these animals as well, statistical comparisons between cells in the non-deprived layers revealed **no significant differences** on any of our dependent measures. Hence, cells from the non-deprived layers were combined.

## The Role of Binocular Competition in Cats Reared With Squint: *Somatic Development.*

***Soma Size: Main Effects of Visual Rearing History.*** Like MD, artificially induced strabismus causes a disruption in the somatic development of LGN cells in animals **reared with an intact optic chiasm.** Thus, in these animals the mean soma size for the squinted layers (i.e., class 1 and class 2 relay cells combined) was significantly smaller than those obtained in the non-deprived layers. By contrast, in animals **reared with optic chiasm section** (SQ-OX), cells in the squinted layer exhibited somatic profiles which were **not** significantly different

from cells in the non-deprived layers. Because chiasm transection eliminates competitive interactions among squinted and non-squinted geniculocortical arbors in cortex, these observations regarding soma size indicate that chiasm section protects LGN cells from the squint-induced reduction in somatic growth by eliminating these competitive interactions. Taken together, these data from squint and SQ-OX reared animals indicate that binocular competition plays an important role in causing the overall reduction in LGN somatic growth (i.e., class 1 and class 2 relay cells combined) in animals reared with squint.

Previous work in squinted animals has provided mixed results with respect to the effects of squint on LGN cell development. Ikeda and colleagues (Ikeda, Plant, and Tremain, 1977; Tremain and Ikeda, 1982) report that animals reared with squint have significantly smaller somata than normally reared animals. Support of Ikeda's observations regarding an arrest of somatic development has been provided by Garraghty et al. (1985) which used unilateral ocular paralysis in order to induce optical misalignment. Crewther et al. (1985), however, have disputed Ikeda's and others results claiming that the aggressive surgical procedures used to elicit strabismus (i.e., unilateral myectomy of both the lateral rectus and superior oblique muscles) were responsible for inducing the reduction in LGN somatic growth rather than visually induced morphological modifications resulting from early squint rearing. Crewther et al. (1985) report that less aggressive surgical procedures which involve tenotomization of the lateral rectus muscle did produce a behaviorally detectable amblyopia for the squinted eye, but

did not yield the kinds of severe physiological and morphological changes noted by Ikeda and colleagues. This claim, however, seems odd in light of Garraghty et al.'s (1985) monocular paralysis preparation which yielded decrease in the size of LGN somata (as reported by Ikeda and colleagues) without the use of surgical procedures which did not directly invade the orbit of the eye itself (i.e., surgical paralysis of the eye was induced by transection of the oculomotor nerves behind the eyesocket *via* a ventral approach).

In light of the controversy described above, the results from the present study are somewhat enigmatic. The surgical methods used in this study involved tenotomization of the lateral rectus muscle. According to Crewther et al. (1985), this surgical procedure should not have produced morphological anomalies in the somatic development of LGN cells. Two possible explanations are offered here to account for the difference between Crewther et al.'s (1985) findings and those reported in this study: (1) methodological differences in sampling; and (2) differences in the timing of the surgical insults.

First, the two studies employed different sampling procedures for defining the population of LGN cells to be studied. Crewther et al. (1985) employed cresyl violet staining procedures in order to obtain estimates of soma size. That method stains both relay and interneurons in the LGN. In this study, only cells identified as relay cells by the intracellular presence of rhodamine microspheres were included in the sample. The role of binocularly competitive mechanisms in guiding LGN relay cell development are likely to be more influential than those

which guide interneuron development. Since LGN interneurons do not send axons into the visual cortex, they cannot be influenced directly by cortical binocular competition. In contrast, LGN relay cells terminate principally in the visual cortex, therefore they are likely to be affected by binocularly mediated competition in ways that LGN interneurons are not. This line of analysis suggests that the ability of Crewther et al.'s (1985) method to detect morphologic differences between cells in the squinted and non-squinted layers may have been compromised by the fact that their sample of LGN cells included two populations, relay cells that are sensitive and interneurons which can not be insensitive to the binocularly mediated competitive interactions introduced by squint.

If squint does induce a differential sensitivity for its effects on interneurons and relay cells in the LGN, then these findings from squint reared animals differ from those obtained in animals reared with MD. That is, the analysis of LGN relay cell somata from this study were consistent with the results of cresyl stained preparations from MD reared animals. Taken together, these observations may indicate that MD reduces the soma size of both LGN interneurons and relay cells in the LGN, whereas squint selectively reduces the size of LGN relay cells without modifying LGN interneurons. Such a conclusion, however, is contingent on acceptance of the idea that these differences in method (i.e., lucifer injections of identified LGN relay cells vs. cresyl violet analysis)

reflect a differential impact of squint on interneurons and relay cells in the LGN as opposed to alternative explanations (see below).

A second possible way to account for the difference between Crewther et al.'s (1985) report and the present study deals with age of onset for the tenotomization. Crewther and colleagues induced esotropia in their tenotomized kittens at about 10 days postnatal, shortly after natural eye opening. This is approximately two weeks prior to the advent of the critical period for the development of central visual pathways in the cat (Hubel and Wiesel, 1970; Mitchell, 1981). In the present study, however, unilateral tenotomization of the lateral rectus muscles was performed at the end of the fourth week postnatal, at a time shortly following the beginning of the visual critical period. Based on previous experience with this surgical procedure, the tendons of these lateral rectus muscles reinsert onto the eyeball and are capable of producing lateral (albeit not full amplitude) eye movements within days after the surgery. It seems possible, therefore, that this recovery of ocular motility in Crewther et al.'s (1985) tenotomized animals may have been reasonably complete by the time in which many of the central visual areas of the brain exhibit an acute sensitivity to perturbations in their visual input. Hence, recovery of ocular motility in the squinted eye may have protected Crewther and colleagues' animals from the severe developmental morphological anomalies which might have arisen had these animals been tenotomized during the critical period, as in the present study.

***Soma Size: Cell Type by Visual Rearing History Interactions.*** In addition to the main effects of squint described above, an analysis of the decrease in somatic growth for each cell class (i.e., class 1 and class 2) was permitted by the significant cell type by visual rearing history interaction noted in the ANOVA for soma size. In animals reared with **intact optic chiasms**, statistical analysis demonstrates that **class 2, but not class 1,** LGN relay cells were significantly different from their corresponding cell types in the non-deprived layers. In animals reared with **optic chiasm transection** (i.e., SQ-OX), squinted class 2 LGN somata remained significantly smaller than class 2 cells in the non-deprived layer, thus suggesting that the abnormal development of squinted class 2 cells was not affected by binocular competition. This finding is curious, in that a similar class 2 effect was not obtained for animals reared with MD. Hence, it appears that the stimulus modifications induced by squint establish conditions which are detrimental to the development of class 2 cells in ways that deprivation does not.

In addition to these effects of squint observed in class 2 cells, some evidence suggests that binocular competition also contributes to the reduction in soma size in squint reared animals. In animals reared with squint alone, the ANOVA revealed that the main effects of squint (i.e., class 1 and class 2 cells combined) yielded a significant decrease in LGN soma size relative to non-deprived controls. Transection of the optic chiasm abolished this statistically significant reduction in soma size, thus suggesting the influence of binocularly

mediated competition. Because the analysis of class 2 cells suggested that these cells were not influenced by optic chiasm transection, it seems that class 1 cells may have been responsible for contributing to these results. Inspection of appendix C (pg. 232) reveals that, although not statistically different, squinted class 1 cells were smaller than class 1 cells from the non-deprived layer, thus suggesting that changes in class 1 cells also contribute to the main effects (i.e., class 1 and class 2 cell combined) of squint. Taken together, these results from the two squint preparations (i.e., squint alone and SQ-OX) suggest that the mechanisms which control somatic development in squinted class 1 cells may be sensitive to the effects of binocular competition, whereas the factors which guide squinted class 2 somatic development are controlled by other mechanisms.

Because of the limited sample size, however, replication of these results is essential in order for more solid conclusions to be drawn regarding the mechanisms which control class 1 and class 2 somatic development. The fact that these squint induced modifications are more pronounced among class 2 cells is somewhat disconcerting given the extensive empirical literature which suggest that perturbations of early experience are primarily noted among Y (class 1) relay cells in the LGN (MD: Friedlander et al., 1982; Sherman et al., 1972; Sherman et al., 1974; dark rearing: Kalil and Worden, 1978; Kratz, Sherman, and Kalil, 1979). However, in some ways this finding of a significant decrease in soma size which is limited primarily to class 2 LGN cells in animals reared with squint is consistent with certain features of strabismic amblyopia. For example,

behavioral testing suggests that the impairment in visual acuity and contrast sensitivity (Cleland, Crewther, Crewther, and Mitchell, 1982; Holopigian and Blake, 1983; Jacobson and Ikeda, 1979) associated with strabismus is typically revealed for the higher spatial frequencies, which is suggestive of a disruption in the X cell pathway (Holopigian and Blake, 1984). Moreover, many of the physiological effects of squint that have been noted both in the retina (Ikeda and Tremain, 1979; where binocular competition could not even occur) and in the LGN (Ikeda et al., 1978; Jones et al., 1984; Mower et al., 1982) indicate that squint yields its effects on the X cell pathway (but see Cleland et al., 1982; Crewther et al., 1985; Crewther and Crewther, 1988). Therefore, the pronounced decrease in the soma size for LGN class 2 cells noted here is consistent with certain amblyopic deficits others have noted in squint reared animals (i.e., at least to the extent that the class 2 cells in this sample correspond to LGN X cells).

The Role of Binocular Competition in Cats Reared With Squint: *Dendritic Development.*

***Qualitative Observations.*** In animals reared with squint, neither class 1 nor class 2 LGN relay cells exhibited tangled, convoluted dendrites like those qualitatively observed among class 1 relay cells in the deprived layers of MD reared animals. As in normally reared animals (Guillery, 1966; Friedlander et al., 1981), the dendrites of squinted class 2 cells possessed grape like clustered appendages located at or near dendritic branch points, and also displayed

dendrites oriented perpendicular to laminar borders. Qualitatively, dendritic processes of class 2 cells in the squinted layers appeared somewhat thinner than their non-deprived counterparts, although quantitative analysis of dendritic segment width did not support this claim. The dendrites of class 1 LGN cells from squinted laminae tended to depart from the soma in a radial fashion and possessed tiny spines located along their dendritic shafts. Moreover, dendritic segments of the squinted class 1 cells appeared relatively straight and free from the tangling noted among class 1 cells from the deprived layers of MD cats. Hence, these qualitative observations suggest that squint does not drastically alter the dendritic form of class 1 or class 2 LGN relay cells.

***Quantitative Effects of Squint on Cumulative Dendritic Length.*** Quantitative analysis of cumulative dendritic length for LGN cells revealed that the dendrites of cells in the squinted layers were significantly shorter those in the non-deprived layers. Unlike the results from the soma size analysis, however, the ANOVA failed to detect a significant cell type by visual rearing history interaction, thus suggesting that the effects of squint on cumulative dendritic length are present in both class 1 and class 2 LGN relay cells. As described in the MD discussion above, this difference between significant and non-significant cell type by visual rearing history interactions for soma size and cumulative dendritic length (respectively) suggests the possibility that the factors governing somatic and dendritic development may be different. Squint rearing, in any case, results in a non-selective reduction in dendritic length for LGN class 1 and class 2 relay cells.

By contrast, the data from animals reared with SQ-OX demonstrate that optic chiasm transection protects the dendrites LGN cells from the detrimental effects of squint. This conclusion is drawn from the observation that dendrites of cells from the squinted layers of chiasm sectioned animals (i.e., SQ-OX) were not significantly shorter than cells in the non-deprived layer; whereas in animals reared with squint (alone) the dendrites of squinted cells were significantly shorter than cells in the non-deprived layer. Therefore, it is clear that cumulative dendritic length is controlled by mechanisms that involve binocular competition for two related reasons: (1) chiasm transection eliminates the possibility for binocularly mediated competition between geniculocortical afferents in cortex; (2) squinted LGN relay cells in animals reared with SQ-OX were protected from the detrimental effects of squint because of the elimination of these binocularly mediated competitive interactions between geniculocortical afferents.

***Factors Involved in the Squint Induced Modifications of Cumulative Dendritic Length.*** As in MD, further analysis revealed that the effects of squint on cumulative dendritic length were due to a decrease in the length of individual dendritic arbors among cells in the squinted layers (see results on average length per dendrite above), but were not due to changes in the number of dendrites. Moreover, this decrease in dendrite length for cells in the squinted layers is attributed to reductions in the amount of dendritic branching, but is not related to changes in the length of individual dendritic segments.

**In animals reared with SQ-OX,** squint did **not** significantly decrease either the length of dendrite arbors or the number of dendritic branch points. Because chiasm section eliminates competitive interactions among squinted and non-deprived LGN arbors in cortex, these observations regarding dendrite length and the amount of dendrite branching indicate that chiasm section also appears to protect LGN dendrites from squint induced reductions in dendrite length and dendritic branching. Taken together, these data from squint and SQ-OX reared animals indicate that **binocular competition** is an important mechanism for controlling these features of morphological development among LGN cells.

Finally, it is important to point out that the decrease in dendrite length was not attributable to modifications in the length of the individual dendritic segments. Therefore, as in MD, squint **does not** decrease the cumulative dendritic length by decreasing segment length, rather it does so by diminishing the number of dendritic segments present in the cell's dendritic network. As described above, these modifications in dendritic length and branching noted among LGN relay cells in both squint and MD reared animals have important functional implications for the performance of these cells in visual processing. Moreover, it is interesting to note that the effects of squint and MD on dendrite morphology appear to be very similar, thus, suggesting the possibility of a common mechanism operating on dendrites in both cases. This idea of a common developmental mechanism is further supported by the fact that chiasm

transection protects LGN cell dendrites from the detrimental effects of both squint and MD.

***Effects of Squint on Dendritic Surface Area.*** Squint also appears to decrease the cumulative dendritic surface area of LGN neurons. As described in the discussion of MD above, differences in dendritic surface area are important because they reveal possible differences in the potential for synaptic space and signal processing characteristics of LGN neurons (Bloomfield et al., 1987). This reduction in dendritic surface area for cells in the squinted layer is consistent with findings from cells in the deprived layers of MD animals. This finding is somewhat puzzling in that, unlike MD, previous research has failed to demonstrate that squint causes the kinds of profound changes in the physiological responsiveness of LGN neurons that are noted with MD (Jones et al., 1984; Mower et al. 1982). Other than reductions in the visual resolution (Ikeda and Wright, 1976; Jones et al., 1984; Tremain and Ikeda, 1982) and contrast sensitivity (Ikeda, Tremain and Einon, 1978; Ikeda and Wright, 1976; Tremain and Ikeda, 1982) among LGN X cells, there is little evidence that squint causes a decrease in the responsiveness of LGN neurons.

This lack of a powerful physiological correlate in squint seems even more odd in light of recent observations which show that retinal Y (Garraghty et al., 1989) and X (Roe, et al., 1990) axon arbors are affected by rearing animals with squint. In MD reared animals, similar findings on Y and X retinal arbors have been used to explain reductions in the recordability of LGN Y cells (see Sherman,

1985) and the presence of class 1, X cells (Friedlander et al., 1982). Therefore, because squint and MD appears to cause similar morphological modifications both in retinal (Garraghty et al., 1989; Roe et al., 1990) and in LGN cells (present data) which do not appear to have direct physiological correlates, these results suggest that other factors in addition to reductions in surface area must be involved in producing the physiological differences between squint and MD rearing.

In squint, unlike MD, the reduction in the cumulative dendritic surface area of squinted cells appears to result from binocular competition. This conclusion in based on the observation that chiasm transection appears to protect LGN neurons from the detrimental affects of squint (i.e., in SQ-OX animals squint and non-deprived LGN cells are not statistically different from each other). Because chiasm transection eliminates the possibility for binocularly mediated competition between geniculocortical afferents in cortex, and because cells in the squinted layer of animals reared with SQ-OX (but not in squint alone) are not statistically different from cells in the non-deprived layer, these observations suggest that the reductions in cumulative dendritic surface area in squinted animals is controlled *via* mechanisms that involve binocular competition. It is interesting to note that the reductions in cumulative dendritic surface area produced with squint appears to arise as a result of binocular competition, whereas the reductions in cumulative dendritic surface area in animals reared with MD do not (see above). This difference between squint and MD suggest the

possibility that the reductions in dendritic surface area are caused by different developmental mechanisms, one which relies on binocular competition (i.e., squint) and the other which does not (i.e., MD).

***Effects of Squint on Sholl Ring Length and Surface Area.*** In animals reared with an intact optic chiasm, squint causes a reduction in the relative proportion of dendritic length and dendritic surface area located at distances > 100 $\mu$ from the center of the cell. As with MD, squint induced modifications in Sholl ring indices were due to the fact that their dendritic length were simply impoverished relative to dendrites in the non-deprived layers. The mean dendritic length and mean dendritic surface area for the first two Sholl rings (< 100 $\mu$) indicated that cells in the squinted layers had less dendritic length and surface area than did cells in the non-deprived layers. Thus, these results suggest that squint produces its effects on radial dendritic extension by merely decreasing the total length of the dendritic tree.

In animals reared with SQ-OX, Sholl ring indices for LGN cells in the squinted layers were not different from those obtained from cells in the non-deprived layers. This finding from SQ-OX animals suggest that binocular competition is likely to be responsible for the reduction in radial dendritic extension observe in animals reared with squint (alone).

***Are Cells in the Unsquinted Layers Also Affected by Squint Rearing?*** One final point worth commenting on here deals with previous claims from the physiological (Chino, Shansky, Jankowski and Banser,1983), anatomical

(Garraghty et al., 1985; 1989), and behavioral (Holopigian and Blake, 1983; 1984) literature which indicate the visual pathway of the unsquinted eye is also affected by rearing animals with either squint or squint-like (see Garraghty et al., 1985) preparations. In the present sample, there was no evidence to the support these claims either for soma size or on any of the dendritic attributes studied (see above). This conclusion is based on two sets of observations. First, the cells in laminae innervated by the non-deviating eye in squinted animals did not differ from that of cells innervated by the non-deprived eye in animals reared with MD. Secondly, cells in the squinted layers typically yielded statistically significant differences from those of cells found in the unsquinted layers. An alternative to concluding from these observations that there is no effect of squint on the non-squinted layer is to recognize that, if such effects occur, they are substantially smaller and less reliable than that seen in cells from the squinted layer.

Comparisons Between the Effects of MD and Squint on LGN Cell Development.

*Somatic Development.* Rearing animals with MD and squint retard the somatic development of class 1 and class 2 cell populations in the LGN differently. These differences in the effects of MD and squint on somatic development suggest that, to some degree, different developmental mechanisms in these rearing histories may have been responsible for producing the observed reductions in cell body size. MD results in a significant decrease in the soma size of class 1, but not class 2 LGN relay cells. Surgical transection of the optic

chiasm blocks the deleterious effects of MD on class 1 cells, thereby indicating that the decreased soma size of class 1 cells in animals reared with MD alone is caused by the effects of binocular competition.

In contrast, results from strabismic cats suggest that two factors (one insensitive and another sensitive to binocular competition) may have contributed to the disruption of somatic development in squinted animals. Analysis of class 2 cells demonstrated that squint resulted in a retarded growth in class 2 cell somatic development which was unaffected by chiasm transection, thus suggesting that this residual effect did not arise from binocular competition. This residual effect of squint is important in that it emphasizes that cell development in class 2 LGN cells is differentially sensitive to the monocular effects of squint in ways which are not apparent in MD, thereby pointing to differences in the mechanisms by which squint and MD induce their effects on somatic development. In animals reared with an intact optic chiasm, statistical analysis of both **squinted class 1 and class 2** LGN cells **combined** resulted in a **significant** reduction in their soma size relative to cells in the non-deprived layer. Furthermore, transection of the optic chiasm, in part, eliminated the reduction in soma size for LGN cells as revealed by a **non-significant** difference in the soma size of cells in the squinted and non-deprived layers of **chiasm sectioned** animals. These results suggest that binocular competition contributed to the effects of squint, possibly by **releasing** class 1 cells (whose somata in the squinted layers,

although not statistically different, were smaller than class 1 cells in the non-deprived layer, see appendix C pg. 232) from the detrimental effects of squint.

***Factors which Establish a Differential Sensitivity of Class 1 and Class 2 Cells to Binocular Competition.*** Support for the notion that class 1 and class 2 cells are differentially sensitive to the effects of binocular competition has been proposed by Sherman and Spear (1982). They point out that, in normal animals, LGN Y cells (i.e., class 1) are more sensitive to the effects of binocular competition because their terminal arbors innervate several different ocular dominance columns in the visual cortex. The projection patterns of LGN X (i.e., class 2) cells, on the other hand, are limited to only one ocular dominance column in cortex (Bullier and Henry, 1979; Ferster and LeVay, 1978; Gilbert and Wiesel, 1979). These anatomical findings regarding geniculocortical connectivity allow one to speculate that the reductions in soma size of class 1 cells in MD reared animals results from the inability of deprived class 1 cells to compete with non-deprived class 1 cells for synaptic connections in the visual cortex. Moreover, the fact that LGN X (i.e., class 2) cells do not maintain large dendritic fields in the visual cortex would allow one to predict, as our data and others (see Friedlander et al., 1982) bear out, that X (i.e., class 2) cell somatic development is relatively insensitive to binocularly competitive processes.

For a detailed description of the possible mechanisms responsible for the squint induced changes in class 2 somata see section entitled: *Effects of MD and/*

*or squint which survived chiasm transection: i. Reduction in soma size for squinted class 2 somata in the LGN.*

***Dendritic Development.*** The present results in both MD and squint reared animals also reveal that dendritic development is highly sensitive to the influence of binocular competition. Cells from the deprived and squinted layers both demonstrated significant modifications in their dendritic morphology which chiasm transection was able to block. It is important to note that the effects of deprivation and squint reported above (e.g., reductions in cumulative dendritic length, decreases in number of dendritic branches) probably result from atrophy or a retraction of dendrites rather than from a failure in the growth process. This follows from Mason's (1983) observations which demonstrate that LGN dendritic morphology is more or less established in both form and extent by the end of the fourth week postnatal. Therefore, because the visual perturbations induced in this study were performed at postnatal day 28 (i.e., after dendritic growth is largely finished), the effects described above underscore the importance of normal visual experience for the **maintenance** of normal LGN dendrites.

Furthermore, it is important to note that the effects of deprivation and squint on LGN dendrites **are not**, for the most part, cell class-specific. The effects of deprivation and squint on LGN cell dendrites differ in this respect from those described for LGN somata. It is important to note however, that, except for the decrease in somatic size in squinted class 2 cells, the majority of the effects of

MD and squint on somatic and dendritic development are caused by mechanisms which involve binocular competition. Hence, the lack of correspondence between the class specific effects of MD and squint on somatic development and the apparent non-class specific effects of MD and squint on dendritic development suggests that binocular competition may yield its effects on LGN somata and dendrites in a manner which is dissociable. Previous results from normally reared animals demonstrate that the somatic development of LGN cells typically reflects a protracted postnatal development which can last until the $140^{th}$ day postnatal. Dendritic development, on the other hand, is relatively complete by the $4^{th}$ postnatal week (Mason, 1983). Thus, these differences imply that the effects on LGN somata of our manipulations result from an interruption in growth, whereas the effects on LGN dendrites result from atrophy or dendritic retraction. Hence, differences in the temporal sequence of somatic and dendritic development and the corresponding differences in atrophy or interrupted growth may offer a possible explanation for the way in which binocular competition induces cell class specific effects on LGN somata but not on LGN dendrites (see discussion of developmental mechanisms below for details).

Finally, it is important to note that the dendrite development in MD and squint reared animals did differ on some of the morphological parameters measured. These differences are important because may reflect differences in the stimulus conditions which control the morphological development of these

various dendritic attributes. One difference noted between MD and squinted LGN cells is that the dendrites of some class 1 cells from the deprived layers of MD reared animals exhibited sinuous and tangled dendritic processes. These same features were not observed in the dendritic trees of deprived cells from chiasm sectioned animals nor were they observed among cells in the squinted layers of strabismic reared animals. Hence, it appears that the factors which contributed to these pathologies in class 1 cell dendrites were sensitive to the effects of binocular competition, and were unique to the stimulus perturbations induced by monocular eye lid suture. Second, the effects of deprivation on cumulative dendritic surface area did not appear to be sensitive to the effects of binocular competition in animals reared with MD. However, cumulative dendritic surface area was shown to be sensitive to the effects of binocular competition in animals reared with squint. It is possible that differences between the effects of squint and MD on dendritic surface area may have arisen as a result of differences in the stimulus modifications introduced by these two manipulations. That is, squint results in defocused (Ikeda and Tremain, 1982) and uncorrelated neural activity between the two eyes, whereas, MD introduces an imbalance in the amount of neural activity generated by the two eyes. Hence, differences in the stimulus perturbations may explain why these two manipulations differentially effect dendritic surface area in LGN neurons. These differences parallel findings in somatic development (i.e., retarded growth of class 2 somata in squint, but not in MD) which indicate that some of the

developmental processes operating in squint differ from those observed in MD. For a more detailed description of the factors which may be responsible for inducing these differences in squint and MD see the discussion on developmental mechanisms below.

Effects of Denervation on LGN Relay Cell Development.

**Somatic Development.** The most detrimental developmental scenario described for LGN cells in the present study was that of retinal deafferentation, which was produced by optic chiasm transection for cells in the A layer. Cells from the denervated A layers of the LGN were significantly smaller than cells obtained from the non-deprived laminae. Because the ANOVA yielded a statistically significant cell type by rearing history interaction for soma size, assessment of the effects of denervation on class 2 cells was permitted. LGN class 1 cells could not be evaluated statistically because only one class 1 cell was obtained from the denervated layers. It is worth noting, however, that the soma of this denervated class 1 cell was much smaller than the average soma size for non-deprived class 1 cells. Statistical analysis revealed that denervated class 2 LGN relay cells were significantly smaller than their counterparts from the non-deprived laminae. Taken together, these findings support the thesis that retinal innervation is quite important for the normal somatic development of LGN cells (Guillery, 1973b; Kalil, 1980; Kupfer and Palmer, 1964).

The results presented here are consistent with previous findings from enucleation studies which have demonstrated that LGN somata are severely

shrunken by removal of their retinal afferents (Guillery, 1973b; Kalil, 1980; Kupfer and Palmer, 1964). In characterizing the retardation of somatic growth in LGN somata, Kalil (1980) discovered that the retinal deafferentation performed at 4 weeks postnatal halts the growth of deafferented cells immediately. Aside from a minor atrophy occurring one day postoperatively, the majority of the cell size changes that develop during the first month after enucleation are attributable entirely to the premature cessation of growth. Furthermore, Kalil (1980) describes a second reduction in denervated LGN cells which occurs at roughly 8 weeks postnatal, when LGN cells should be completing the bulk of their normal growth (Kalil, 1978). This second period of soma size reduction is attributed to atrophy and involves a precipitous shrinkage of LGN somata that occurs over the third postnatal month.

***Dendritic Development.*** In addition to the detrimental effects of denervation on somatic development, the dendrites of denervated LGN relay cells were also drastically affected by retinal deafferentation. Statistical analysis revealed that the dendrites of denervated LGN cells were impoverished relative to the non-deprived cell dendrites on every dependent measure that was assessed. The effects of denervation on LGN cell dendrites (i.e., based on comparisons to dendrites of non-deprived cells) include: 1) shorter dendrites (as assessed by cumulative dendritic length and average length per dendrite); 2) fewer dendritic branches and dendritic segments; 3) lower estimates of cumulative dendritic surface area; and 4) a shorter radial extension of their dendritic arbors (as

measured by Sholl ring procedures). In addition, the dendritic fields of class 2 cells exhibited orientations **atypical** for normal class 2 cells (i.e., many of their dendritic arbors were oriented parallel to laminar borders).

These findings regarding the effects of retinal deafferentation on LGN dendrites are particularly interesting in light of Mason's (1983) scenario for normal dendritic development in the LGN. According to her account, the majority of dendritic growth is complete by the fourth week after birth and that changes occurring during the second postnatal month are merely modifications in form (e.g., formation of spines and subsequent pruning, molding of grape like clusters) rather than extension of length. In as much as the surgical transection of the optic chiasm was not performed until the end of the fourth postnatal week in the present study, it would appear that the effects of denervation on LGN cell dendrites observed in the present study are due at least to atrophy of the dendritic processes rather than in a failure of growth. Furthermore, in some instances remodelling is so extensive that it is possible that more than atrophy is occurring (see section below).

These results from cells in the denervated layers suggest a very important role of retinally mediated activity in the development and/or maintenance of central neural connections in the visual system. Furthermore, the importance of retinal afferents to LGN cell development is supported by an emerging literature which demonstrates that pharmacological blockade of retinal activity (i.e., using tetrodotoxin, TTX) can disrupt many features of geniculate organization,

including: the maturation of retinogeniculate synapses (see Kalil and Dubin, 1988), the growth and maintenance of LGN somata (Kuppermann and Kasamatsu, 1983); dendritic differentiation (Hsiao, Dubin, and Kalil, 1987); and the establishment of normal geniculocortical connections (Stryker and Harris, 1986). These findings are particularly interesting in that they suggest such an important role of retinal input for LGN organization despite the fact that retinal synapses account for only 10% of the total synaptic input to the LGN (Sherman and Koch, 1986). Taken together, these two bodies of literature (i.e., retinal deafferentation and pharmacological activity blockers) underscore a paradox in geniculate cell development, namely that such a small proportion of the LGN's synaptic neuropil which derives from retina could be so crucial for the structure and function of LGN relay cells and their development.

The Role of Retinal Afferents in the Dendritic Orientation of LGN Class 2 Relay Cells.

One unexpected finding from the present study was that optic chiasm transection causes modifications in the dendritic orientation of LGN class 2 neurons. Non-vertically oriented dendritic trees were observed in a relatively high proportion of cells obtained from the A (60%) and A1 (45%) layers in cats reared with optic chiasm transection. By contrast, in animals reared with intact optic chiasms, every class 2 LGN relay cell possessed vertically oriented dendritic arbors. In addition, these modifications in dendrite orientation for chiasm sectioned animals did not appear to be related to the LGN cell's deprivation

history (i.e., MD-OX, SQ-OX, or Non-deprived-OX). Moreover, there were no other features of the dendritic morphology among cells in the A1 layers which appeared to correlated with these disruptions of dendrite morphology. That is, class 2 relay cells in the A1 layer often maintained elaborate dendritic processes, while also exhibiting abnormal dendritic orientations. As pointed out above, however, the dendrites of cells in the A laminae of chiasm sectioned animals were retinally deafferented, therefore the effect of chiasm transection on dendrite orientation in A layer cells is inextricably confounded by the devastating effects of denervation. Finally, it is important to note that the inappropriately oriented class 2 cells in chiasm sectioned animals were not typically located near laminar borders and were clearly within either the A or A1 layers. Therefore, it is unlikely that these cells were either interlaminar cells or class 4, relay cells from the C laminae, both of which have been shown to possess dendritic arbors elongated parallel to laminar boundaries (Guillery, 1966, Friedlander et al., 1981; Stanford et al., 1983). Taken together, these results indicate that the disruption in dendritic orientation for some class 2 relay cells in the LGN is caused by optic chiasm transection, and does not result from either experimentally induced deprivations (i.e., MD or squint) or misclassification of LGN cell types.

These findings are puzzling in that they suggest that the normal retinal innervation of the A laminae of the LGN is necessary for the maintenance of normal class 2 cell dendritic orientation. Developmentally, retinogeniculate

fibers enter the LGN at embryonic day 37 (Shatz, 1983) in the cat, and are segregated into their appropriate LGN lamina by birth (Sretevan and Shatz, 1986). The retinal fibers enter the ventral (C) laminae of the LGN on the lateral margin of the nucleus and course their way medially in the optic tract (Sanderson, 1971). When they reach their appropriate targets they exit from the optic tract and project dorsally into their respective termination zones in the LGN. Fibers which project into the A laminae pass through the A1 layer in a columnar fashion oriented more or less perpendicular to the laminar borders of the LGN (Bowling and Michael, 1980; 1984; Friedlander et al., 1983; 1985; Sur and Sherman, 1981; Sur, Esguerra, Garraghty, Kritzer, Sherman, 1987). At the time in which the optic chiasm was transected (i.e., 28 days postnatal) in this study, the retinogeniculate fibers have reached their appropriate termination zones, they exhibit adult-terminals (i.e., with respect to size, shape and arrangement) and are beginning a period of synapse maturation in which glial cells form glomerular arrangements with retinal axons and LGN appendages (Mason, 1982a; 1982b). In addition, by the fourth postnatal week the LGN cell dendrites of both class 1 and class 2 relay cells exhibit their respective mature orientations (Friedlander, 1982; Mason, 1983). Therefore, it seems that the anomalies in dendritic orientation noted among some class 2 LGN neurons must result from a remodeling phenomenon since LGN cell dendrites exhibit relatively mature profiles at the time in which the optic chiasm surgery was performed.

The present results regarding dendritic remodeling suggest that retinal afferents to the A layer serve two roles regarding dendritic orientation of class 2 relay cells in the LGN. First, it appears that these A layer projecting retinal axons play an important role in maintaining the orientation of the dendritic processes of class 2 cells in the A layer of the LGN. It is possible that these results may be accounted for by the elimination of retinally driven afferent activity. As pointed out above (see discussion of denervation), retinally mediated activity is important for maintaining the morphological viability of LGN somata and dendrites. Hence, the modifications of dendrite orientation in the denervated A layers may possibly reflect this loss of retinally mediated activity. Secondly, it appears that retinal afferents which project to the A layers of the LGN also play an important role in maintaining the appropriate orientation of class 2 neurons in the A1 layer. This finding is somewhat surprising in that synaptic linkages between retinal axons projecting to the A lamina are not formed with A1 cell dendrites. Thus, this observation implies that changes in dendritic orientation for cells in the A1 layer can not be accounted for by activity-driven processes directly (although it may be that this modification of dendritic orientation for the A1 layer class 2 cells may be introduced by some indirect means resulting from a lack of activity occurring in the adjacent A lamina).

It should be pointed out that the modifications in class 2 cell morphology occur after the elongation of the dendritic processes in the vertical (i.e.

perpendicular to laminar borders) axis has already taken place (Friedlander, 1982; Friedlander, 1984; Mason, 1983), hence these changes in dendrite orientation must reflect a remodeling of the dendritic tree. There are two basic ways by which chiasm transection might produce this remodeling of dendritic orientation for class 2 cells from the A1 lamina. The first way would involve a wholesale remodeling of the dendritic tree which would entail a retraction of vertically oriented dendrites followed by a subsequent regrowth of dendrites in the inappropriate orientations. Montague and Friedlander (1991) have recently demonstrated that denuded (i.e., cells which have experimentally had their dendrites removed) retinal ganglion cell's can be induced to regrow their dendrites in cell culture. Their findings indicate, therefore, that remodeling of a cell's dendritic tree in young but differentiated cells is possible under the ideal conditions provided by the cell culture media (e.g., presence of developing neurites). One would think, however, that such a remodeling process would require a rather profound modification in the plasticity of the developing system in order for such a significant change in dendrite orientation to be observed.

The second possibility for explaining the orientation shifts in dendrites from class 2 cells in the A1 layer is that chiasm transection induces a rotation of the cell within the nucleus. In this regard one might speculate that dendrites of class 2 LGN cells use the columnar organization of the retinal afferents projecting to the A layer as a guide for orienting their dendritic processes perpendicular to laminar borders. Therefore, when optic chiasm transection is performed this

would cause a loss of the retinal guides, thereby, resulting in a relatively passive modification in dendritic orientation of cells in the A1 layer. It is recognized that the explanations provided for the observed changes in dendritic orientation are speculative, however, the phenomena which were observed here are quite interesting in that they suggest unexplored features of LGN cell development and plasticity which, warrant further experimental investigation.

Possible Developmental Mechanisms Underlying LGN Cell Development.

*Dimensions of Binocular Competition: Balanced Binocular Input, Correlation in Binocular Activity, and Their Impact on LGN Cell Development.* The experimental account of binocular competition described above is not developmental by nature, meaning that it does not afford a detailed assessment of the mechanisms by which the development of central visual pathways is controlled. Competition, as an interpretive structure, is recognized if a predetermined set of experimental outcomes is achieved. In the case of binocular competition, it is thought that the development of ocular dominance columns in layer IV of the striate visual cortex reflects the outcome of an activity-dependent competitive process, whereby geniculocortical projections receiving their inputs from either the right or left eyes compete for synaptic connections with cortical cells. Hence, the formation of regularly spaced, repeating patches of ocular representation (i.e., ocular dominance columns) is believed to indicate an epigenetic process resulting from two important features of visually driven activity in early development: (1) the relative amount of activity from each eye is

believed important for determining the degree of cortical representation of a given eye; (2) the sculpting of visual cortex into gradually changing columns of ocular dominance linked patches of binocularly sensitive cells is thought to result from correlated neural activity produced by the two eyes converging on similar visual targets; thus the cortical maps of ocular representation that are formed are believed to derive from this correlated retinally driven activity. Therefore, correlated binocular input of roughly equal proportions is necessary for the development of symmetrical ocular dominance stripes in visual cortex.

Monocular deprivation causes a binocular imbalance in the retinally driven activity, such that the activity of the sutured eye is depressed and less correlated with the activity produced by the non-deprived eye. The physiological signatures of this imbalance in early visual input are a reduction in the number of cells responding to the deprived eye, a corresponding increase in number of cells responsive to the nondeprived eye, and a pronounced decrease in the number of binocularly responsive units (Kratz, Spear, and Smith, 1976; Singer, 1977; Wiesel and Hubel, 1963; 1965; Wilson and Sherman, 1977). Injections of anterograde tracers into the deprived eye reveal thinner than normal ocular dominance bands, whereas injections into the non-deprived eye result in thicker than normal bands of the ocular dominance (Hubel, Wiesel, and Stryker, 1978; Shatz and Stryker, 1978; Shatz et al., 1977). Moreover, recent evidence by Friedlander et al. (1991) have demonstrated that the arbors of deprived geniculate cells are smaller and possess far fewer synaptic boutons than do the

arbors of non-deprived cells. Finally, it is widely believed (for review see Guillery, 1988; Sherman and Spear, 1982) that the changes in geniculocortical connectivity reported above are responsible for inducing many of the morphological changes in LGN cells noted in the deprived layers of MD animals.

Squint, as compared to MD, causes a disturbance in geniculocortical connectivity by merely producing uncorrelated activity between the two eyes. In squint the retinal activity of both eyes is roughly the same, however, the degree of correlation in activity of the two eyes is compromised by misalignment of the ocular axes. Physiological recordings of cortical cells in squinted animals reveal a roughly balanced proportion of cells that are sensitive to the squinted and unsquinted eyes, although few cortical cells respond to binocular stimulation (Blakemore and Van Sluyters, 1974; Hubel and Wiesel, 1965; Mower et al., 1982). Anatomical studies have demonstrated that squint produces roughly equal representations for the two eyes in visual cortex, however, the borders between ocular dominance bands appear sharper than normal (Kalil, 1982; Shatz et al., 1977). Taken together, these physiological and anatomical findings indicate that strabismus induces a severe disruption in cortical binocularity. Hubel and Wiesel (1965) first described the abnormal binocular interactions in strabismic animals as a process which entails competition without advantage. They postulate that uncorrelated activity between the two eyes is likely to result in a symmetrical retraction of geniculocortical afferents from cortical regions where significant binocular interactions might occur (i.e., at the marginal

portions of the ocular dominance bands). Hence, this model suggests that disruptions in geniculate cell development may result as a by-product of axonal retraction occurring in the geniculocortical arbors of LGN relay cells (Tremain et al., 1982).

In chiasm sectioned animals, whether squint or MD these binocular competitive processes between geniculocortical afferents are eliminated. Therefore, residual effects of either MD or squint in chiasm sectioned animals could be accounted for by four possible explanations: (1) they could reflect binocular interactions introduced by callosal projections in the visual cortex (i.e., callosal projections from the non-deprived cortex may cause abnormal binocularly mediated competition with deprived geniculocortical afferents, thereby resulting in modifications of LGN cell morphology) (see Cynader, Lepore and Guillemot, 1981); (2) they may reflect non-binocular competitive interactions between X and Y retinal axon terminals (Garraghty et al., 1986; Sherman, 1985); (3) they may reflect the contribution of non-competitive processes (i.e. the effects of deprivation or squint *per se*); (4) they may indicate the contribution of non-visual competitive interactions arising from mismatches in binocular proprioception (i.e., in squints and possibly in MD animals proprioceptive signals regarding differences in eye position for the two eyes may by some indirect route modify the development of cells in the LGN). Thus, for those effects of deprivation or squint which survived optic chiasm section (see below) further experiments are warranted.

***Effects MD and/or Squint which Survived Chiasm Transection:***

***i. Reduction in Soma Size for Squinted Class 2 Somata in the LGN.*** One of the squint induced effects which could not be reduced for by elimination of binocular competition between geniculocortical fibers is the reduction in soma size of squinted class 2 cell somata. It is unlikely, however, that binocular competition between callosal and squinted geniculocortical terminals could account for the smaller class 2 cell somata for the following reasons. In cats, morphological changes in the extent of connections made by callosal neurons occur during the early phase (2-4 weeks postnatal) of the critical period for visual development (Elberger and Smith, 1985). At birth the corpus callosum interconnects the primary visual cortical regions of the two hemispheres corresponding to all portions of the visual field. By the end of the second postnatal week the projections to cortical regions representing the peripheral visual field have decreased, and by the end of the first postnatal month the adult pattern of projection emerges with almost no callosal connections outside of the cortical regions representing central visual fields (0°-5°: area 17; 0°-10° area 18) (Innocenti, 1978; Segraves and Rosenquist, 1982). The organization of geniculocortical projections into columns of ocular dominance has been shown to occur between postnatal weeks 2-6 (LeVay et al., 1978; Van Sluyters et al., 1990), thereby indicating that the period of overlap in callosal and geniculocortical pathway development occurs between postnatal weeks 2-4.

Binocular competition between callosal projections and squinted geniculocortical afferents could not account for the observed reductions in squinted class 2 somata in the LGN for two reasons. First, squint was not induced until after the development of callosal pathway was complete. Therefore, unless squint extends the period of plasticity for callosal connections, competitive interactions between geniculocortical and callosal projections cannot account for the observed changes. Second, in normally reared animals, callosal projections between cortical hemispheres are restricted to central visual fields (Choudhury, Whitteridge and Wilson, 1965; Segraves and Rosenquist, 1982; Shatz, 1977). The present sample of squinted LGN neurons, however, were not restricted to regions of the LGN which represent central visual fields. Hence, unless squint induces an expansion of the non-deprived callosal projection after its normal development is complete, it is unlikely that the present population of squinted cells from this sample would have undergone competition with callosally projecting fibers for space in the visual cortex. Thus, the decreased growth of squinted class 2 somata cannot be attributed to this form of binocular competition.

Moreover, it is unlikely that non-binocular competition between X and Y retinal terminals could account for the observed effects on squinted class 2 cells in the LGN. Garraghty et al. (1989) reported that Y axons from both the squinted and non-deviating eyes of strabismic cats possess impoverished terminal arbors with fewer boutons than normal Y retinal axons. Later, reports by Roe et

al. (1990) indicate that centrally projecting (i.e., 0°-5°) X arbors from either the squinted or non-deviating retinae possess larger than normal terminal fields in the LGN. Taken together, these findings and their sequelae in the MD literature have been interpreted to reflect the operation of a non-binocular competitive processes thought to control many features of LGN cell development (see Sherman, 1985). This mechanism does not, however, explain the present findings since it is mainly used to describe disruptions in the development of Y (i.e., class 1), but not X (i.e., class 2), cells in the LGN.

The third mechanism which does not involve binocular competition between geniculocortical afferents is that of the noncompetitive effects of squint *per se*. Ikeda and Wright (1974) have previously suggested that squint induces accommodation errors which result in defocused retinal images. Since X (i.e., class 2) cells are noted for their sensitivity to high spatial frequencies (see Blake, 1979; Lennie, 1980), it seems likely that their development might be compromised by defocused retinal images in ways which would not affect LGN Y cells which are more sensitive to low spatial frequencies. Ikeda (Ikeda and Wright, 1975; 1976; Ikeda et al., 1978) and others (Holopigian and Blake, 1983; Jones, et al., 1984; Mower et al., 1982, but see Crewther, et al. 1988) have noted that the acuity of squinted LGN X cells is significantly decreased. Moreover, Ikeda and Tremain (1979) have reported similar results for retinal X cells (but see Crewther et al., 1985). Hence, Ikeda and colleagues have speculated (1978) that a defocused image may be responsible for selective

decrease in somatic growth, which may account for small class 2 cells in the squinted layers of the LGN.

There is, however, a problem with Ikeda and colleagues' theory because it is not clear how the brain is able to identify which retinal image is provided by the misaligned eye. Accommodation (i.e. lens refraction) and vergence (i.e., positional eye movement) are visual operations thought to be controlled by a neural mechanism which computes the binocular disparities in fused retinal images in order to appropriately regulate the amount of refraction and ocular convergence needed for focusing on objects in depth (Duke-Elder, 1969; Miles, 1985). Since squint and SQOX reduce binocularity in the visual cortex, this eliminates the opportunity for an assessment of binocular disparities. Thus, it unclear why accommodation errors would be observed only for the deviated eye. Moreover, it is not clear how the brain of squinted animals is able to determine which of the two eyes is deviating simply on the basis of visual information derived from the two retinae.

The last possibility to be addressed for determining the disruptions in class 2 somatic development for squinted animals is the possibility that non-visual (i.e., proprioceptive) signals regarding ocular misalignment are mediating the effects. Work in adult cats has demonstrated that the activity of LGN cells is affected by both static (Lal and Friedlander, 1990a) and dynamic (Donaldson and Dixon, 1980; Lal and Friedlander, 1990b) shifts in eye position. The source of inflowing non-visual afferent signals regarding eye position has been traced to

proprioceptors in the extraocular muscles (Batini, 1979) whose sensory afferents enter the brain along the ophthalmic branch of the trigeminal nerve (Alvarado-Mallart et al., 1975; Batini et al., 1975; Porter and Spencer, 1982). Moreover, work from this lab has shown that the encounter rate for LGN X (i.e., class 2) cells can be decreased by chronic monocular paralysis (ChMP) in adult cats (Brown and Salinger, 1975; Garraghty et al., 1982; Salinger et al., 1977), and this decrease can be reversed by unilateral transection of the trigeminal nerve (Guido et al., 1988). Therefore the observations from this lab indicate that aberrant proprioceptive signals caused by ChMP contribute to the decrease in X cell encounter rates.

The route by which proprioceptive signals influence LGN cell activity is unclear. However, results from this lab (Moore et al., in prep.) have demonstrated that either reversible cryogenic blockade of the ipsilateral visual cortex or surgical lesions of the cerebellum are capable of reversing the suppression of LGN X cell encounter observed in ChMP animals. It is also not known when in development the proprioceptive influences on LGN cells can be manifested. In this regard, however, several investigators have demonstrated that the proportion of binocularly activated neurons in visual cortex of kittens is substantially lower than normal when proprioceptive afferents are interrupted by unilateral transection of the trigeminal nerve (Berardi et al., 1981; Maffei, 1979; Trotter et al., 1981).

The advantage in using ocular proprioception as a mechanism for explaining how the brain can distinguish the squinted from the nonsquinted eye is that the computational information required for this discrimination is present. That is, the oculomotor system simply needs to compare the difference in motor signal output for eye movements with the proprioceptive feed back provided by the extraretinal afferents (i.e., stretch receptors). The result of this neural computation would allow for a direct assessment of the oculomotor status of each eye. Such a system, therefore, could by some as yet unspecified circuit result in a differential arrest in the development of squinted class 2 somata for animals reared with squint or SQOX.

*ii. Dendritic Surface Area in Deprived LGN Cells.* The only other effect which survived chiasm transection was that of the deprivation-induced reduction in cumulative dendritic surface area. Assessment of the effects of binocular competition between non-deprived callosal and geniculocortical projections could not be directly addressed in the present experiment. However, as described above, such a mechanism is unlikely to account for reductions in cumulative dendritic surface area for two reasons. First, competitive interactions between geniculocortical and callosal fibers for cortical connections normally occur before the time deprivation was performed in the present experiment. Thus, unless MD induced a significant delay in the retraction of the callosal projection, it is not likely that deprived geniculocortical and callosal fibers competed for cortical targets. Second, one would predict that the consequence of deprivation should

reveal an eccentricity-specific effect on deprived LGN cells. The compilation of deprived layer cells in the present study was taken over a wide range of eccentricities and there was no detectable difference between centrally or peripherally located LGN neurons. Therefore, it would seem that binocularly mediated competition between callosal and deprived thalamic projections are not a likely explanation for the results obtained here.

It is possible that the deprivation induced reduction in cumulative dendritic surface area could, in part, be accounted for by non-binocular competitive interactions between X and Y retinal terminals in the LGN. Such a mechanism would predict that deprived Y (i.e. class 1), **but not X (i.e., class 2)**, cells should exhibit reductions in dendritic surface area. Inspection of the data suggests that the effect of deprivation on dendritic surface area appears more reliable among class 1 LGN cells than among class 2 cells (see appendix C pg. 255). This is suggested by the fact that the distribution of deprived and non-deprived class 1 cells are non-overlapping whereas considerable overlap exists for this measure of dendritic surface area in the class 2 cell distribution. One might speculate, therefore, that two mechanisms may be at work in producing the reductions in dendritic surface area, one which operates exclusively and more completely on LGN class 1 cells (i.e., possibly non-binocular competition between X and Y retinal terminals in the LGN), and another which variably effects class 2 LGN cells (unknown).

Third, it is possible that non-competitive factors (i.e., deprivation *per se*) could account for the deprivation induced decrease in dendritic surface area. Squint induced reductions in dendritic surface area, however, appear to be related to binocular competition. Differences between the stimulus modifications operating in MD and squint may be responsible for differentially controlling this morphological feature in LGN cells. One might hypothesize, for example, that a depression in retinally generated signals accompanying MD may result in a reduction in dendritic surface area for deprived LGN cells.

Finally, it is possible that non-visual (i.e., proprioceptive) signals could produce the reductions in dendritic surface area of deprived LGN cells. MD is thought to cause a misalignment in the deprived eye which could contribute by some indirect circuit (see subsection above) result in a differential modification in dendritic surface area for cell in the deprived lamina. It is not likely, however, that ocular misalignment could be used to explain this phenomenon given that similar effects were not observed in squinted animals.

***Effects of MD and Squint Which Involve Binocular Competition.***

***i. Differences Between the Effects of Competition on LGN Somata and Dendrites.*** The present study demonstrates that binocular competition plays a crucial role in the somatic and dendritic development of neurons in the LGN. Moreover, data from the present study indicate that binocular competition may operate differently on LGN somata than on LGN dendrites. That is, the effects of binocular competition on LGN somata appear to be focused on class 1 LGN cells,

whereas the effects on LGN dendrites are not class specific, with the exception of the sinuosities observed in class 1 cells of MD animals. Although it is possible that two mechanisms, both of which are sensitive to the effects of binocular competition, may be operating on LGN somata and dendrites differentially, it seems that a two-mechanism model is not required. A more plausible explanation is that the traditional notion of binocular competition between LGN axon terminals is responsible for mediating both the differential effects on both LGN somata and dendrites. In this regard there are three explanations which could account for the differential manner by which binocular competition operates.

First, it is possible that unsuccessful competitive interactions among deprived LGN axon terminals in cortex result in the failure of somatic growth which, in turn, subsequently produce changes in the dendrite structure of deprived LGN relay cells. This possibility is not likely, however, since the observed changes in MD reared animals suggest that only class 1 cell somata are affected by MD. Hence, this explanation could not account for the nonspecific effects of binocular competition on LGN dendrites (i.e., competition affects dendrites of both class 1 and 2 cells).

Secondly, temporal differences in somatic and dendritic development may account for differences in the effects of binocular competition in animals reared with either MD or squint. Previous studies have demonstrated that much of the somatic growth in LGN neurons (Kalil, 1978) happens after the bulk of dendritic

growth has occurred (Mason, 1983). In the present study, either MD or squint was performed on animals at a time (28 days postnatal) **during** which significant growth is occurring in LGN somata, and **after** which the majority of dendritic elongation has already taken place. These differences imply, therefore, that the effects of binocular competition on LGN somata result from an interruption in growth, whereas the effects on LGN dendrites result from atrophy or dendrite retraction. Hence, differences in the temporal sequence of somatic and dendritic development may offer a possible explanation for the way in which binocular competition induces cell class specific effects on LGN somata (*via* interrupted growth) but not on LGN dendrites (*via* atrophy or dendrite retraction).

Finally, it is possible that binocular competition works *via* a two stage process driven exclusively by competitive interactions between deprived and non-deprived geniculocortical afferents in the visual cortex. According to this possibility, interruption in the somatic development of deprived class 1 LGN cells could result as a secondary consequence of unsuccessful competition for the deprived axon terminals in the visual cortex. Whereas the retraction of LGN dendrites may result from a reduction in cortical binocularity which could influence LGN dendrites secondarily by functionally disconnecting the **corticogeniculate** pathway whose input into the LGN is not cell class specific (see second subsection below for details). This later explanation has the advantage of offering a method which explains the class specific effects of binocular competition on LGN somata while at the same time offering a mechanism by

which binocular competition could modify LGN dendrites in a manner that is not class specific. In addition, this later explanation is consistent with the temporal gradient for the somatic and dendritic development of LGN neurons reported above (i.e., see second explanation above). The following two sections explore this third explanation more thoroughly.

***ii. Binocular Competition: Class Specificity and the Relationship Between Terminal Retraction and the Reduction in Somatic Growth.*** The reduction in the soma size of deprived class 1 LGN cells of MD animals is related to the fact that class 1 terminal arbors are unable to effectively compete with the terminal arbors of their non-deprived counterparts in the visual cortex. Moreover, deprived class 1 cells are more affected by biased competition than deprived class 2 cells. Presumably, this differential sensitivity to binocular competition in deprived class 1 and class 2 cells is due to the fact that LGN class 1 arbors in the visual cortex are larger and, therefore, are forced to compete over a broader region of the visual cortex (Bullier et al., 1979; Ferster et al., 1978; Gilbert and Wiesel, 1979). Thus, the class specific effects of binocular competition on somatic development appear to be related to the amount of cortical space over which the competitive interactions are distributed.

Comparisons between the effects of MD and squint on the formation of ocular dominance columns support the idea that the reduction of somatic growth for deprived class 1 cells is determined, in part, by the degree of disadvantage in the competitive process. In MD, the retinally driven output from the deprived

eye is both depressed and uncorrelated with that coming from the non-deprived eye. This places LGN cells innervated by the deprived eye at a competitive disadvantage, thus, resulting in a retraction in their geniculocortical afferents (Friedlander et al., 1991; Hubel, Wiesel, and Stryker, 1978; Shatz and Stryker, 1978; Shatz et al., 1977). In squint there is **no** imbalance in the retinal output of the two eyes, although there is a decided lack of correlated activity between the two retinae. According to Hubel and Wiesel (1965) squint results in a form of binocular competition in which neither ocular projection possesses a competitive advantage and, therefore, results in a bilateral retraction of geniculocortical afferents from the binocular regions of the visual cortex. In the present study, **deprivation induced a significant reduction** in the cell body size of class 1 LGN cells which was dependent on binocularly competitive interactions (i.e., chiasm transection blocked the effects of MD on class 1 cells). In **strabismic** animals, squinted class 1 somata were, on average, smaller but not significantly different from non-deprived class 1 somata (see discussion on comparisons between MD and squint above). This difference between the magnitude of the effects of MD or squint on the development of class 1 somata indicates that the more profound imbalance in binocularity introduced by MD (i.e., asymmetrical and less correlated binocular input) caused a greater reduction in the soma size of class 1 cells than that produced by squint, which merely introduces uncorrelated binocular input. These findings are consistent with anatomical studies which show that deprivation induces a greater retraction of deprived

geniculocortical afferents than observed in the visual cortex of squinted animals. These observations lead one to speculate, therefore, that the degree of retardation in somatic growth for class 1 neurons is directly related to the relative competitive success of their terminal arbors in cortex, which itself is dependent on the degree to which retinal activity is balanced and correlated during early visual development.

***iii. Binocular Competition: Factors Which Could Mediate Dendrite Retraction.*** The observation that LGN cell **dendrites** are affected by binocular competition is somewhat surprising, since there is no clear-cut relationship between factors which control **axon terminal** development and factors which affect LGN dendrite morphology. The idea that binocular competition between axon terminals in cortex controls the development of LGN somata is well accepted (Garraghty et al., 1984; Guillery and Stelzner, 1970; Guillery, 1972; Kuppermann and Kasamatsu, 1983), since the axonal arbor of a cell is responsible for determining the metabolic requirements of the soma, and as a consequence can control the size of the soma needed to meet those metabolic requirements. However, a dendrite's development and its subsequent maintenance is generally thought to be controlled both by genetic factors (Berry and Sadler, 1988; Montague and Friedlander, 1991) and by the influence of afferent contacts (Berry, McConnel and Sievers, 1980; Hsiao, et al., 1987; Kapfhammer and Raper, 1987a; 1987b). Hence, the relationship between factors which control the size of a cell's axon

terminal arbor (i.e., competitive interactions) and factors which control dendrite form is not readily apparent.

It is possible, however, that disruptions in LGN dendrite morphology could result as a secondary consequence of abnormal binocular competition and its potential effect on the corticogeniculate projection. Anatomical studies have shown that the corticogeniculate pathway is present at birth (Stein and Edwards, 1979) and originates from cells in layer 6 of the striate and extrastriate visual cortex (Gilbert and Kelly, 1975; Updyke, 1977; 1975). In adult cats, it is estimated that this cortical projection may account for as much as 40-45 % of the total number of synapses in the A and A1 layers of the LGN (Sherman and Koch, 1986). Recent studies by Weber and Kalil (1987) have demonstrated that RSD terminals from corticogeniculate axons can be observed as early as 2 weeks of age postnatal, and during the interval from 2 through 12 weeks the number of corticogeniculate synapses doubles. After 12 weeks, there appears to be little change in either the length, density, or number of corticogeniculate synaptic contacts, which suggests that the morphological development of this pathway is essentially complete at this age. The role which this projection plays in the development of LGN cells has not been assessed to date.

Both MD and squint introduce disruptions in the binocularity of visual cortex, which may have functional consequences for the output of this massive corticogeniculate projection. In the present study, it is clear that the effects of deprivation or squint on LGN dendrites are present in both classes of LGN

neurons, and are introduced after dendritic differentiation has already occurred (Mason, 1983). Disturbances in the activity level of this massive cortical projection may introduce changes in the dendritic structure of LGN neurons. Some evidence in support of this idea is provided by observations regarding the effects of MD and squint on Sholl ring length and surface area. In normal animals, the corticogeniculate projection innervates X and Y LGN cells at more distal sites (i.e., >100 $\mu$ from the cell's geometric center) along their dendrites (Wilson et al., 1984). Since the present results in Sholl ring length suggest that the dendrites of deprived and squinted LGN neurons do not typically extend beyond 100 $\mu$ microns from the center of the somata, these findings suggest that corticogeniculate synapses may be affecting the retraction of LGN cell dendrites. One might speculate, for instance, that both MD and squint produces a functional deafferentation of the corticogeniculate projection, which in turn may result in a loss of dendritic length and surface area in the more distal portions of the dendritic tree of deprived and squinted LGN cells. Such a mechanism would account for the lack of a class specific effect on LGN cells (i.e. since there is no evidence that the corticogeniculate projection differentially effect LGN X and Y cells), and would point to the importance of activity in the **maintenance** of dendritic morphology. Clearly, however, more experimental evidence is required.

***Effects Which Were Not Sensitive to Modifications in Early Experience.*** In addition to those features of LGN morphology which demonstrated sensitivities to modifications of early experience, there were also a few features of dendritic

morphology which did not exhibit experience dependent changes. These features include: (1) numbers of dendrites per cell; (2) average length per dendritic segment; (3) average width per dendritic segment; and (4) changes in the Rall's 3/2 power rule for dendritic branch points. It seems that these aspects of cell morphology must be controlled by other epigenetic mechanisms which do not involve early visual experience (i.e. at least with respect to the paradigms used here).

An important observation from the present study is that the dendritic branching patterns of cells followed Rall's 3/2 power rule, thereby meeting one geometric preconditions for modelling dendritic arbors as equivalent cylinders. Although the measures for the present study revealed some scatter, this scatter bore no relationship to the visual rearing conditions under study here. Moreover, it seems quite surprising that, in spite of the many features of dendritic morphology that were affected by early visual experience, there was no apparent alteration in this geometric pattern of dendritic branching.

Bloomfield et al. (1987) have suggested two general classes of explanations to account for the adherence of LGN cell dendrites to the 3/2 power rule: (1) growth related phenomenon; and (2) represents pattern of development highly canalized by genetic constraints in order to maximize efficient current flow. First, they suggest that this branching pattern simply reflects the structural or hydraulic needs of the dendritic arbor. For instance, such a branching pattern may be an epiphenomenon of growth, or may reflect the most efficient structure

for the internal transport of various substances. Data from the present study suggest that if this explanation is true, then the growth processes responsible for regulating this branching pattern are relatively insensitive to modifications of early visual experience, despite the fact that many other features of dendrite development do reveal sensitivities to modifications in early visual experience. Secondly, Bloomfield and colleagues suggest that this branching pattern may represent an important means of controlling electrotonic signal transmission within a dendritic arbor. Recent computer models indicate that the 3/2 power rule exhibit the least attenuation of electrotonic conduction in both directions, and that any other branching pattern can maximize such conduction in only one direction. Thus, dendritic branching patterns that obey the 3/2 power rule not only permit efficient electrotonic conduction of post-synaptic potentials towards the soma, but they also permit efficient conduction towards the distal dendrites for currents generated at the soma or proximal dendrites (Bloomfield et al., 1987). As a result, action potentials initiated in the soma or axon hillock, as well as synaptic potentials generated proximally, can invade the more distal dendrites and influence the further generation of synaptic potentials there. In this manner, the state of activity of a LGN cell can regulate synaptic activation of signals coming from other parts of the dendritic arbor.

The preservation of Rall's power rule indicates that deprivation induced modifications in the physiological responsiveness of LGN neurons (Derrington and Hawkin, 1981; Friedlander et al., 1982; Geisert et al., 1980; Mangel, et al.,

1983; Mower et al., 1981; Sherman et al., 1972; Zetlan et al. 1981) cannot be attributed to modifications in this important feature of dendritic electrotonus. Since the 3/2 power rule establishes an anatomical condition for determining the direction of the passive flow of electrical currents in the dendritic tree, these observations from the present study suggest that retinogeniculate transmission in deprived LGN cells is reduced by factors which do **not** involve alterations in this branching rule. Alternatively, deficits in retinogeniculate transmission may reflect either static changes in the structure of retinogeniculate connections (e.g., modifications in the number and/or placement of synaptic inputs on LGN dendrites [Garraghty et al., 1986; Sur and Sherman, 1982]), **or** in the more dynamic features of synaptic transmission (e.g., modifications in receptor number and/or sensitivity, changes in the membrane capacitance and/or permeability to different ion species). In order to determine the relative contribution of these possible factors in the disruption of retinogeniculate transmission, more physiological and anatomical investigation is needed.

Implications for Visual Development and Vision.

***Dendritic Plasticity in the LGN: Regrowth vs. Structural Torsion.*** An extensive body of research has been performed to determine the limits of the sensitive period for visual development in cats (Cynader et al., 1980; Hubel and Wiesel, 1970; Mitchell, 1981; Olson and Freeman, 1980). In general, this period of susceptibility is thought to correspond roughly to the completion of the formation of ocular dominance columns in the visual cortex. Thus, between

postnatal weeks 2-8 the visual system demonstrates its highest degree of sensitivity to perturbations of visual experience (see Refs above). Data from the present study revealed two rather interesting examples of plastic changes in the structure of LGN cell dendrites.

***i. Pathologies Among Deprived Class 1 Cells.*** The first example of plasticity in LGN cell dendrites is the sinuous and tangled distal segments of one of the deprived class 1 cell dendrites in animals reared with MD (see figure 3B). These observations are consistent with published reports of Friedlander et al. (1982) and appear to result from the deleterious influence of binocular competition because they were not observed in the deprived layers of MD-OX animals. The interesting feature of this result is that the formation of these dendritic pathologies must have been induced after a time in which the majority of dendritic elaboration has already occurred (Mason, 1983). Thus they must reflect either new growth in the distal segments of the dendrites or a structural change of the distal segments introduced by deprivation as the growth and expansion of the LGN occurs during early postnatal development.

If new growth is involved in the introduction of these sinuosites it is interesting to note that the developmental rules mediating this regrowth process are not the same ones that govern the initial growth process. For example, distal dendritic segments seem to expand in close proximity to their neighboring segments and in some instances actually wrap themselves around each other. If regrowth is responsible for the tangled distal segments, then, it is clear that the

factors controlling this growth are not the same as those which contributed to the initial differentiation of the dendritic tree.

Alternatively, if these twisted deprived class 1 dendrites reflect the influence of structural torsion operating on the distal dendritic segments, it is interesting to note that this effect only appears in class 1 cells. Although it is difficult to imagine why these dendritic sinuosities would exhibit cell class specificity, they may be related to factors which cause the disruption in growth noted among deprived class 1 cell somata. In addition, these class-specific effects may reflect a structural weakness in the design of radially extending arbors such that torsion introduced by nuclear expansion may differentially result in sinuosities for deprived class 1 dendrites. Hence, this combination of factors (i.e., disruptions in somatic growth and structural susceptibility of radial dendrites) may contribute to the pathologies noted among class 1 neurons in the deprived layers.

***ii. Alteration in Dendritic Orientation for Class 2 LGN Cells.*** Another example of dendritic plasticity revealed in these data was the alterations in dendritic orientation for class 2 neurons in chiasm sectioned animals. As with the dendritic pathologies in deprived class 1 cells noted above, these effects on dendrite orientation offer another instance in which the LGN cell dendrites appear to undergo a remodelling process (this is suggested by the fact that transection of the optic chiasm was performed at a time in which the dendrites have already elongated their dendritic processes in the vertical axis). As

described in the subsection above, this phenomenon may have resulted from either an active remodelling process (i.e., retraction of existing dendrites and regrowth of new ones), or from a passive one (i.e., structural shift in dendritic orientation due to torsional factors operating as nucleus expands during development). If the former (i.e., active remodelling) of these possibilities is true, then it is interesting to note that the regrowth of these dendritic arbors did not demonstrate the kinds of sinuosities noted in deprived class 1 cell dendrites. Alternatively, if the orientation shifts in class 2 cell dendrites result from a structural change induced by optic chiasm transection, this possibility would suggest an important role of the retinal projections to the A layer in producing vertically oriented class 2 dendrites in normally reared animals (see section entitled: *The Role of Retinal Afferents in the Dendritic Orientation of LGN Class 2 Relay Cells*). In either case, it is interesting to note that plastic change in LGN dendrites is possible even after the initial form and extent of LGN dendrites is established.

Taken together, these two examples of neural plasticity (i.e., creation of torturous dendrites in class 1 cells, and shifts in dendrite orientation for class 2 cells in chiasm sectioned animals) suggest that early visual experience serves not only a role in the induction of LGN dendrites but also serves an important role in their maintenance as well.

*Questions Raised for Further Study:*

*i. What are the functional implication of class 2 dendrites oriented in the inappropriate axis?* Modifications in the dendritic orientation of LGN class 2 cells are likely to correspond to functional changes in their receptive field properties. The receptive fields of LGN X (i.e., class 2) cells are round, demonstrate strong center/surround antagonism, and subtend to less than 1° of visual angle (Bullier and Norton, 1979; Hoffmann et al., 1972; So and Shapley, 1981). There is evidence that retinal ganglion (Hammond, 1974; Levick and Thibos, 1980; 1982; Scodak, 1987) and LGN (Chino and Kaplan, 1988; Shou et al., 1986; Soodak et al., 1987; Vidyasagar, 1984; Vidyasagar and Urbas, 1982) cells also display a weak orientation selectivity in their response to visual stimulation. Leventhall and Schall (1983) report that the orientation selectivity in retinal ganglion cells is determined by the orientation of their dendritic arbors. These observations (i.e., Leventhall and Schall, 1983) in retina, therefore, raise the possibility that changes in dendritic orientation for class 2, LGN cells in chiasm sectioned animals (observed in this study) may also result in corresponding shifts in orientation selectivity of these cells in the LGN. If the orientation selectivity of class 2 relay cells is affected by the observed modifications in dendrite orientation, one would predict that orientation sensitivity in the horizontal axis would be enhanced relative to the vertical axis. This is suggested by the fact that LGN retinotopy in the horizontal plane is represented in the axis parallel to LGN laminar borders (Sanderson, 1971). Moreover, it would be interesting to

assess the impact of these dendritic modifications of class 2 LGN cells on both the orientation bias of cells in the visual cortex (Vidyasagar and Heide, 1984), and on the orientation discrimination abilities of animals reared with optic chiasm transection.

***ii. Are retinal arbors affected by dendrite development in the LGN?*** A significant body of literature on LGN cell ontogeny suggests that competition between X and Y retinal terminals play a critical role in the morphological development of LGN cell dendrites (see Garraghty et al., 1986; Friedlander et al., 1982; Sherman, 1985). This thesis is based on two sets of experimental observations: (1) the differential gradient in the development of retinal X and Y arbors in the LGN (i.e., retinal X arbors mature faster than retinal Y arbors [Sur et al., 1984]); and (2) on the effects of MD (Sur et al., 1982) and squint (Garraghty et al., 1989; Roe et al., 1990) on their respective development. The data presented here, however, suggest many features of dendrite development in the LGN are influenced by binocular competition between geniculocortical afferents in the visual cortex, and not by competition between X and Y retinal arbors. Hence, the findings from this study suggest two possibilities that could account for effects of MD and squint on retinal X and Y axon terminals in the LGN: (1) these effects are due to competition between X and Y retinal arbors and this competition is independent of dendrite development in the LGN; (2) the development of X and Y retinal arbors is secondarily affected by the dendritic development of LGN cells. To determine which of these two possibilities are

more likely, it would be interesting to know whether the X and Y retinal terminal arbors in animals reared with MDOX or SQOX are affected by deprivation or squint, respectively. If the effects of deprivation or squint on LGN dendrite development are independent of the effects on retinal X and Y arbors, then one would predict that the X and Y arbors of the retinal ganglion cells would continue to exhibit disruptions in their morphological development. Alternatively, if LGN dendrites guide the retinal terminal development, then one would predict that the X and Y arbors would appear normal in animals reared with MDOX and SQOX (i.e., since optic chiasm protects these LGN cells from the majority of the effects of MD and squint).

**iii. What is the role of corticogeniculate afferents in the development of LGN cells?** One of the enduring mysteries in LGN physiology and cell development is the role extraretinal afferents. As described above, the present study suggests that the massive corticogeniculate projection from layer VI may play an important role in the maintenance of LGN cell dendrites during the later phase of the sensitive period for visual development. Hence, the idea that cortically mediated activity is important for LGN cell development has not been explored experimentally due to the difficulties in modifying cortical responsiveness without directly affecting the geniculocortical afferents. By using a daily infusion of neural activity blockers (e.g., tetrodotoxin, ibotenic acid) applied during the later phase of the visual sensitive period, one might directly address the possibility that the activity provided by the corticogeniculate

projection is critical for the normal development and/or maintenance of LGN cell dendrites.

***iv. What is the functional significance of these structural changes of LGN cell dendrites for neural integration in MD and squinted animals?*** The kinds of morphological anomalies observed in the dendrites of both deprived and squinted LGN relay cells are likely to correspond to functional modifications in synaptic integration. Normal LGN cell dendrites are contacted by both retinal and a host of other nonretinal sources (for review see Sherman and Koch, 1986; Sherman, 1988). These different sources of synaptic inputs produce either excitatory or inhibitory responses on the postsynaptic membrane of the LGN cells. Moreover, these different excitatory and inhibitory signals from retinal and nonretinal sources are distributed along the dendritic arbors in a highly organized fashion (see Wilson et al., 1984). For example, excitatory retinal terminals typically form synaptic contacts with inhibitory terminals from LGN interneurons on either the shafts (in LGN Y cells) or dendritic appendages (LGN X cell) of the proximal dendrites, whereas excitatory inputs from corticofugal terminals are generally found distal to these retinal/interneurons synapses (see Sherman, 1988). When this kind of spatial mosaic of synaptic inputs is revealed in nervous system it is likely to serve an important function with regards to synaptic integration. All other things being equal, the relative electrotonic distance of synaptic inputs to the soma and axon hillock determine the relative weight of the synaptic input).

The reductions in dendrite arbor length and decrease in dendritic segments observed in squinted and deprived LGN cells might affect synaptic integration in two ways. By shortening the dendritic arbors this decreases the total electrotonic distance of the distal segments thereby possibly enhancing the relative influence of distal synapses in the process of synaptic integration. In this regard, it would be important to assay anatomically the relative location of retinal and non-retinal inputs in deprived and squinted LGN cells and document their electrotonic length relative to the somata and/or axon hillock. This information could then be used in conjunction with Rall's equivalent cylinder theory (see Rall, 1959;1970;1977) to model synaptic integration in deprived and squinted LGN neurons. Secondly, it seems likely that decreasing the amount of dendritic surface area could also decrease the number of synaptic inputs to a given neuron. If a reduction in synaptic input were realized, it would be interesting to know whether the loss of synapses is equally distributed among the different retinal and nonretinal inputs, or whether the loss would selectively be observed among these different sources of input to LGN cell dendrites.

***Clinical Implications.*** Amblyopia is a clinical condition which affects approximately 2-5% of the population (Duke-Elder and Wybar, 1973; Marg, 1982) and is marked by a suppression of visual information in one or both eyes in addition to an impairment in binocular vision (Sireteanu, 1982; Sireteanu, Fronius and Singer 1981; von Noorden, 1985). The condition is further characterized by a decrease in visual function, particularly for higher spatial

frequencies (Flom and Bedell, 1985; Levi and Klein, 1982; 1983; 1985), and is believed to result from problems occurring during early visual development (Hess, 1983; 1980; von Noorden, 1985). In order to obtain a more complete understanding of the mechanisms responsible for inducing human amblyopias, it is essential that one understands the role of early experience in the development of central visual pathways.

Two popular animal models commonly employed in vision research for studying the ontogeny of visual amblyopias are **monocular deprivation** (for review see, Blakemore, 1978; Ganz, 1978; Mitchell, 1982; 1990) and **artificially induced squint** (Cleland et al., 1982; Holopigian and Blake, 1983; Jacobson and Ikeda, 1979; Mitchell et al., 1984). These two models are used to mimic developmental conditions which are believed responsible for the induction of anisometropic (i.e., MD) and strabismic (i.e., squint) amblyopia in humans. The visual system of the cat provides a useful model in this regard given the wealth of experimental investigation into the ontogeny of visual pathways in this species (see Blakemore, 1978; Hirsch and Leventhal, 1978 Sherman and Spear, 1982).

The present study further demonstrates the importance that early visual experience on the self-organizing features of central visual pathways. Moreover, it is clear that abnormal competitive interactions in the visual cortex between the ocular pathways for the two eyes induce abnormal development of LGN cell morphology. The morphological findings presented here are relevant for the clinical study of amblyopia for three reasons: (1) they underscore the importance

of balanced and correlated binocular visual activity for the normal growth and maintenance of central structures in the developing visual system; (2) they point to a subcortical source (i.e. LGN) as a possible neural locus for mediating amblyopic deficits; and finally (3) they suggest that binocular competition is likely to be an important developmental mechanism for the induction of visual amblyopias.

Unfortunately, neither the physiological responsiveness of these LGN neurons nor the visual function of these animals were tested prior to this anatomical study. However, given the robust effects of experience on LGN cell morphology reported here, it seems that these experimental models are likely to offer useful insights into the neurological etiology of amblyopic deficits observed in cats reared with either MD (Giffin and Mitchell, 1978; Hoffmann and Lippert, 1982; Maffei, Fiorentini and Cenni, 1990; Timney, Mitchell and Cynader, 1980; van Hof-van and Duin, 1977; 1976) or squint (Cleland et al., 1982; Holopigian and Blake, 1983; Jacobson and Ikeda, 1979). The experiments described here demonstrate that early modifications in binocular stimulation and possibly mismatched ocular proprioception result in a significant disruption for the normal morphological development of cells in the LGN. Furthermore, these changes in the form of LGN somata and dendrites are likely to indicate corresponding modifications in LGN function which may, in part, contribute to visual impairments experienced by human and non-human amblyopes.

## Summary

In summary, MD and squint modify the nature of binocular stimulation to the developing brain. These changes in early visual experience have important consequences for establishing the epigenetic developmental pathways which determine the morphological features of neurons in the LGN. Moreover, it is clear that binocular competition is an important and powerful mechanism for establishing the experience-induced changes noted in LGN cell morphology. Although these results from the developing visual system of cats are not directly comparable to the developing visual system of humans (Marg, 1982, Odom, 1983), the findings amassed here offer an important step in defining the mechanisms responsible for determining the self-organizing principles of nervous system, and therefore provides useful information for making predictions about the formation of central visual pathways under conditions in which early visual experience is distorted (e.g., anisometropia, strabismus).

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# APPENDIX A

## Groups, Histology Procedures, and Quantitative Measures

**Table A-1.**

Table reflects the number of animals present in each of the various experimental conditions.

| Experimental Condition | Number of Cats |
|---|---|
| 1. Monocular Deprivation | 2 |
| 2. Monoc. Dep. + OX [f] | 3 |
| 3. Squint | 4 |
| 4. Squint + OX | 4 |
| 5. OX | 2 |

[f] OX indicates that the optic chiasm has been transected.

**Table A-2**

Staining procedure for cressyl violet used to determine layer borders of the LGN (Myers, 1971)

I. Remove coverslips from slides

II. Tissue processing:

| | |
|---|---|
| A. 100 % Alcohol . . . . . | 5 min |
| B. Xylene. . . . . | 20-60 min |
| C. 100 % Alcohol . . . . . | 5 min |
| D. Water Rinse | |
| E. Cresyl Violet Solution. . . . . | 5-10 min |
| (0.1% aqueous cresyl violet acetate solution) | |
| F. Acid Alcohol (to Decolorize). . . . . | 2-5 min |
| (1 part 10% actic acid solution) | |
| (9 parts absolute alcohol) | |
| G. Water Rinse | |
| H. 70 % Alcohol. . . . . | 3 min |
| I. 90 % Alcohol. . . . . | 3 min |
| J. 100 % Alcohol . . . . . | 2 min |
| K. 100 % Alcohol . . . . . | 2 min |
| L. 100 % Alcohol . . . . . | 2 min |
| M. Xylene. . . . . | 4 min |

III. Cover slip applied using mounting medium (Permount)

Table A-3.

Description of the Various Experimental Measures Used for the Analysis.

| Dependent Measure | Description |
|---|---|
| 1. Soma Size | Only cells containing both lucifer yellow and the rhodamine microspheres were sampled from in this study. The cross sectional area of each soma containing a clear nucleolus was obtained by using a computer analysis system. |
| 2. Cumulative Dendritic Length | Each dendritic segment length was measured *via* a software package from Jandel (Java 3.1). The segment lengths were then added together across the dendritic tree with a computer and the resulting sum was used as the estimate of cumulative dendritic length. |
| 3. Average Length per Dendrite | To obtain this estimate the cumulative dendritic length was divided by the number of dendrites for the cell. |
| 4. Average Width per Dendritic Segment | An average estimate of width was obtained for each dendritic segment. The diameter ($d$) of each dendritic segment was measured every 5-8 $\mu$ and an average width for each segment was obtained. These segment widths were then summed and divided by the total number of segments to obtain an estimate of segment width. |
| 5. Cumulative Dendritic Surface Area | To calculate this measure dendritic segments were assumed to be cylinders with a length of $l$ and a radius of $d/2$. Estimates of dendritic surface area were obtained for each dendritic segment using the formula: $A = (2\pi r^2) + (2\pi rl))$. These estimates of surface area for each dendritic segment were then added together with a computer and the resulting sum was used as the estimate of dendritic surface area. |

Table A-3 (continued).

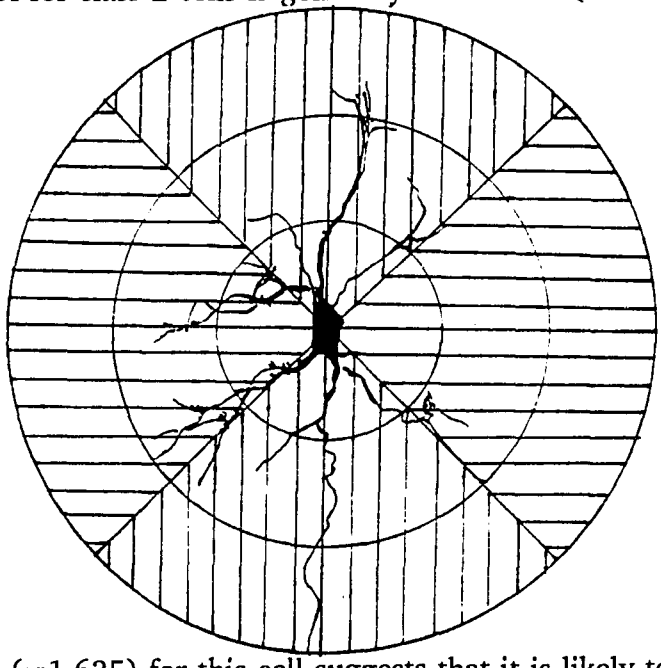
Description of the Various Experimental Measures Used for the Analysis.

| Dependent Measure | Description |
|---|---|
| 6. Number of Dendrites. | The number of dendrites were simply counted from the camera lucida drawings. |
| 7. Number of Dendritic Segments | The number of segments were simply counted from the camera lucida drawings. |
| 8. Number of Dendritic Branch Points | The number of branch points were simply counted from the camera lucida drawings. |
| 9. Sholl Ring Index Length | The ratio of the % of dendritic length in the outer: inner Sholl rings was obtained (outer rings: 3-8); rings 1-2). See Sholl ring diagram in A-4. |
| 10. Sholl Ring Index Surface Area | Same ratio as for length except estimates of surface area were used instead of estimates of length. |
| 10. Orientation Index | See Appendix A-4 (below). |

***All quantitative measures were taken from an analysis of the camera lucida drawings. The images of these drawings were captured with a video camera interfaced to a computer. No attempt was made to characterize variation in dendritic morphology in the Z axis.***

**Appendix A-4.**

Sholl ring analyses are useful for classifying cells into one of Guillery's morphological categories according to the relative degree of dendritic orientation. The figure demonstrates the Sholl ring template which, in this example, includes 3 concentric circles (a template containing 8 circles were used in the present study) spaced 50 $\mu$m apart and centered on the soma. The circles are sectioned into vertical (vertical hatching) and horizontal quadrants (horizontal hatching) with respect to laminar borders of the LGN. In the analysis of dendritic orientation, the number of vertical(n=26 for cell below), and horizontal (n=16) intersections were counted. The number of vertical relative to horizontal intersections provides an index of the cell's orientation bias. Since class 2 cells are generally vertically oriented, their orientation index (OI) is generally > 1 whereas the OI for class 2 cells is generally less than 1 (see Friedlander et al., 1981)



The OI (=1.625) for this cell suggests that it is likely to be of the class 2 type.

## APPENDIX B

## Statistical Summary Tables

**Table B-1**

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Soma Size** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 1.2650 | 0.09731 | 13.80 | 0.0001 |
| Error | 27 | 1.1547 | 0.00705 | | |
| T.C. | 40 | 1.4554 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.869222 | 3.414161 | 0.0839617 | 2.4592 |

Dependent Variable: **Soma Size**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 0.22694 | 0.03782 | 5.37 | 0.0009 |
| CELL Type (CT) | 1 | 0.55592 | 0.55591 | 78.86 | 0.0001 |
| HIS * CT | 6 | 0.17201 | 0.02866 | 4.07 | 0.0050 |

**Table B-2**

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Cumulative Dendritic Length** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 1.9061 | 0.14662 | 3.71 | 0.0019 |
| Error | 27 | 1.0660 | 0.03948 | | |
| T.C. | 40 | 2.9721 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.641326 | 7.072356 | 0.1987029 | 2.8096 |

Dependent Variable: **Cumulative Dendritic Length**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 1.18371 | 0.19728 | 5.00 | 0.0015 |
| CELL Type (CT) | 1 | 0.07765 | 0.07765 | 1.97 | 0.1722 |
| HIS * CT | 6 | 0.18394 | 0.03066 | 0.78 | 0.5954 |

**Table B-3**

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Total Number of Dendrites** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 0.1790 | 0.01377 | 1.38 | 0.2310 |
| Error | 27 | 0.2691 | 0.03948 | | |
| T.C. | 40 | 0.4482 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.399443 | 12.46344 | 0.0998439 | 0.8011 |

Dependent Variable: **Number of Dendrites**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 0.04599 | 0.00766 | 0.77 | 0.6009 |
| CELL Type (CT) | 1 | 0.02180 | 0.02180 | 2.19 | 0.1508 |
| HIS * CT | 6 | 0.04401 | 0.00734 | 0.74 | 0.6252 |

Table B-4

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Average Length per Dendrite** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 1.1288 | 0.08683 | 2.90 | 0.0094 |
| Error | 27 | 0.8074 | 0.02990 | | |
| T.C. | 40 | 1.9363 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.583006 | 8.547379 | 0.1729314 | 2.0232 |

Dependent Variable: **Average Length per Dendrite**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 0.79725 | 0.13287 | 4.44 | 0.0030 |
| CELL Type (CT) | 1 | 0.01281 | 0.01281 | 0.43 | 0.5182 |
| HIS * CT | 6 | 0.14480 | 0.02413 | 0.81 | 0.5734 |

Table B-5

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Segment Length** for LGN Cell Dendrites

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 0.25867 | 0.01989 | 1.79 | 0.09 |
| Error | 27 | 0.29975 | 0.01110 | | |
| T.C. | 40 | 0.55843 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.463221 | 8.49091 | 0.1053660 | 1.2409 |

Dependent Variable: **Dendritic Segment Length**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 0.3982 | 0.0663 | 1.68 | 0.1655 |
| CELL Type (CT) | 1 | 0.0530 | 0.05303 | 2.19 | 0.1508 |
| HIS * CT | 6 | 0.0189 | 0.0189 | 0.48 | 0.8176 |

Table B-6

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Total Number of Segments** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 1.0440 | 0.08031 | 2.51 | 0.0212 |
| Error | 27 | 0.8649 | 0.03203 | | |
| T.C. | 40 | 1.9090 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.546898 | 11.41025 | 0.1789863 | 1.5686 |

Dependent Variable: **Total Number of Dendritic Segments**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 0.71378 | 0.11896 | 3.71 | 0.0080 |
| CELL Type (CT) | 1 | 0.00605 | 0.00605 | 0.19 | 0.6673 |
| HIS * CT | 6 | 0.14621 | 0.02434 | 0.76 | 0.6069 |

Table B-7

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Number of Dendritic Branch Points** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 1.5794 | 0.12149 | 2.79 | 0.0119 |
| Error | 27 | 1.1777 | 0.04361 | | |
| T.C. | 40 | 2.7569 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.572865 | 17.38708 | 0.2088423 | 1.20113 |

Dependent Variable: **Number of Dendritic Branch Points**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 1.18043 | 0.19673 | 4.51 | 0.0027 |
| CELL Type (CT) | 1 | 0.00118 | 0.00118 | 0.03 | 0.8703 |
| HIS * CT | 6 | 0.23772 | 0.03962 | 0.91 | 0.5037 |

**Table B-8**

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Cumulative Dendritic Surface Area** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 2.3517 | 0.18091 | 4.61 | 0.0004 |
| Error | 27 | 1.0591 | 0.03923 | | |
| T.C. | 40 | 3.4109 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.689489 | 6.21133 | 0.1980575 | 3.1886 |

Dependent Variable: **Cumulative Dendritic Surface Area**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 1.4205 | 0.23676 | 6.04 | 0.0004 |
| CELL Type (CT) | 1 | 0.1249 | 0.12492 | 3.18 | 0.0856 |
| HIS * CT | 6 | 0.2788 | 0.04647 | 1.18 | 0.3438 |

**Table B-9**

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Sholl Ring Index (Length)** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 0.01285 | 0.00099 | 3.69 | 0.0020 |
| Error | 27 | 0.00723 | 0.00026 | | |
| T.C. | 40 | 0.02008 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.639895 | 1.59773 | 0.0163661 | 1.0243 |

Dependent Variable: **Sholl Ring Index (Length)**

| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 0.00739 | 0.00123 | 4.60 | 0.0024 |
| CELL Type (CT) | 1 | 0.00045 | 0.00045 | 1.71 | 0.2023 |
| HIS * CT | 6 | 0.00241 | 0.00040 | 1.50 | 0.2144 |

Table B-11

Analysis of Variance (ANOVA) Table on the Effects of Visual History, Cell Type and History by Cell Type on the **Sholl Ring Index (Surface Area)** of LGN Cells

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 13 | 0.03480 | 0.00037 | 3.12 | 0.0061 |
| Error | 27 | 0.00231 | 0.00009 | | |
| T.C. | 40 | 0.02008 | | | |

| R-Square | C.V. | Root MSE | Total Length Mean |
|---|---|---|---|
| 0.600281 | 0.91452 | 0.0092649 | 1.0131 |

Dependent Variable: **Sholl Ring Index (Surface Area)**

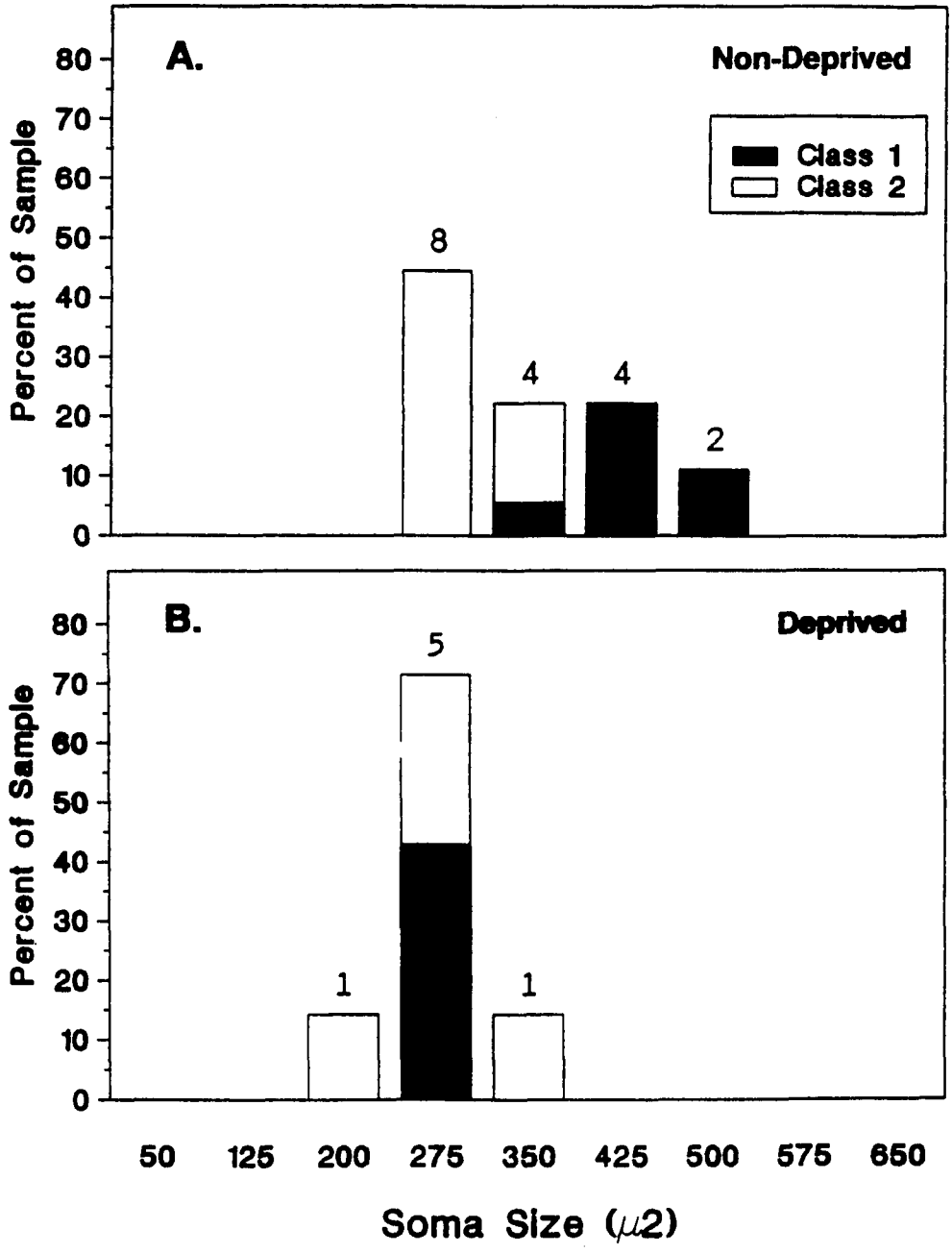
| Source | DF | Type IV SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| HISTORY (His) | 6 | 0.00223 | 0.00037 | 4.34 | 0.0034 |
| CELL Type (CT) | 1 | 0.00007 | 0.00007 | 0.78 | 0.3845 |
| HIS * CT | 6 | 0.00053 | 0.00009 | 1.04 | 0.4228 |

# APPENDIX C

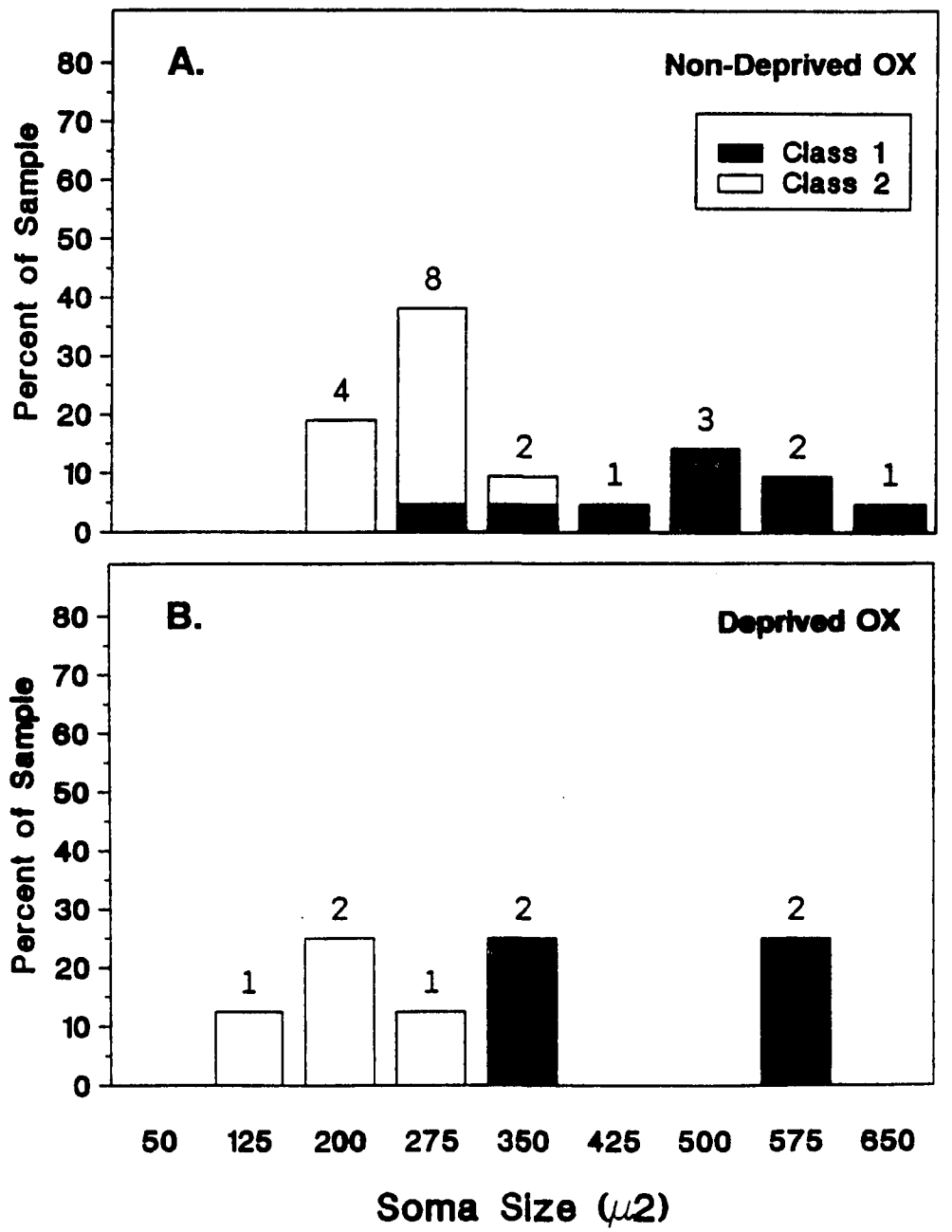
## Individual Data: Presented in a Frequency Histogram Format

Numbers above bars represent the number of cells.

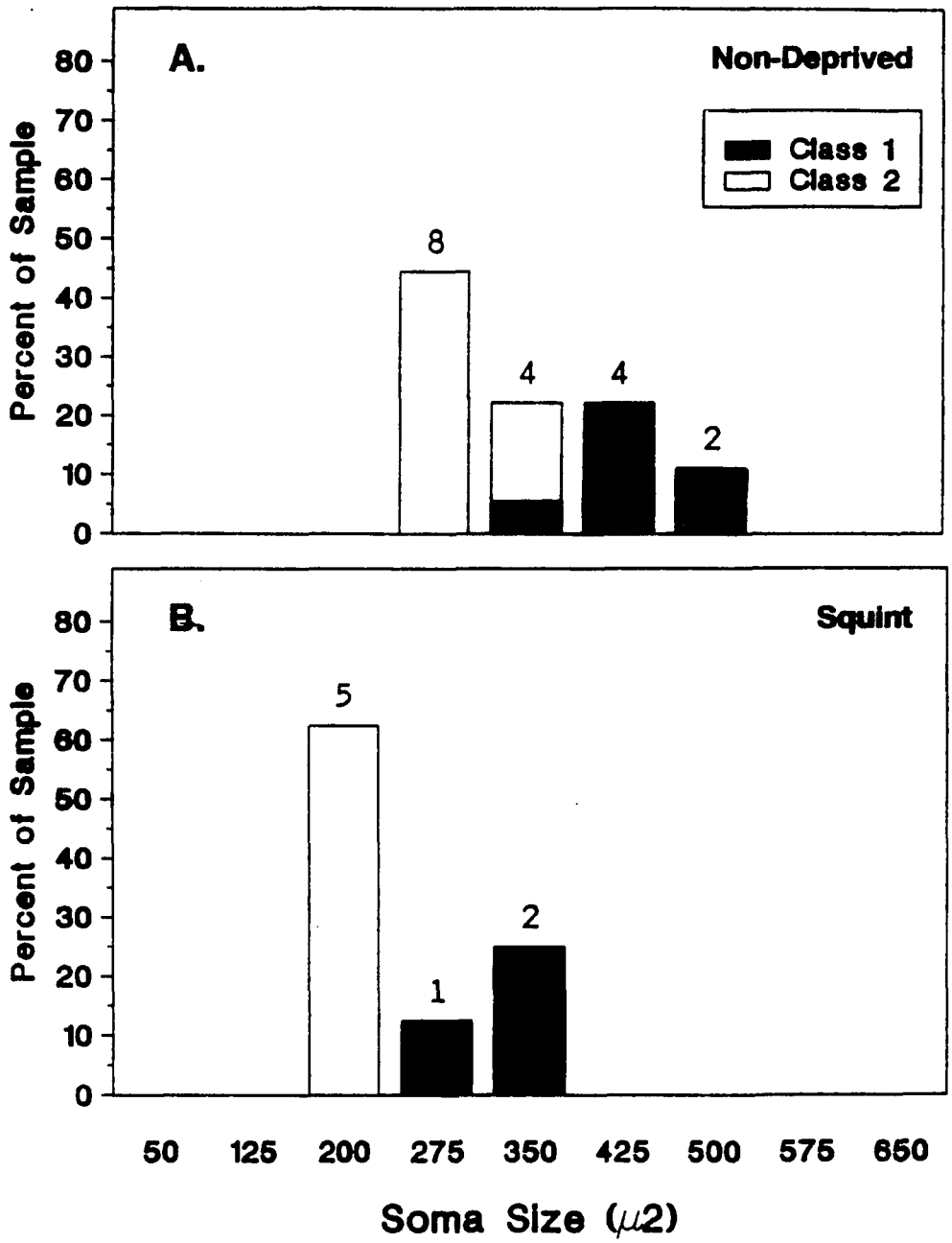
**Effect of Deprivation on Soma Size in Class 1 And Class 2 LGN Cells From Cats with an Intact Optic Chiasm**



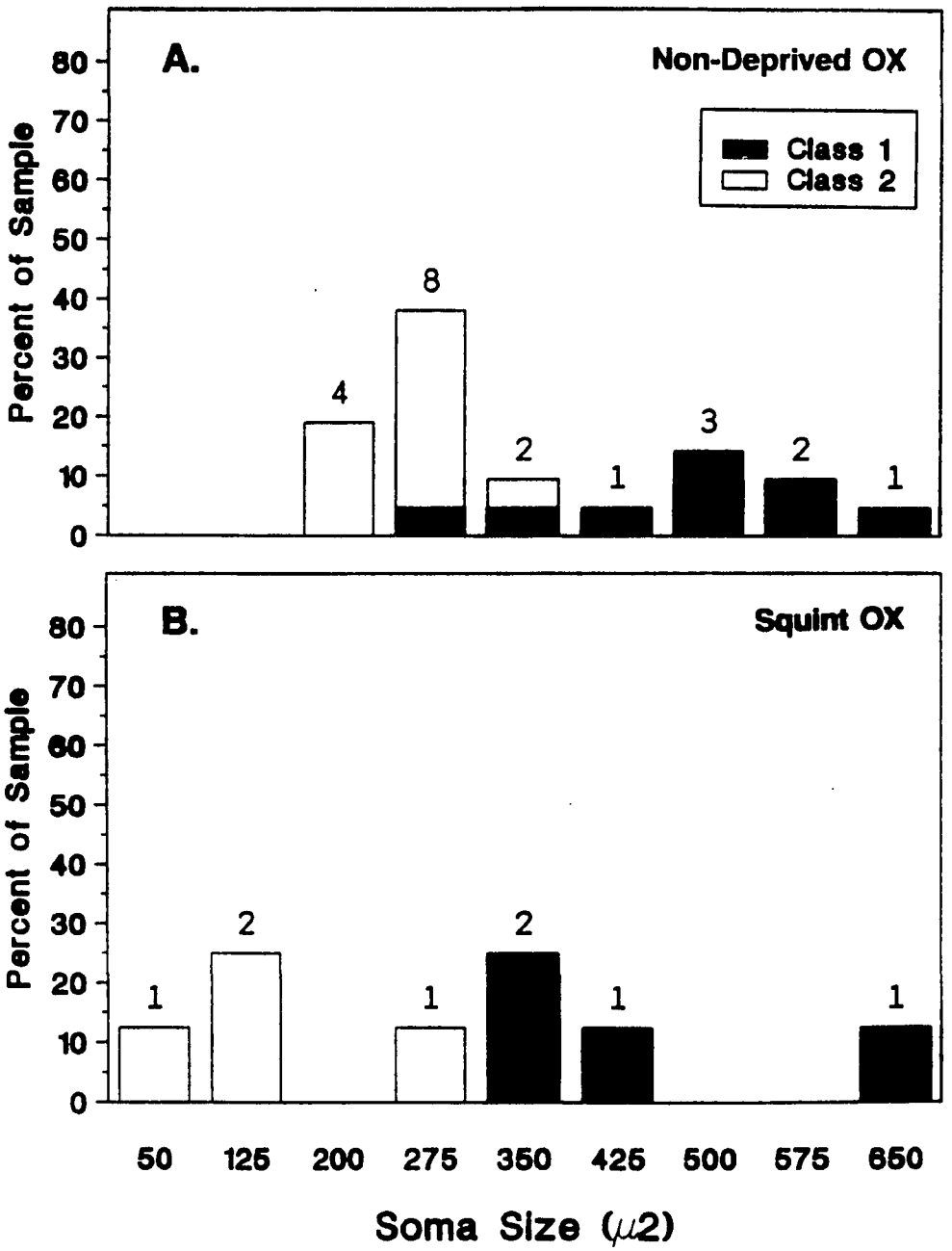
**Effect of Deprivation and Chiasm Section Combined On the Soma Size of Class 1 and Class 2 LGN cells**



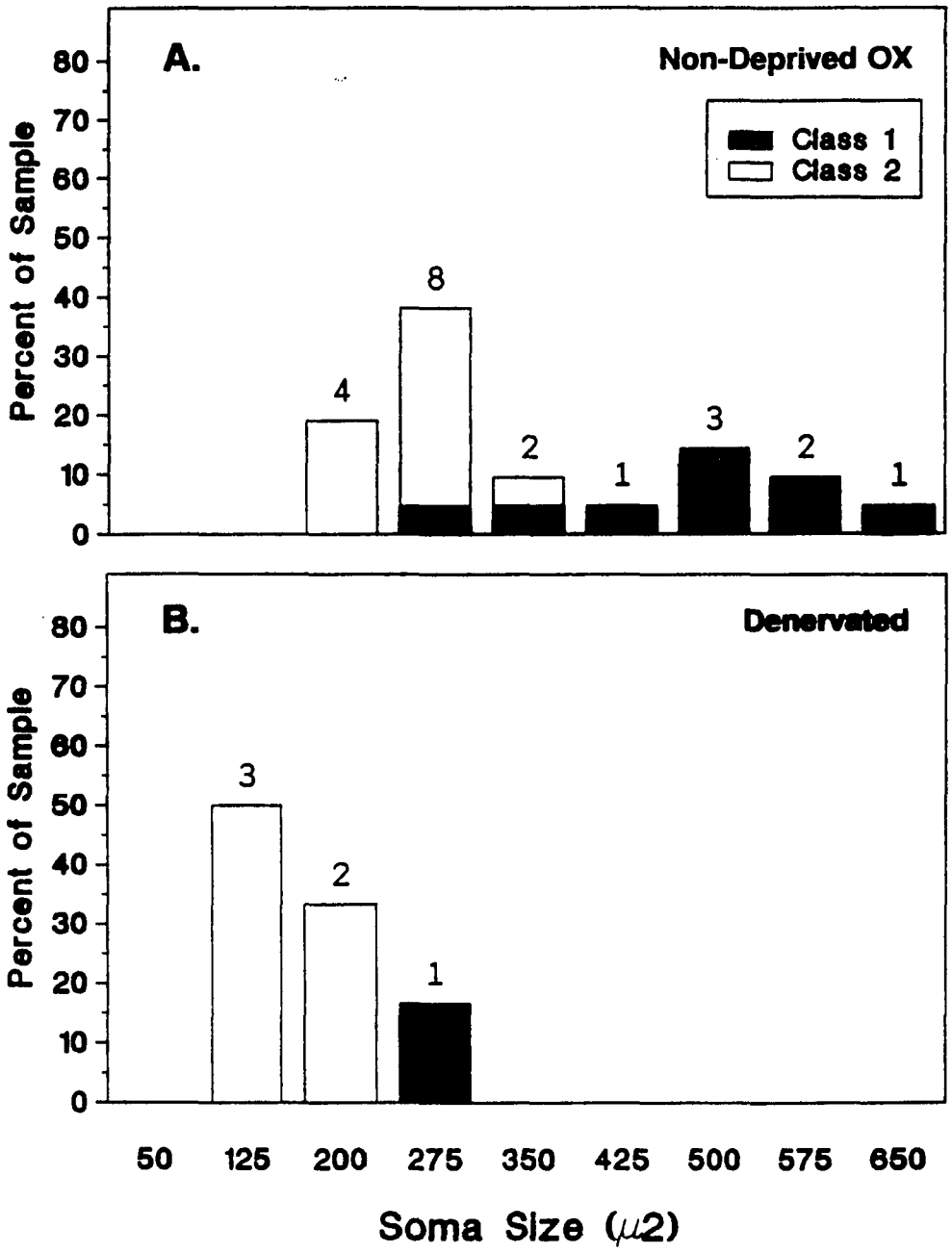
## Effect of Squint on Soma Size in Class 1 and Class 2 LGN Cells From Cats with an Intact Optic Chiasm



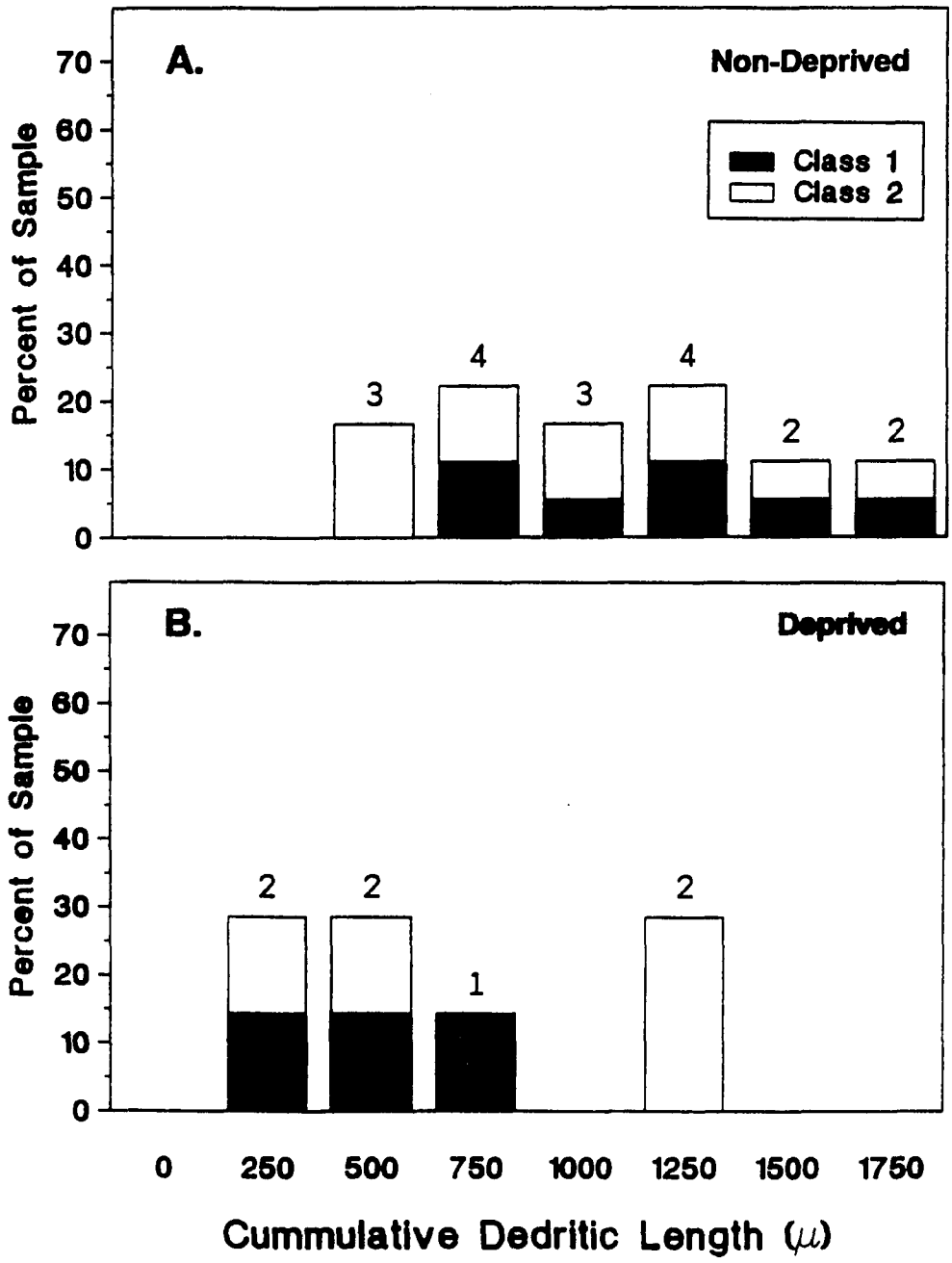
**Effect of Squint and Chiasm Section Combined On the Soma Size of Class 1 and Class 2 LGN cells**



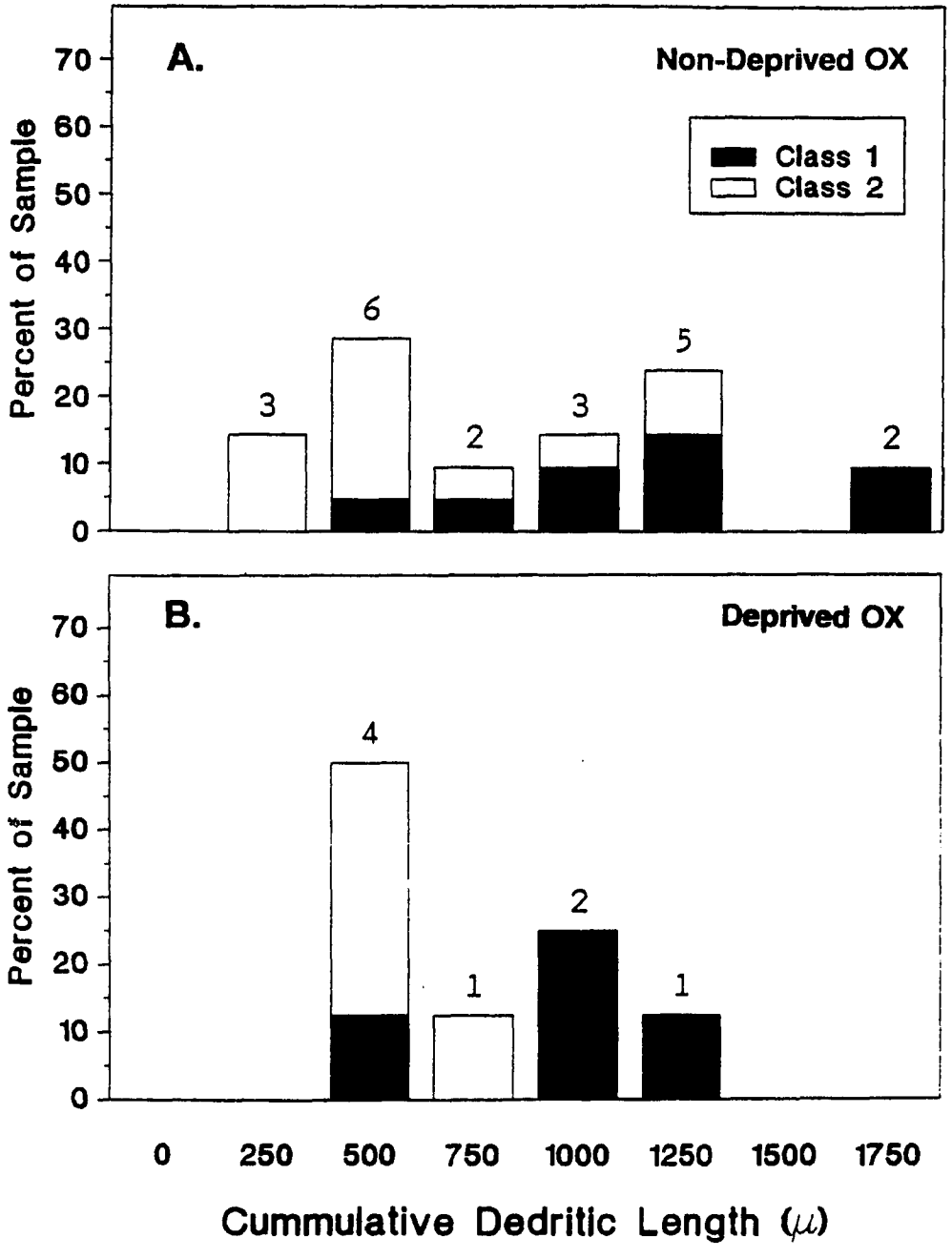
## Effect of Denervation on the Soma Size of Class 1 and Class 2 LGN cells



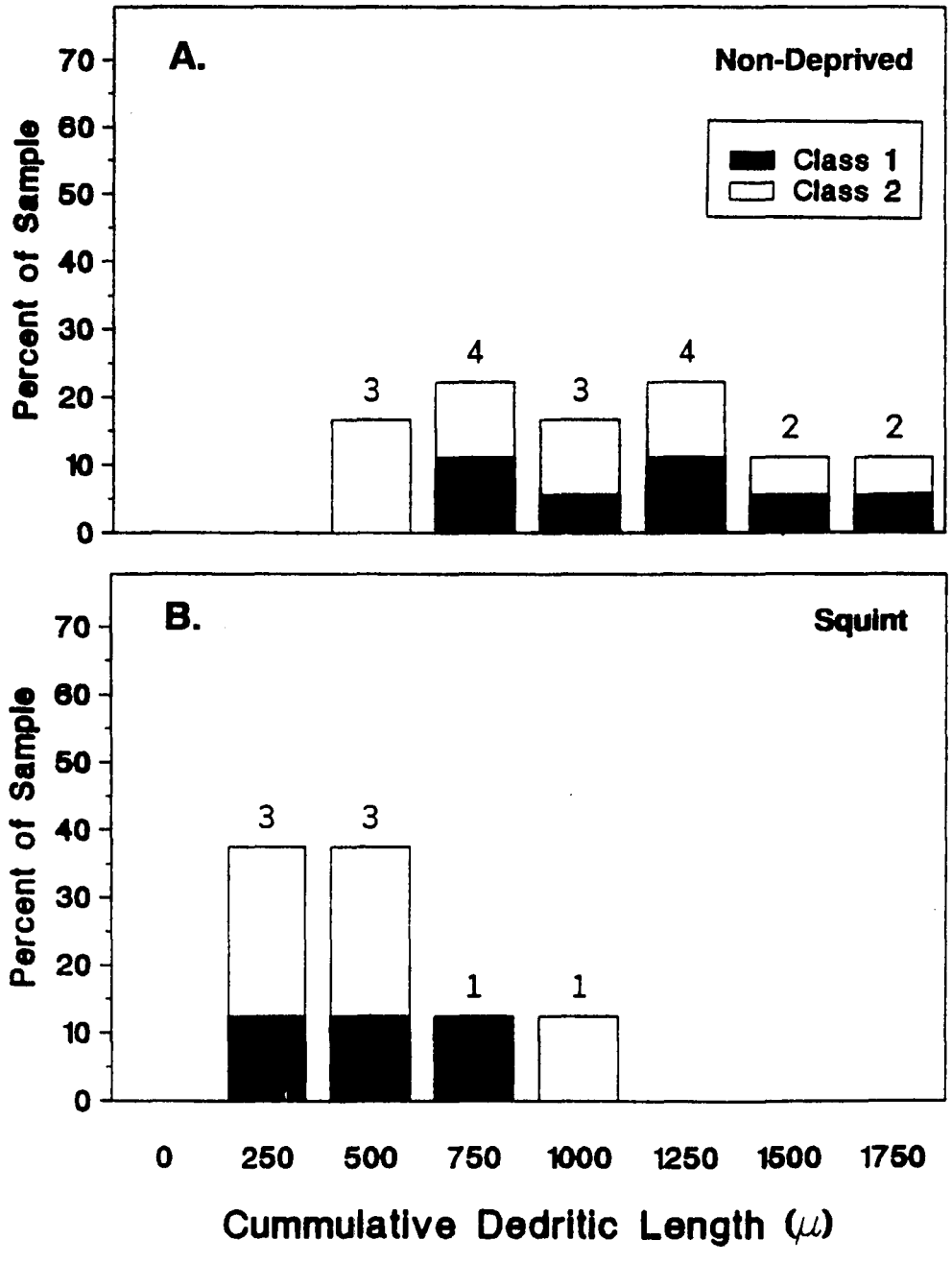
**Effect of Deprivation on Cumulative Dendritic Length in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



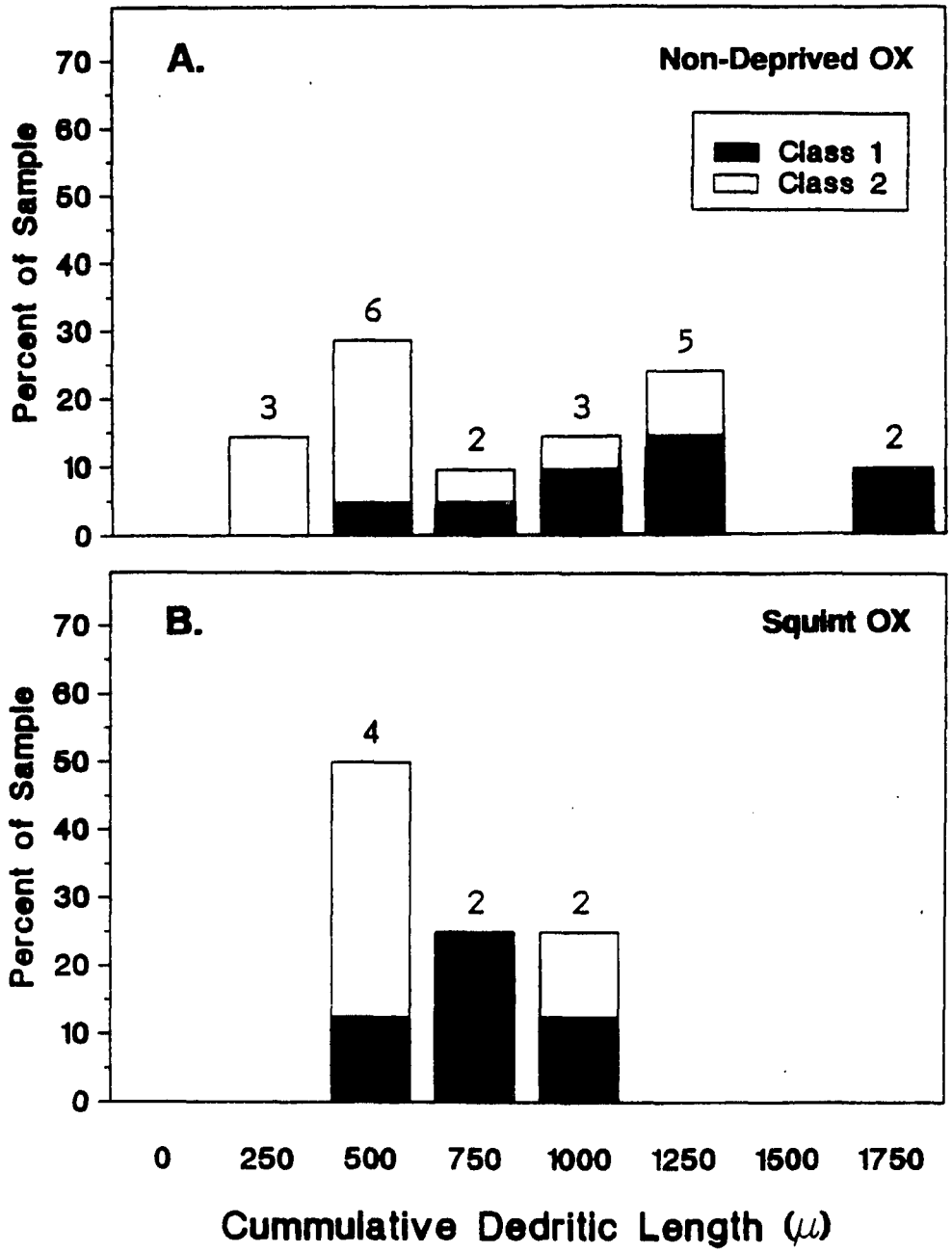
## Effect of Deprivation and Chiasm Section Combined On Cumulative Dendritic Length in Class 1 and Class 2 LGN Cells



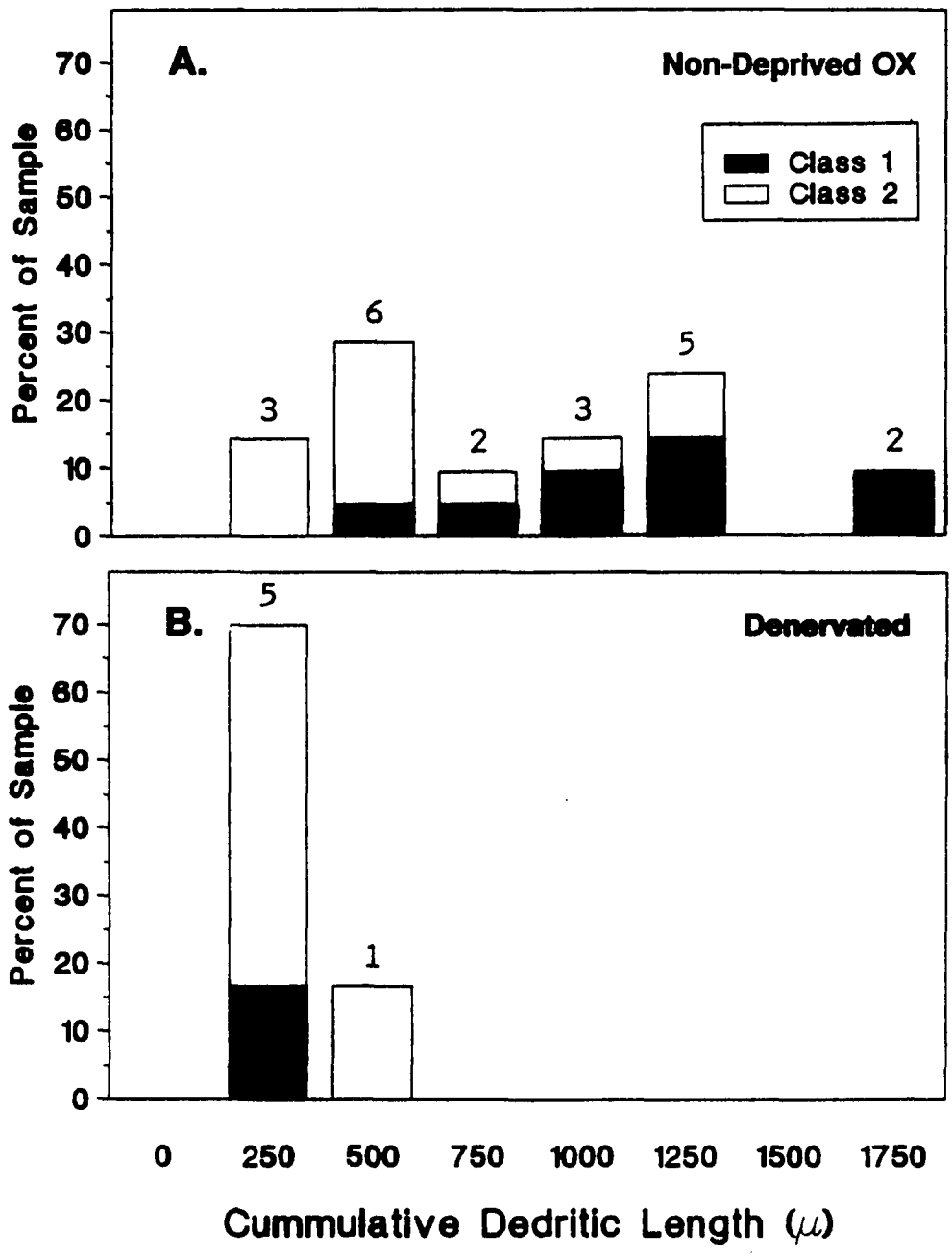
**Effect of Squint on Cumulative Dendritic Length in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



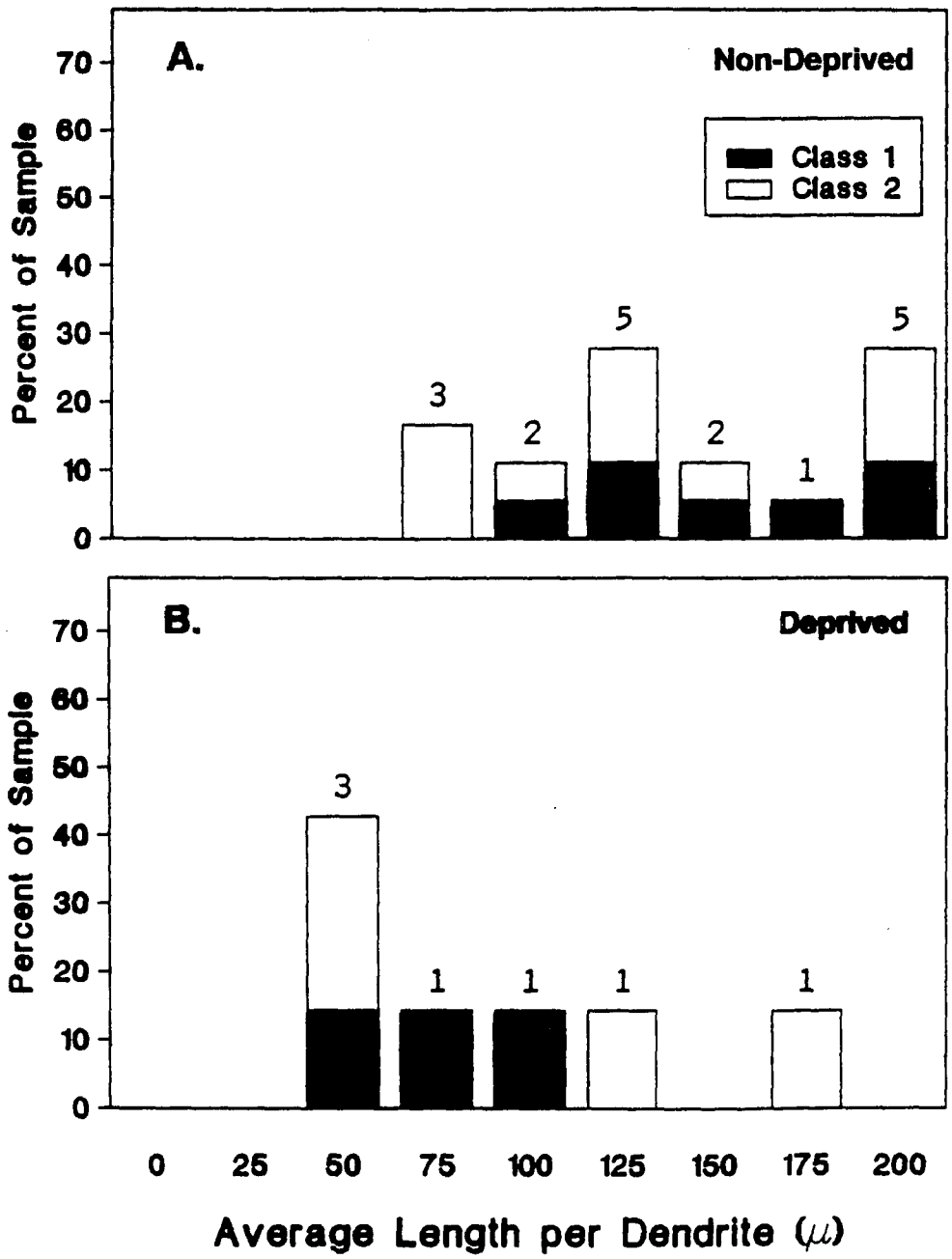
**Effect of Squint and Chiasm Section Combined on Cumulative Dendritic Length in Class 1 and Class 2 LGN Cells**



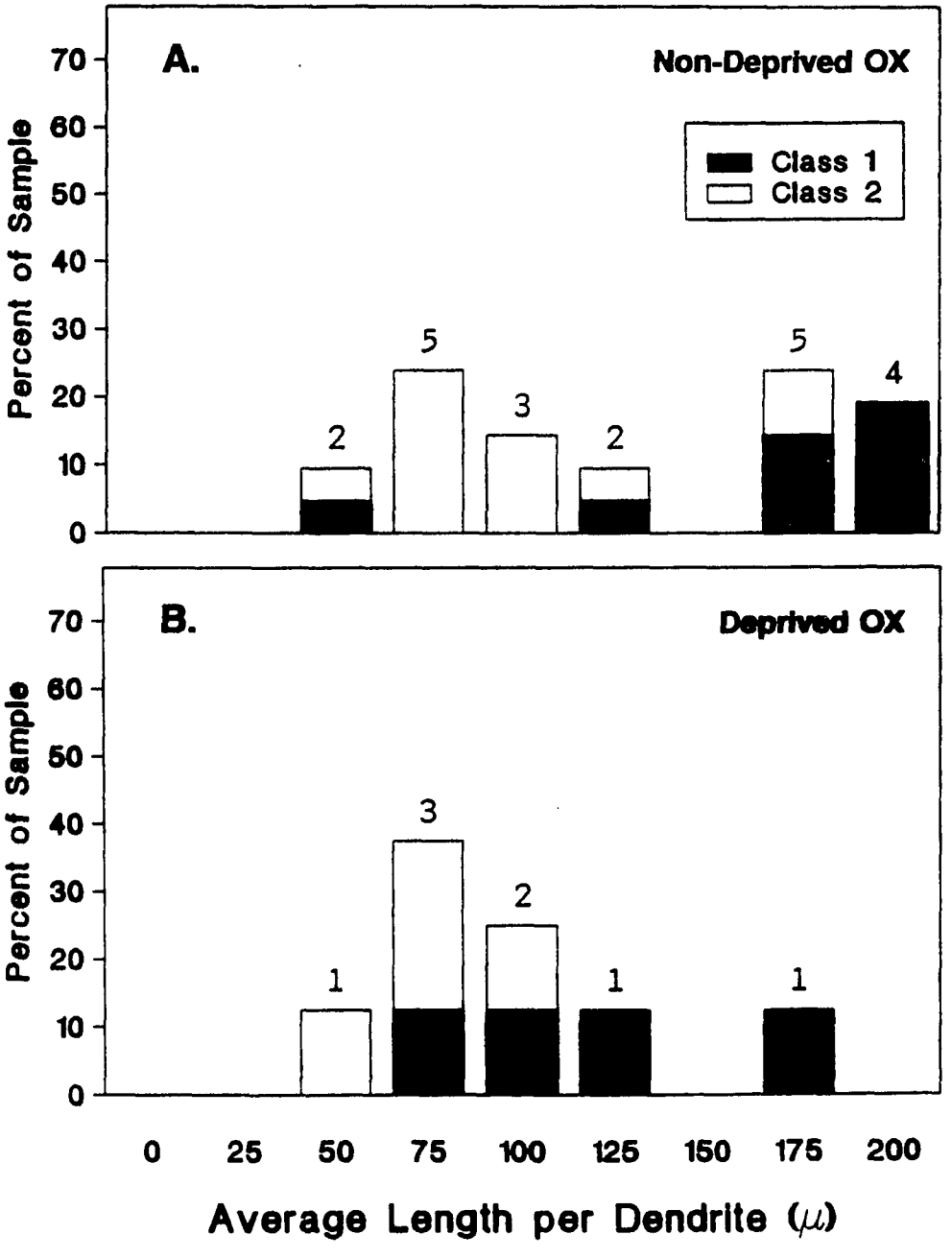
## Effect of Denervation on the Cumulative Dendritic Length in Class 1 and Class 2 LGN Cells



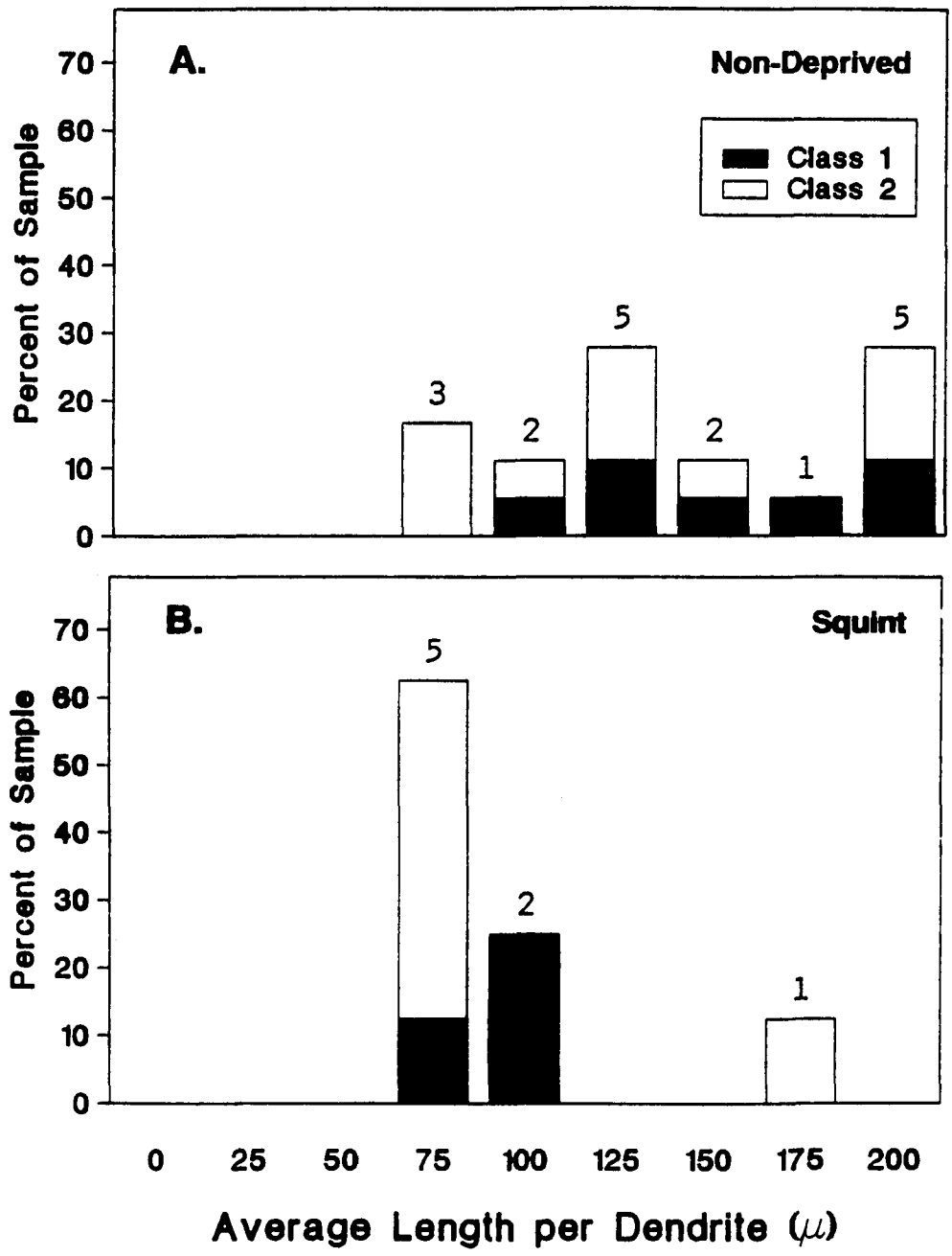
**Effect of Deprivation on the Average Length per Dendrite in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



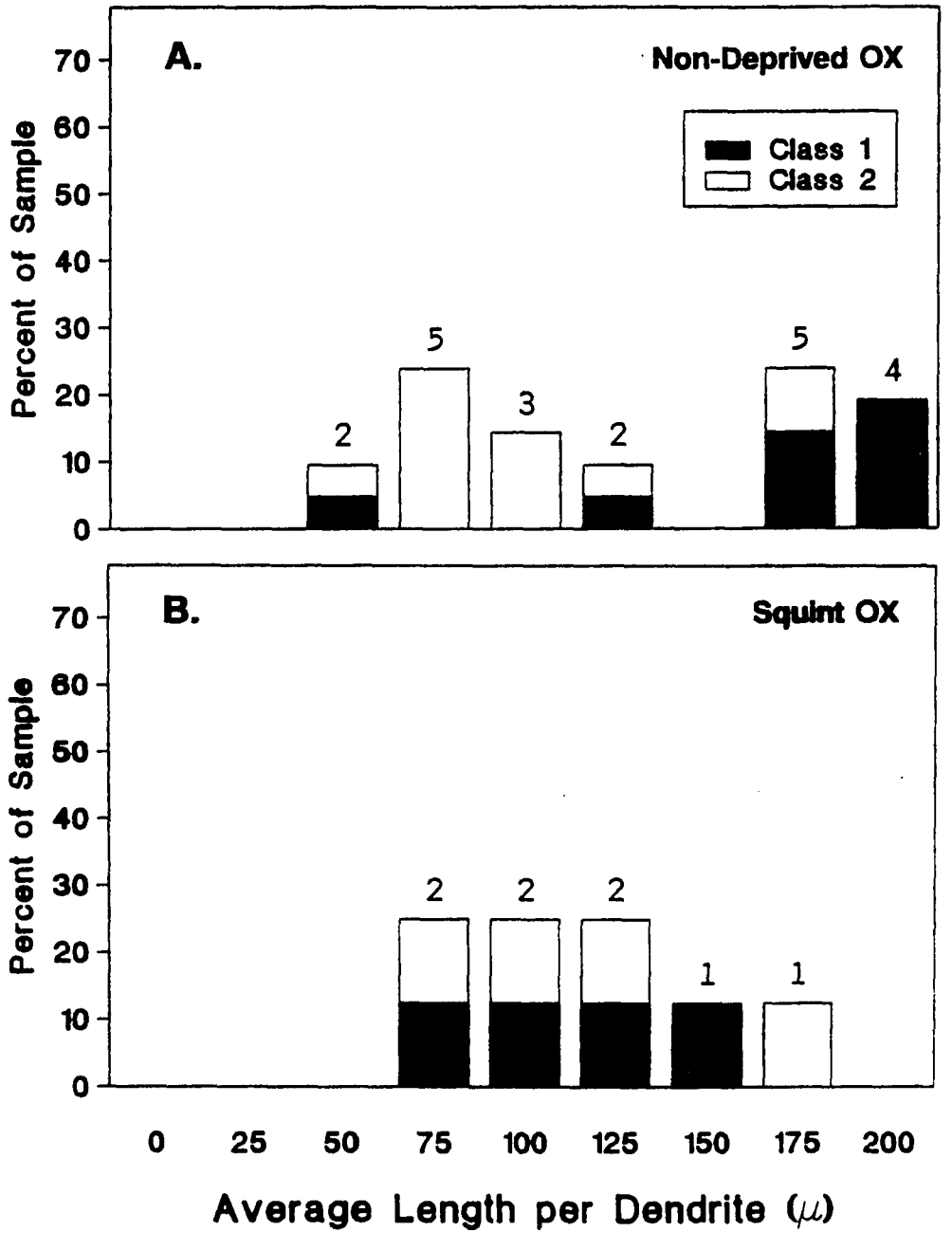
**Effect of Deprivation and Chiasm Section Combined On the Average Length per Dendrite in Class 1 and Class 2 LGN Cells**



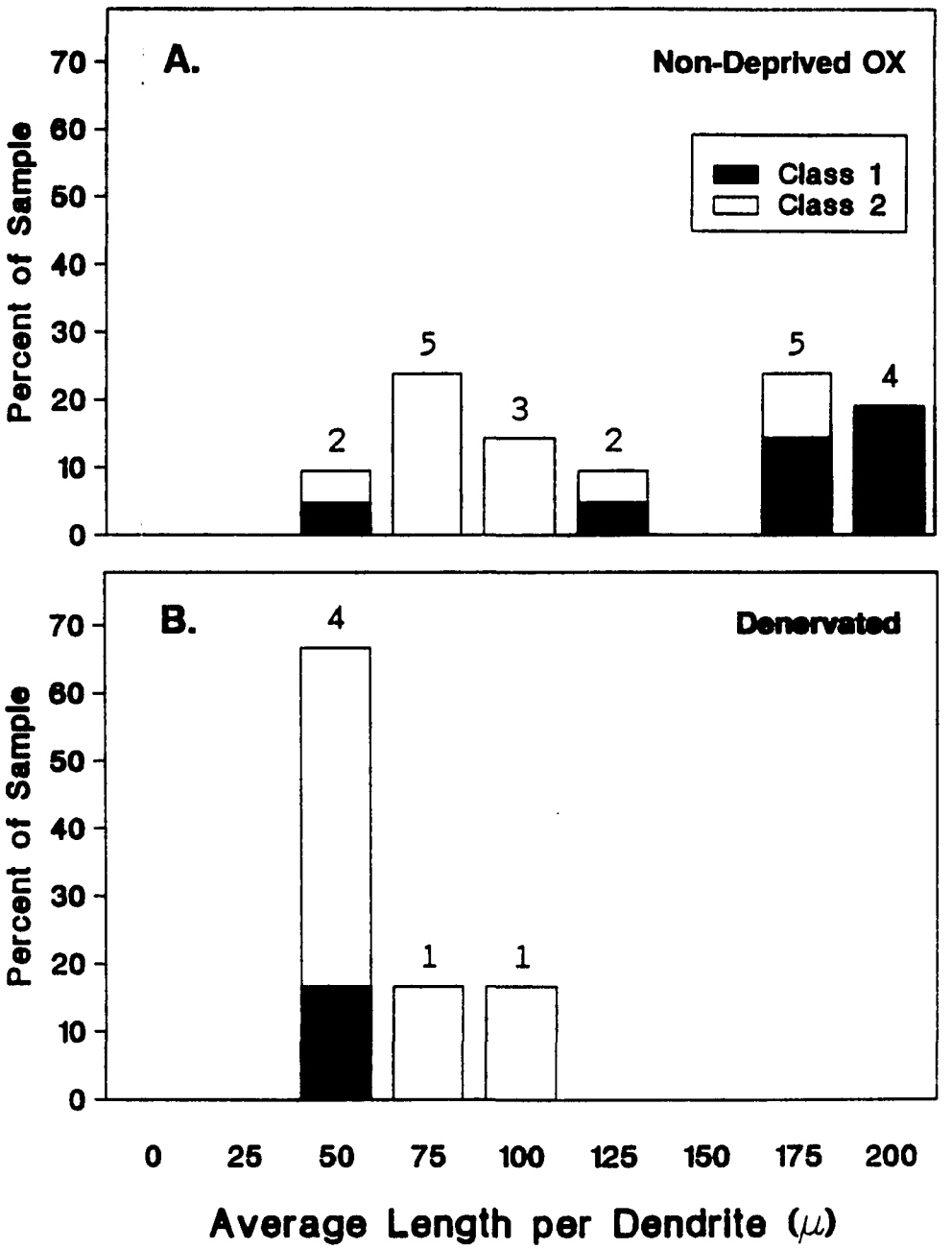
**Effect of Squint on the Average Length per Dendrite in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



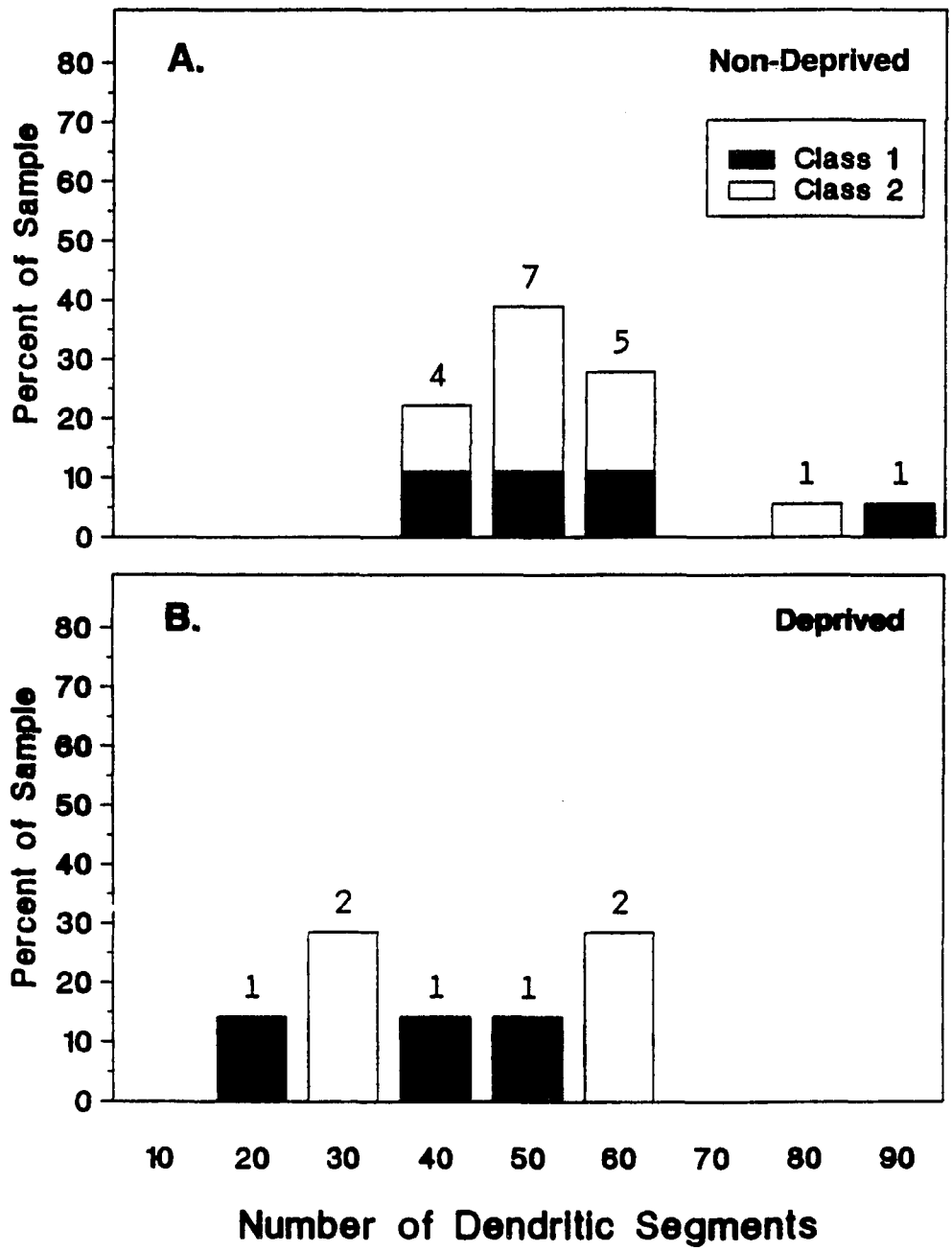
**Effect of Squint and Chiasm Section Combined On the Average Length per Dendrite in Class 1 and Class 2 LGN Cells**



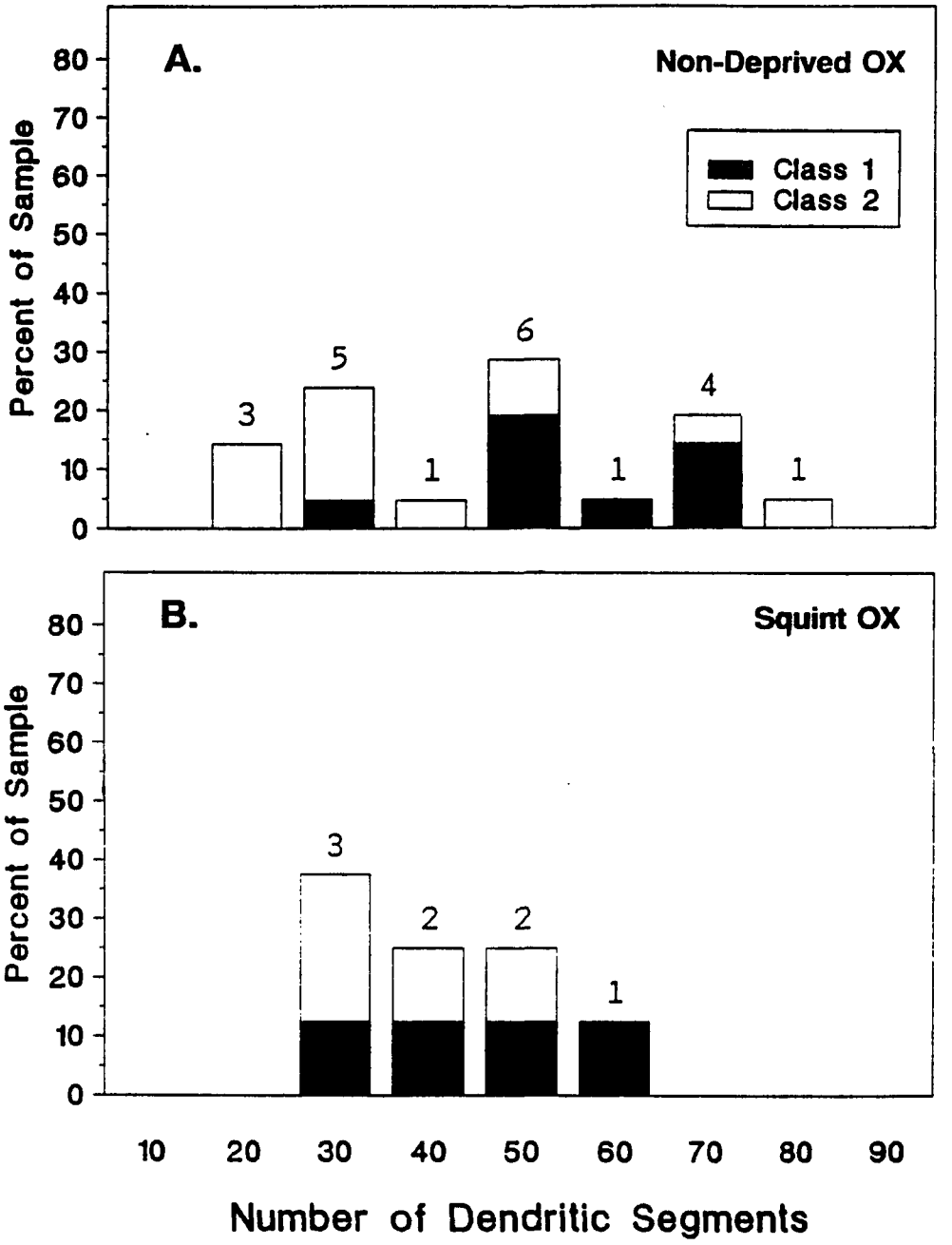
## Effect of Denervation on the Cumulative Dendritic Length in Class 1 and Class 2 LGN Cells



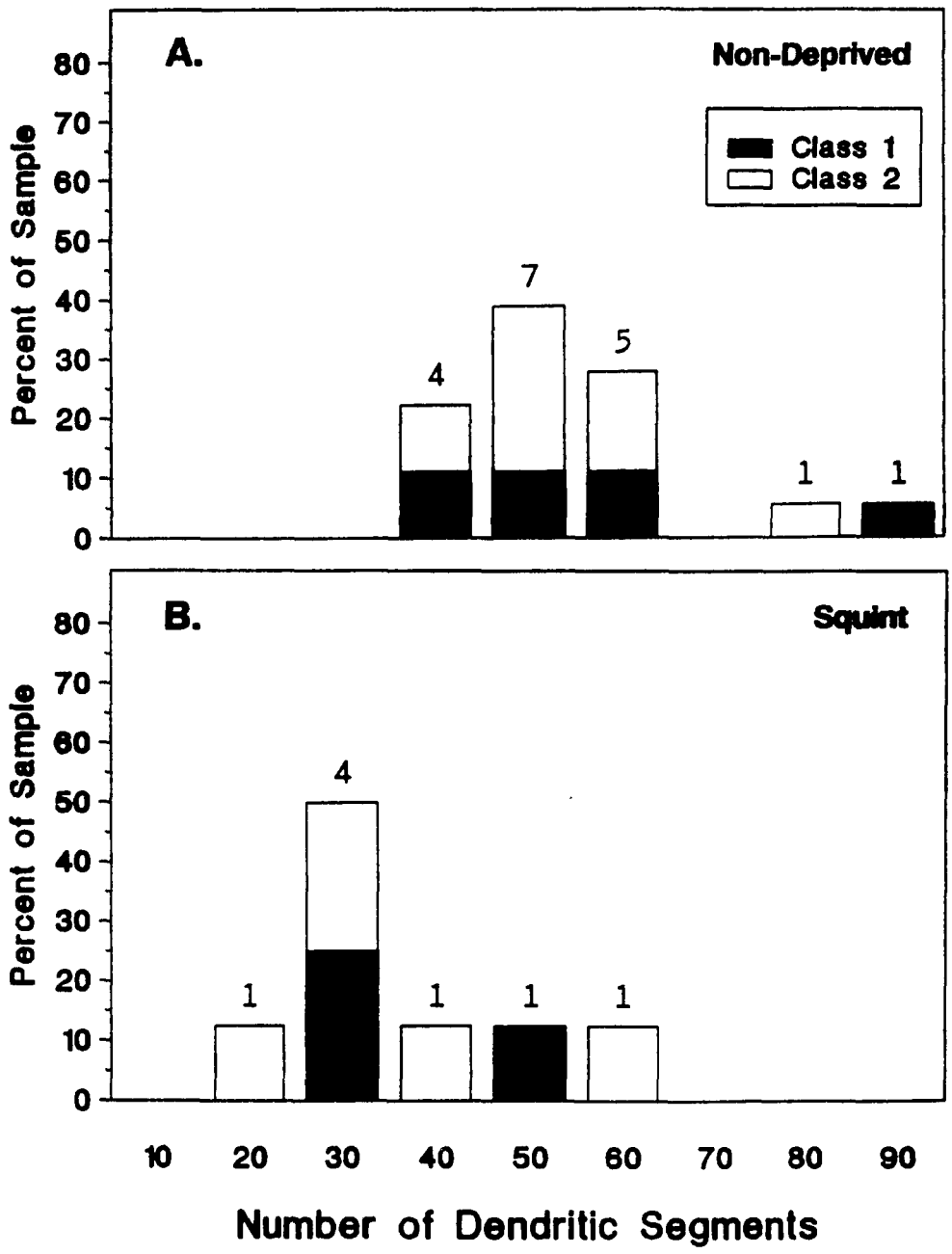
**Effect of Deprivation on the Number of Dendritic Segments in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



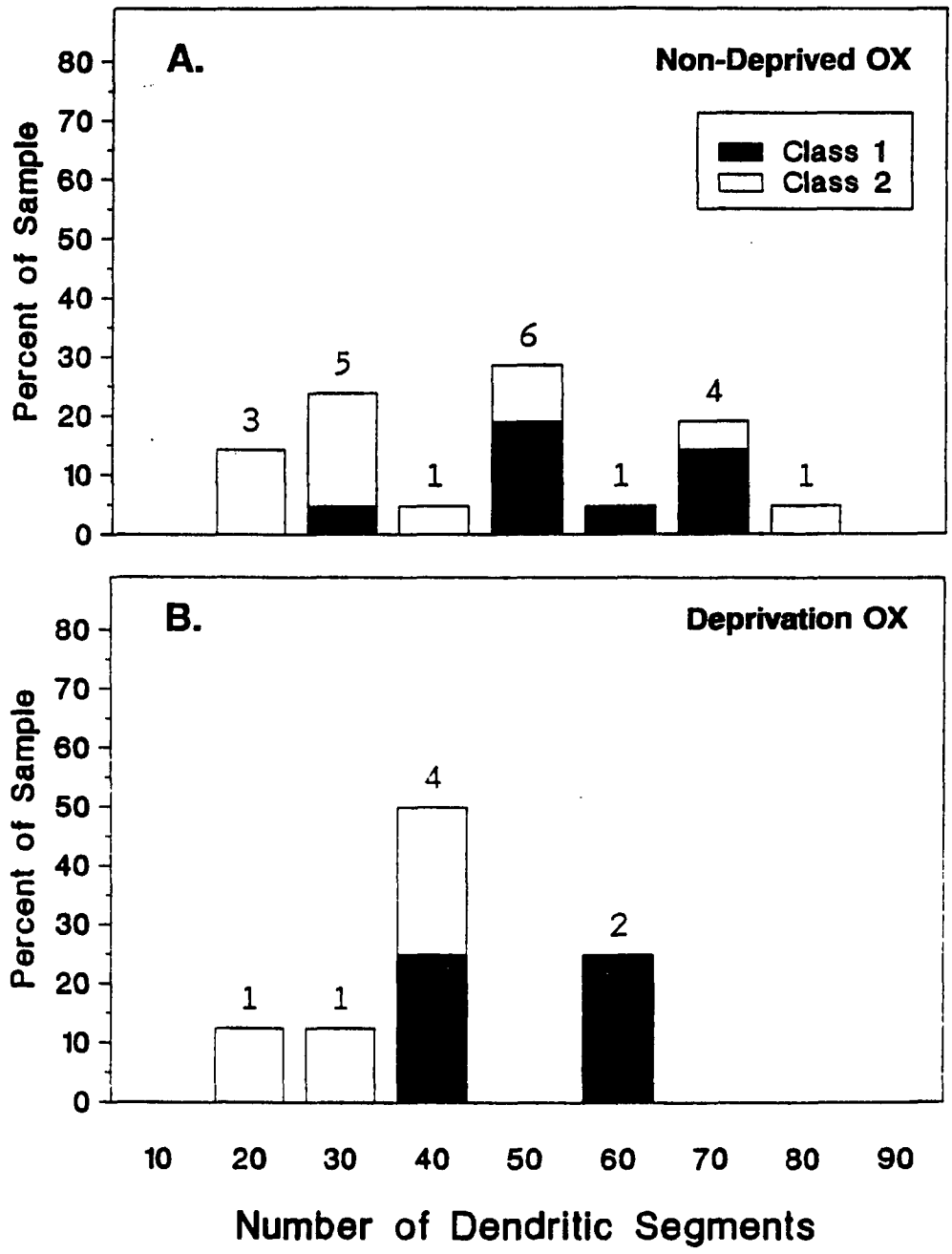
**Effect of Squint and Chiasm Section Combined On The Number of Dendritic Segments in Class 1 and Class 2 LGN Cells**



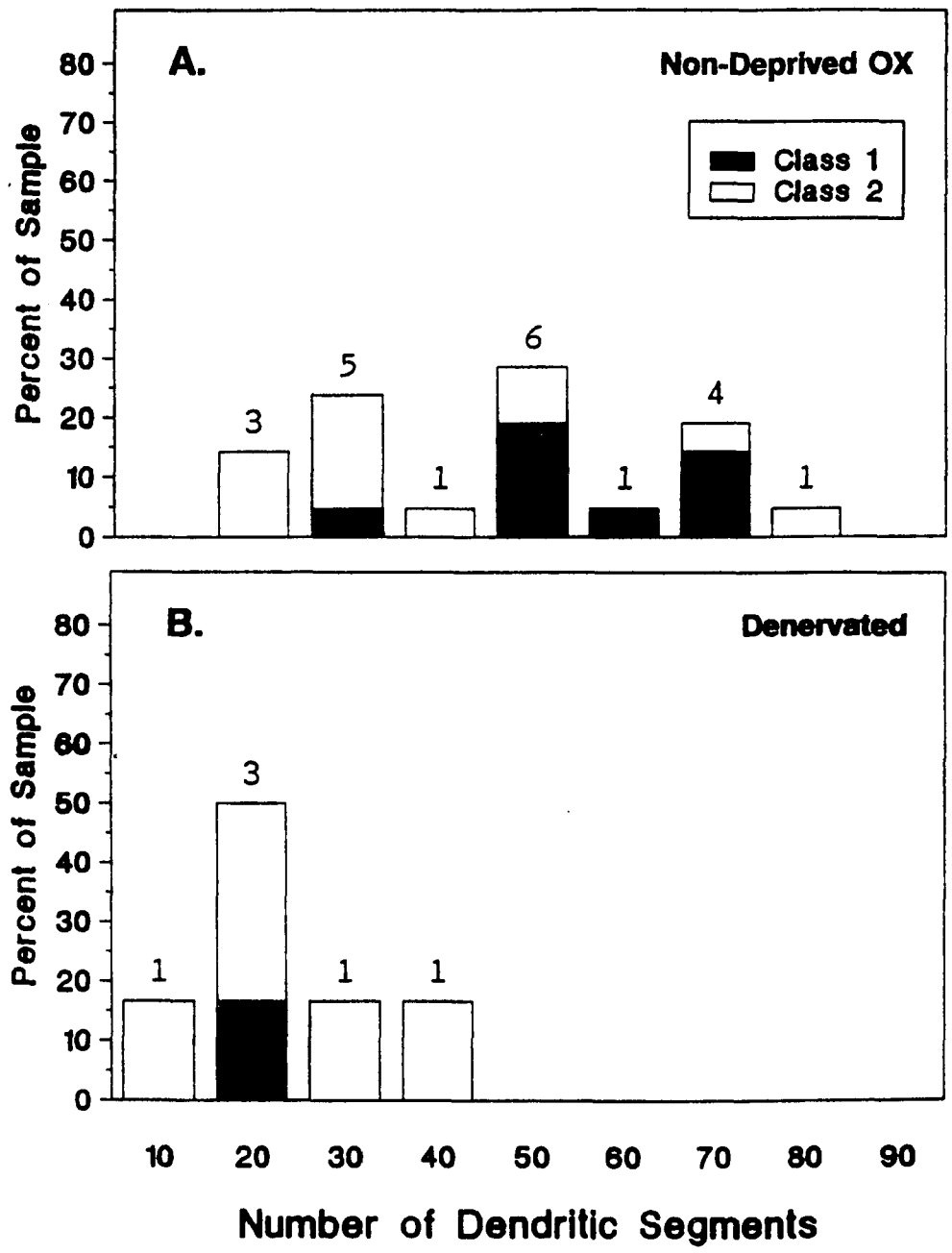
**Effect of Squint on the Number of Dendritic Segments in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



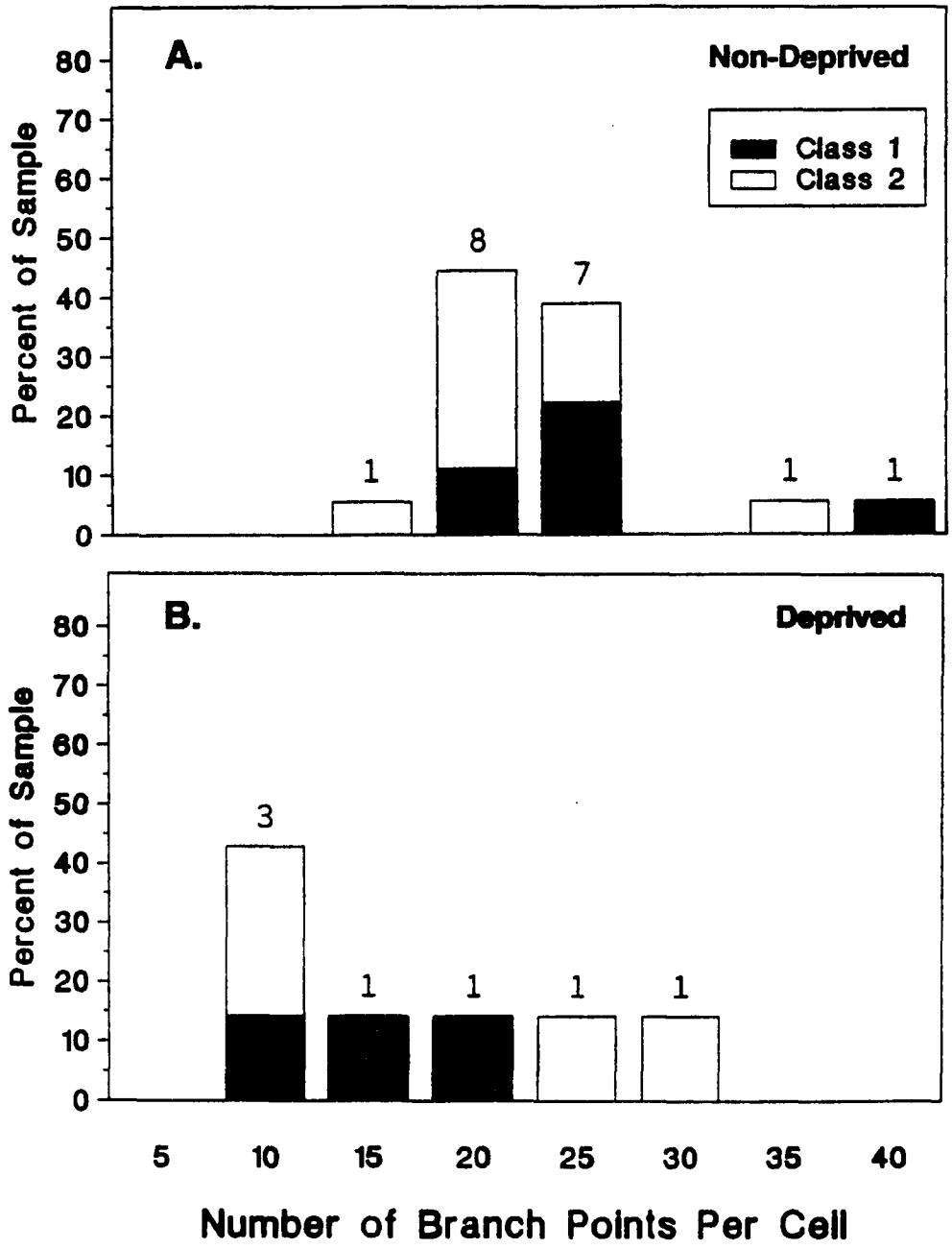
**Effect of Depivation and Chiasm Section Combined On The Number of Dendritic Segments in Class 1 And Class 2 LGN Cells**



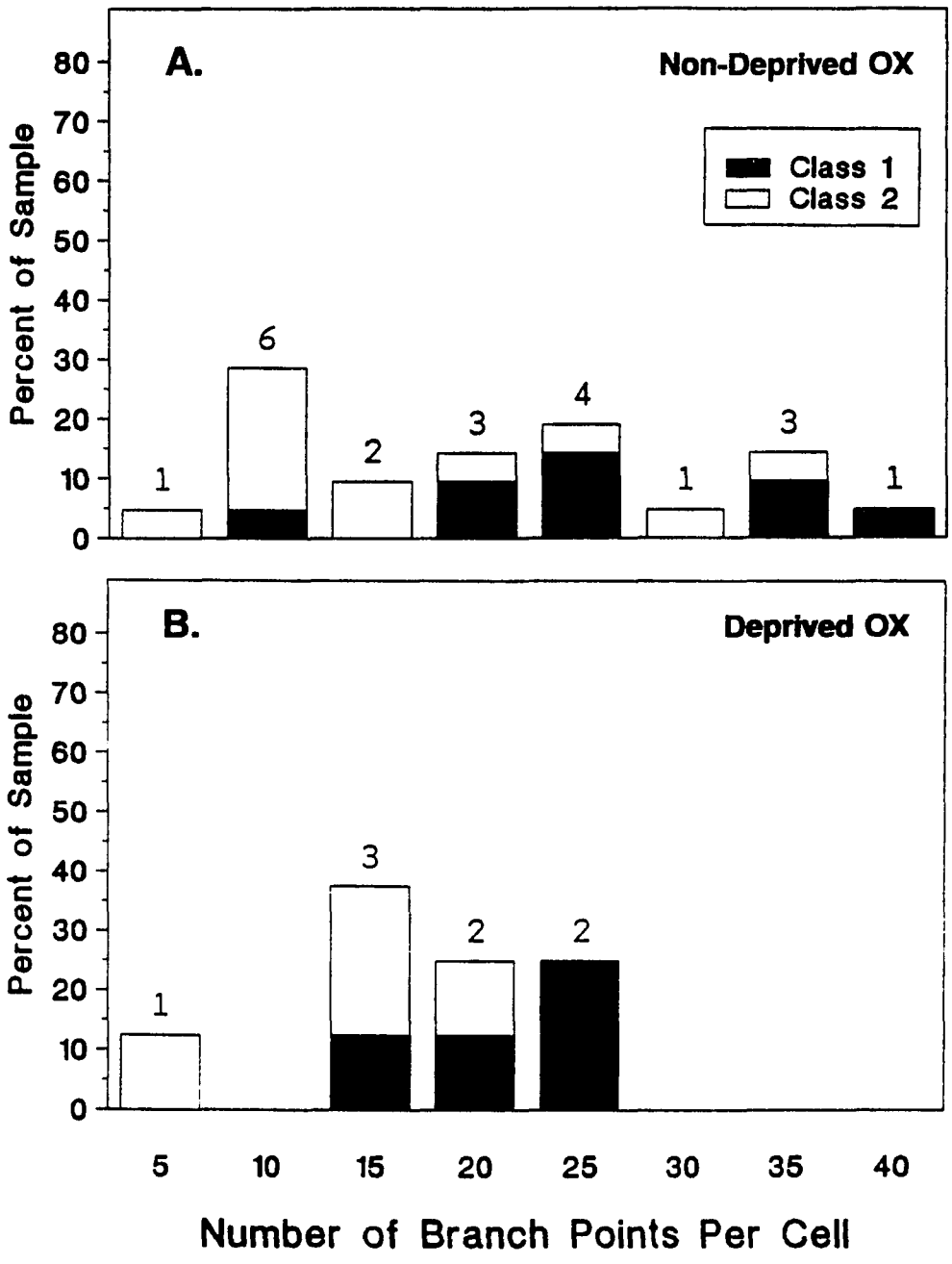
## Effect of Denervation on the Number of Dendritic Segments in Class 1 and Class 2 LGN Cells



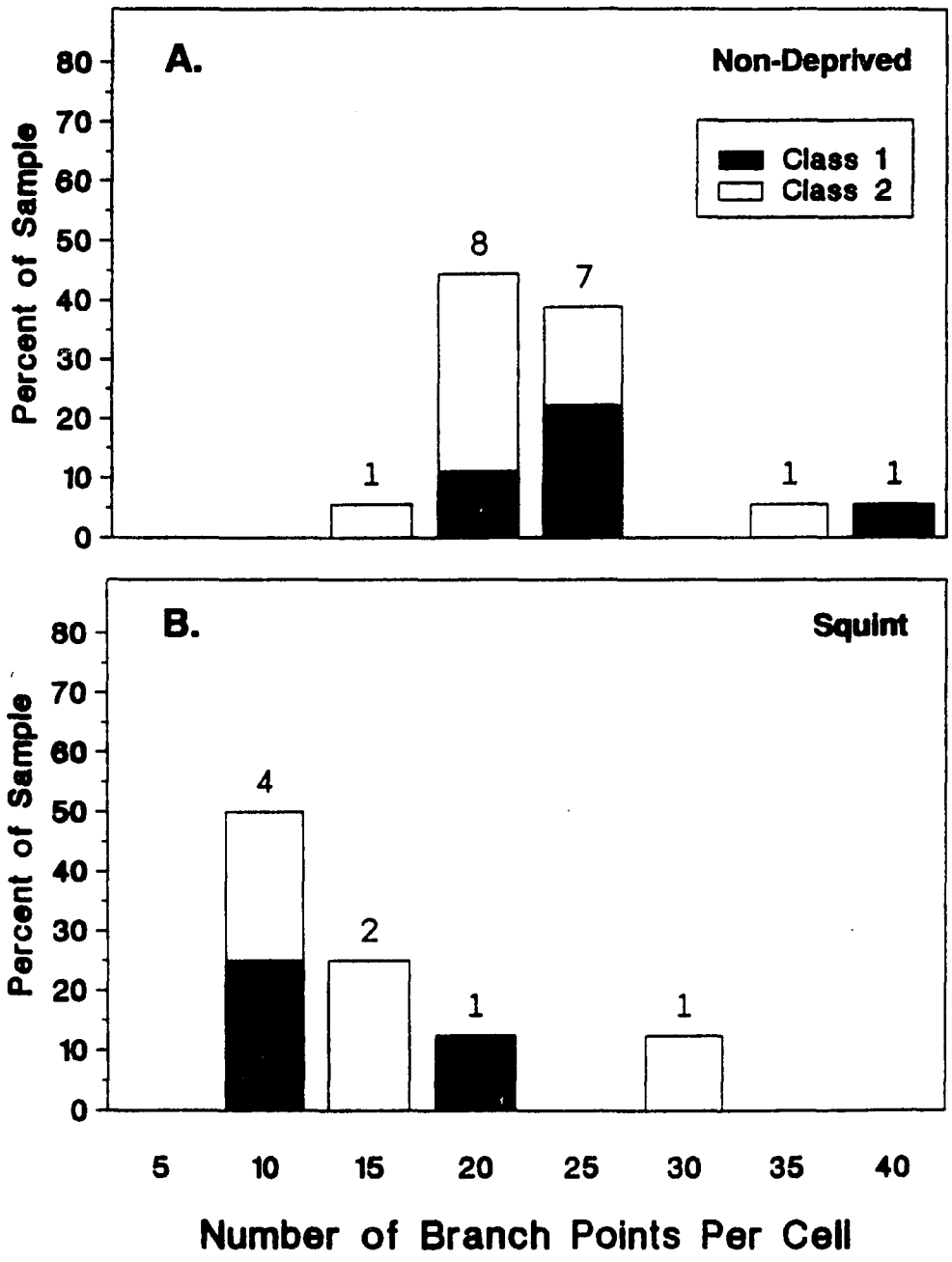
**Effect of Deprivation on the Number of Branch Points in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



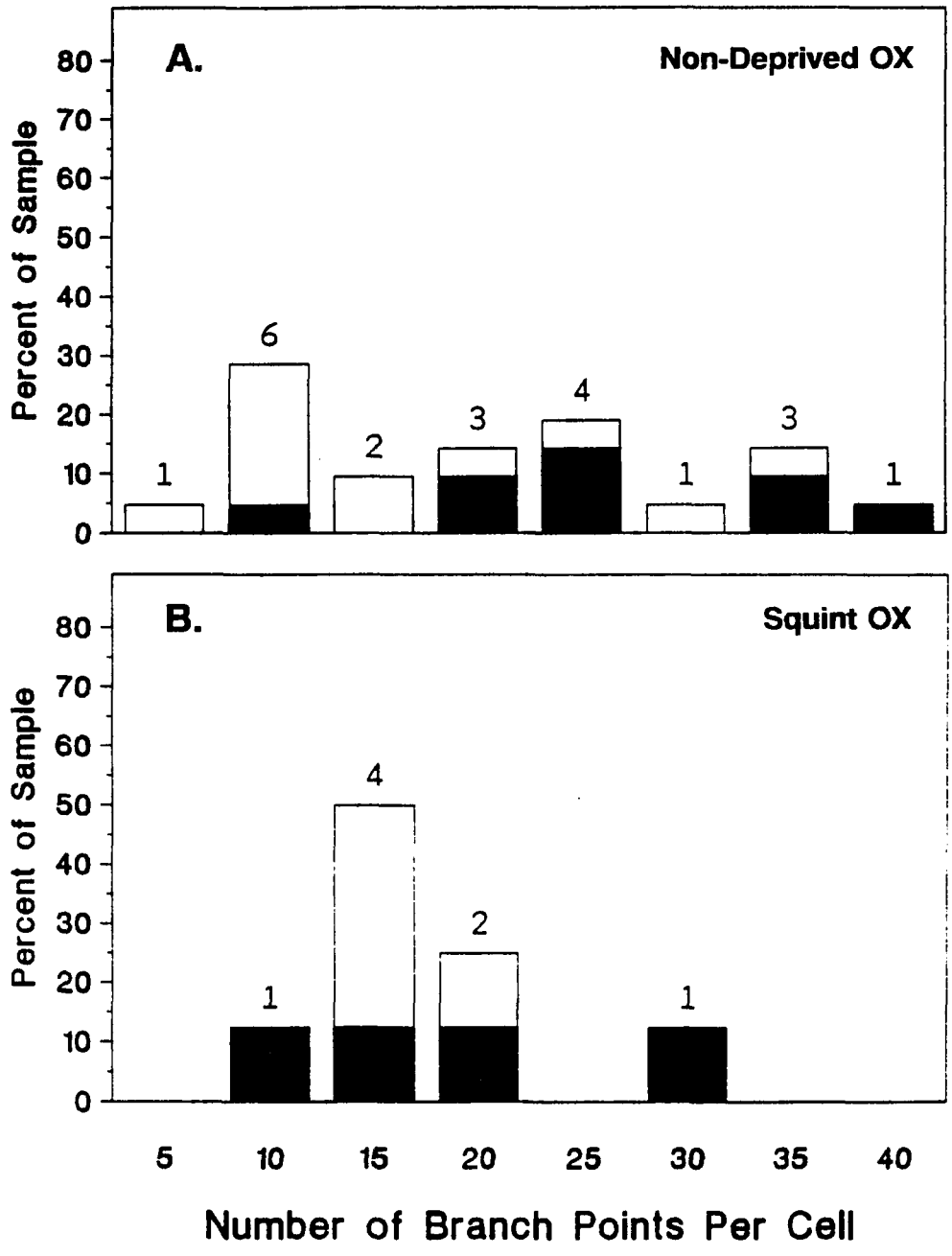
**Effect of Deprivation and Chiasm Section Combined On The Number of Branch Points in Class 1 and Class 2 LGN Cells**



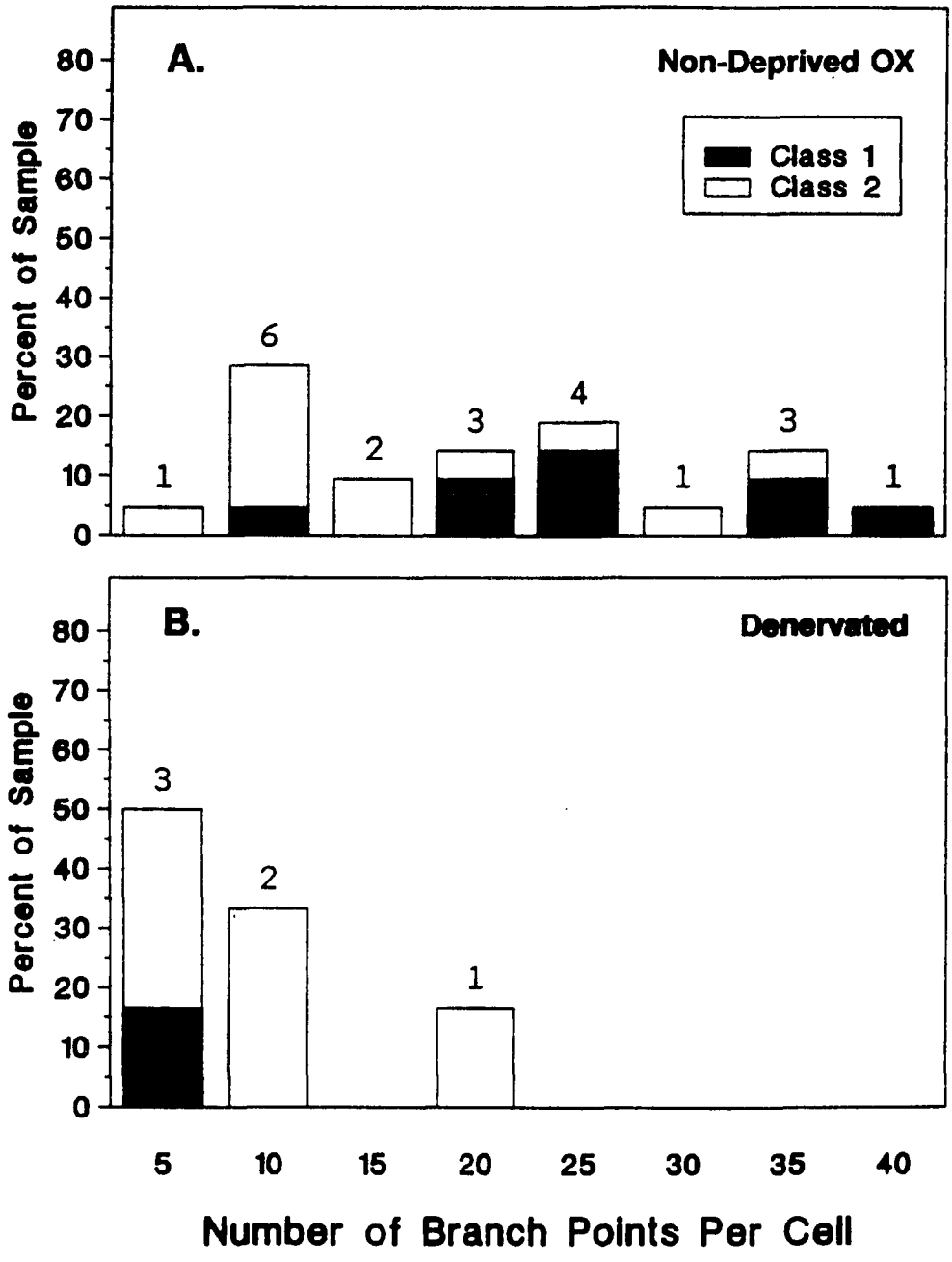
**Effect of Squint on the Number of Branch Points in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



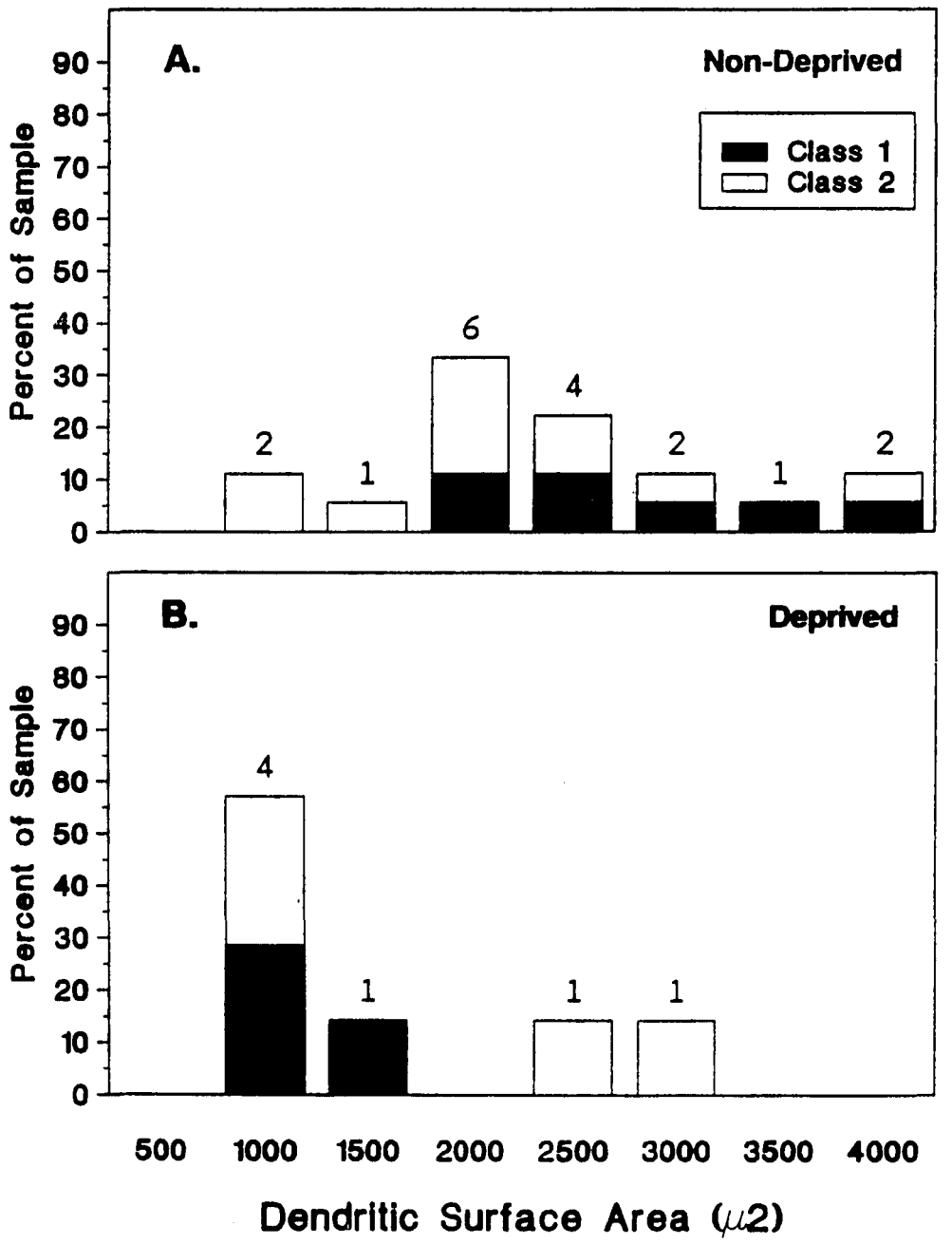
## Effect of Squint and Chiasm Section Combined On The Number of Branch Points in Class 1 and Class 2 LGN Cells



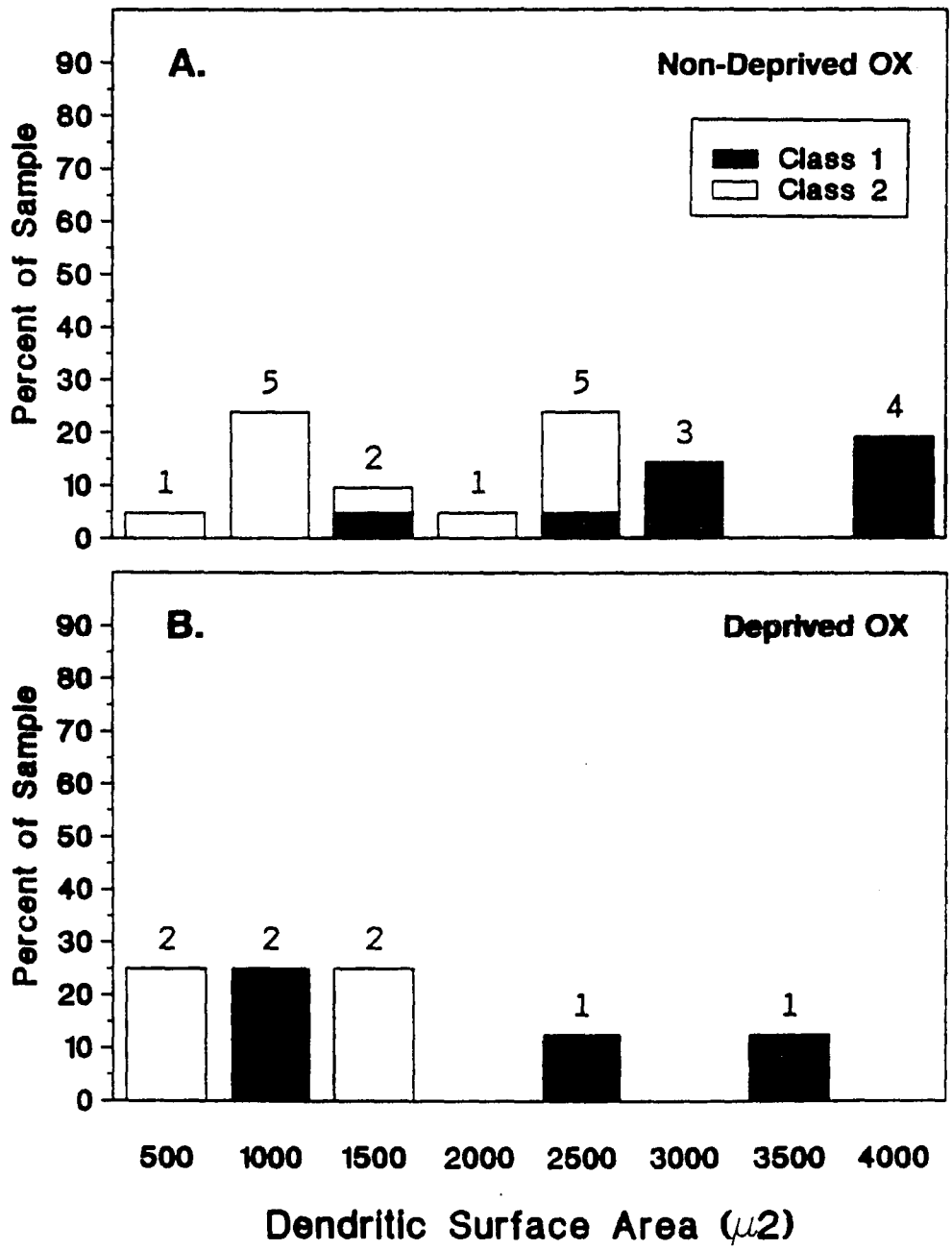
## Effect of Denervation on the Number of Branch Points in Class 1 and Class 2 LGN Cells



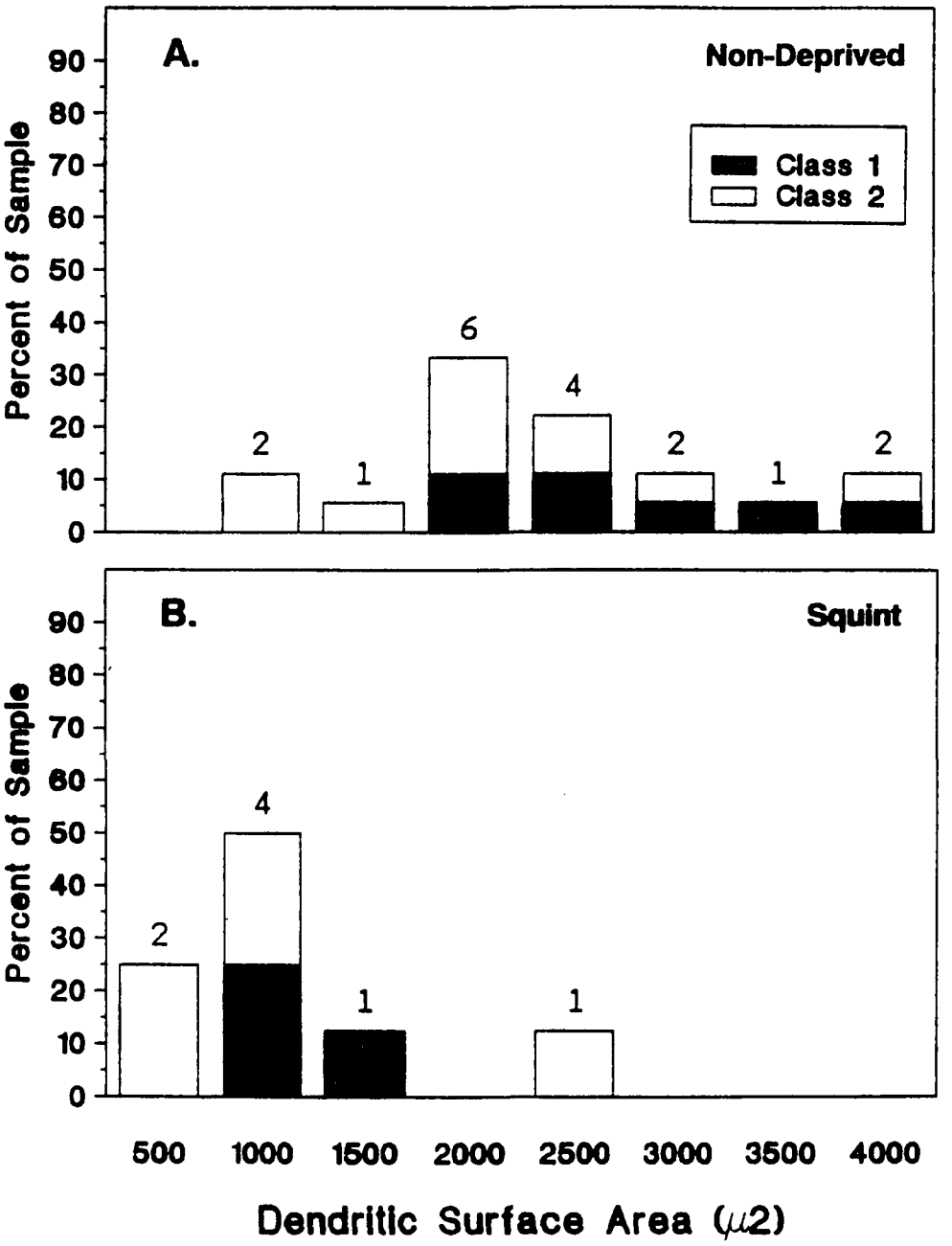
**Effect of Deprivation on Cumulative Dendritic Surface Area in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



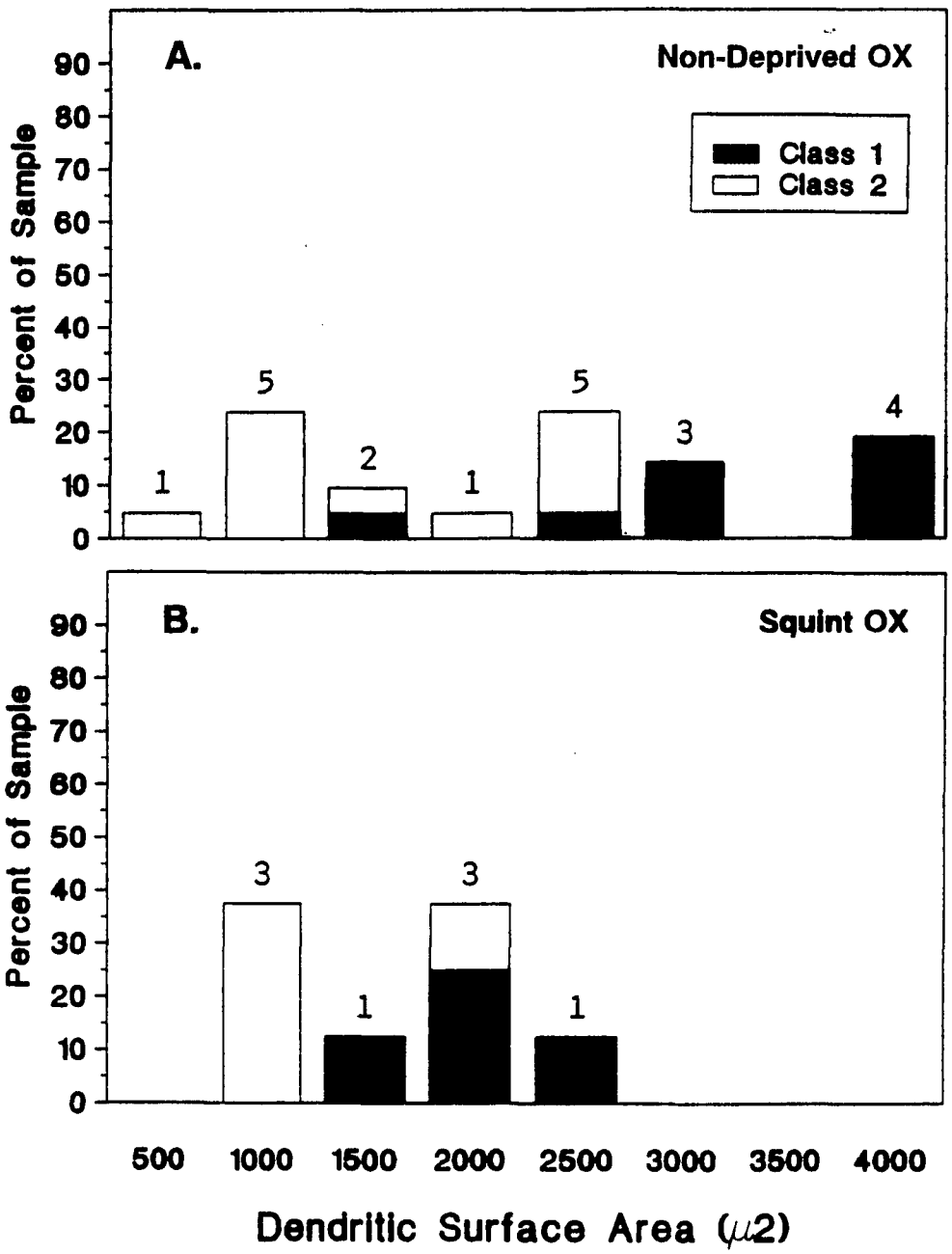
## Effect of Deprivation and Chiasm Section Combined On Cumulative Dendritic Surface Area in Class 1 And Class 2 LGN Cells



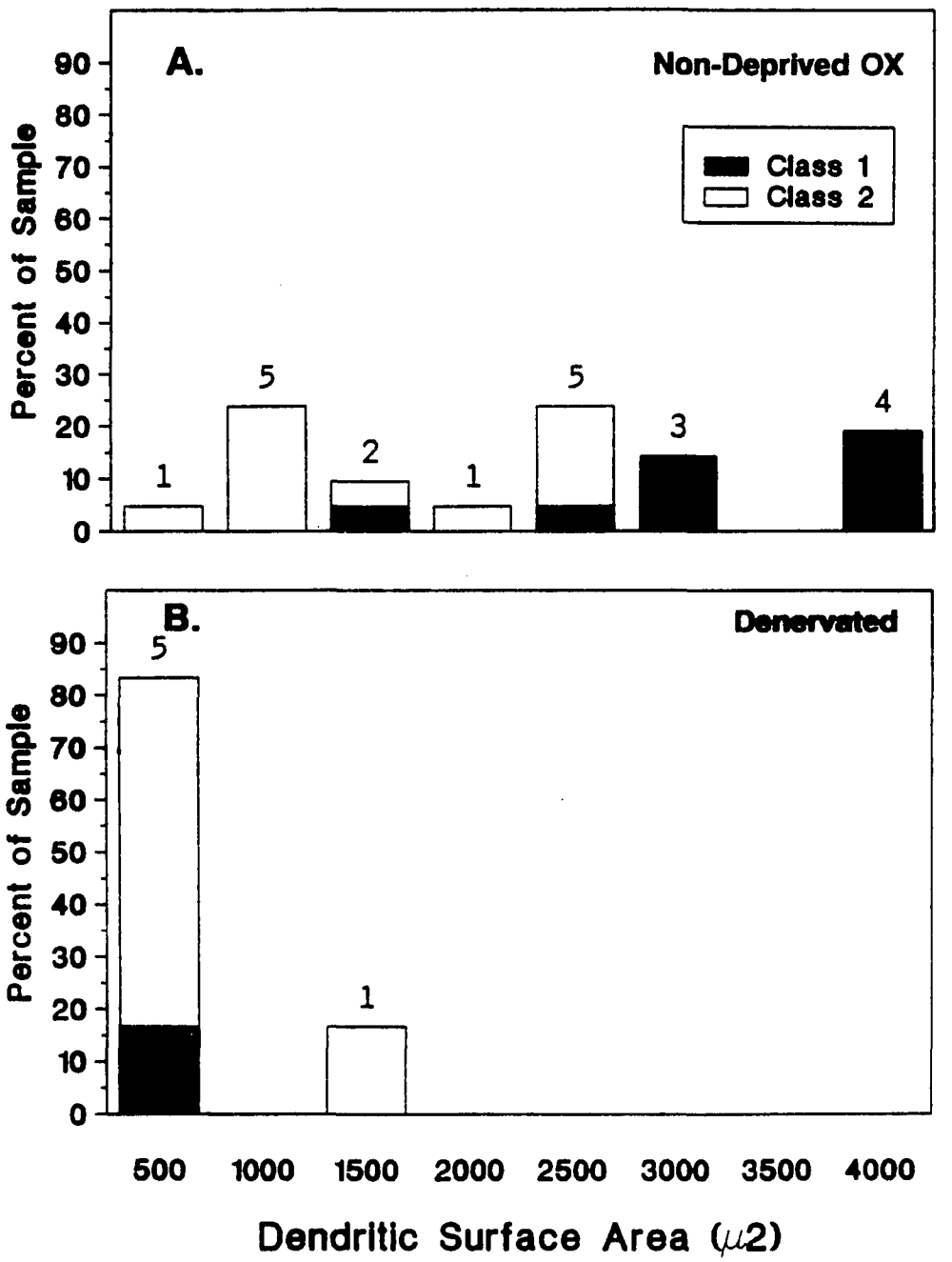
**Effect of Squint on Cumulative Dendritic Surface Area in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



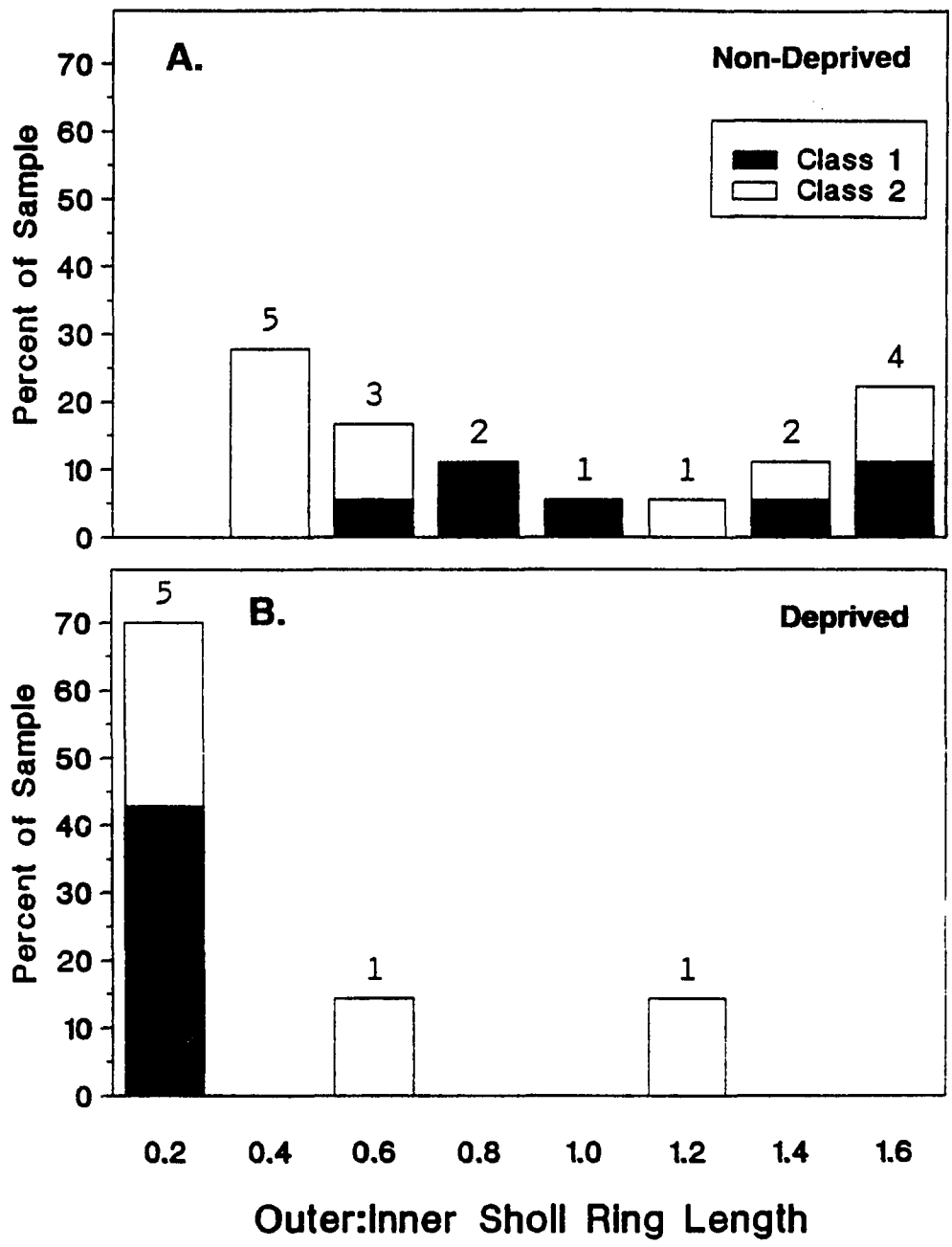
## Effect of Squint and Chiasm Section Combined On Cumulative Dendritic Surface Area in Class 1 And Class 2 LGN Cells



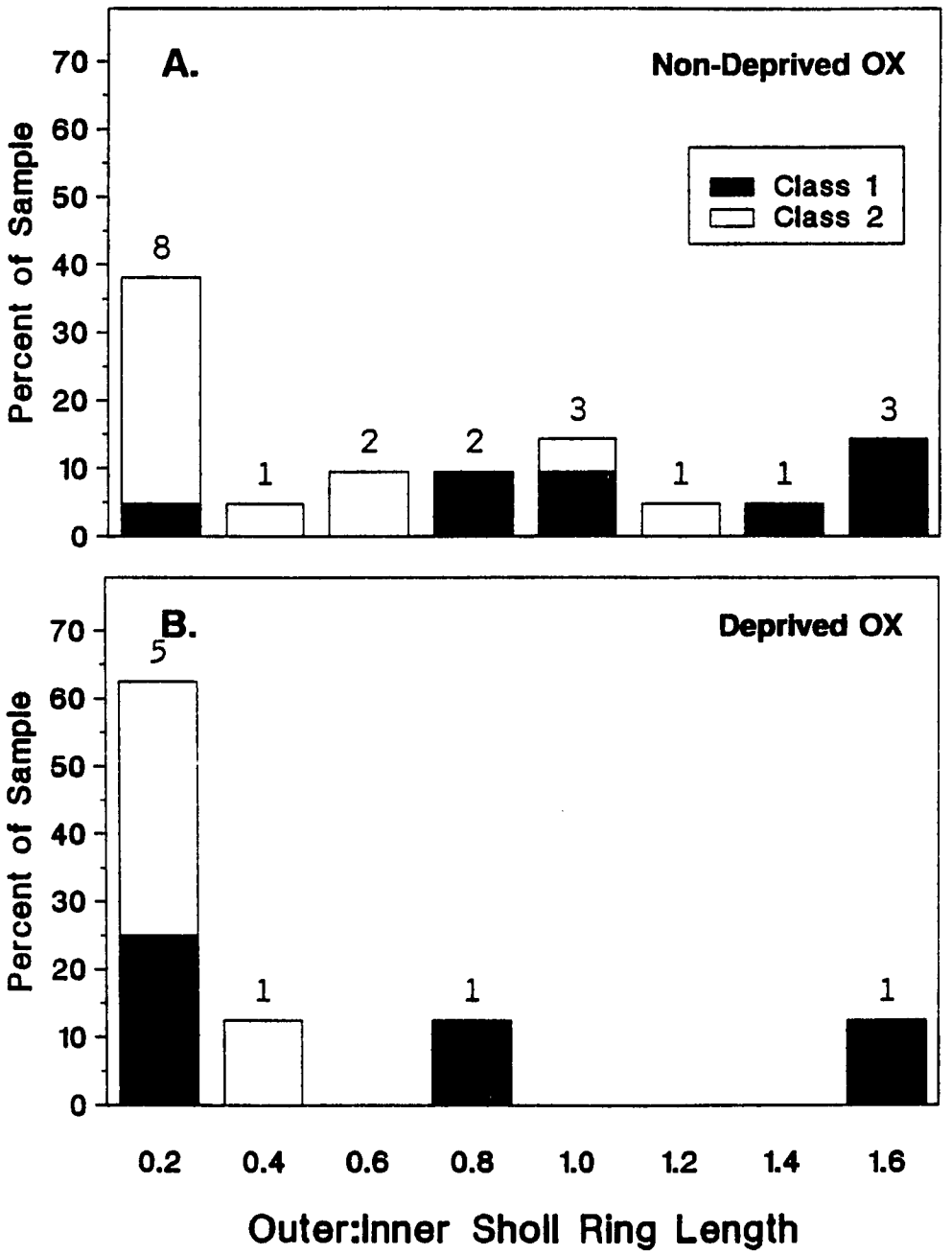
## Effect of Denervation on the Cumulative Dendritic Surface Area in Class 1 and Class 2 LGN cells



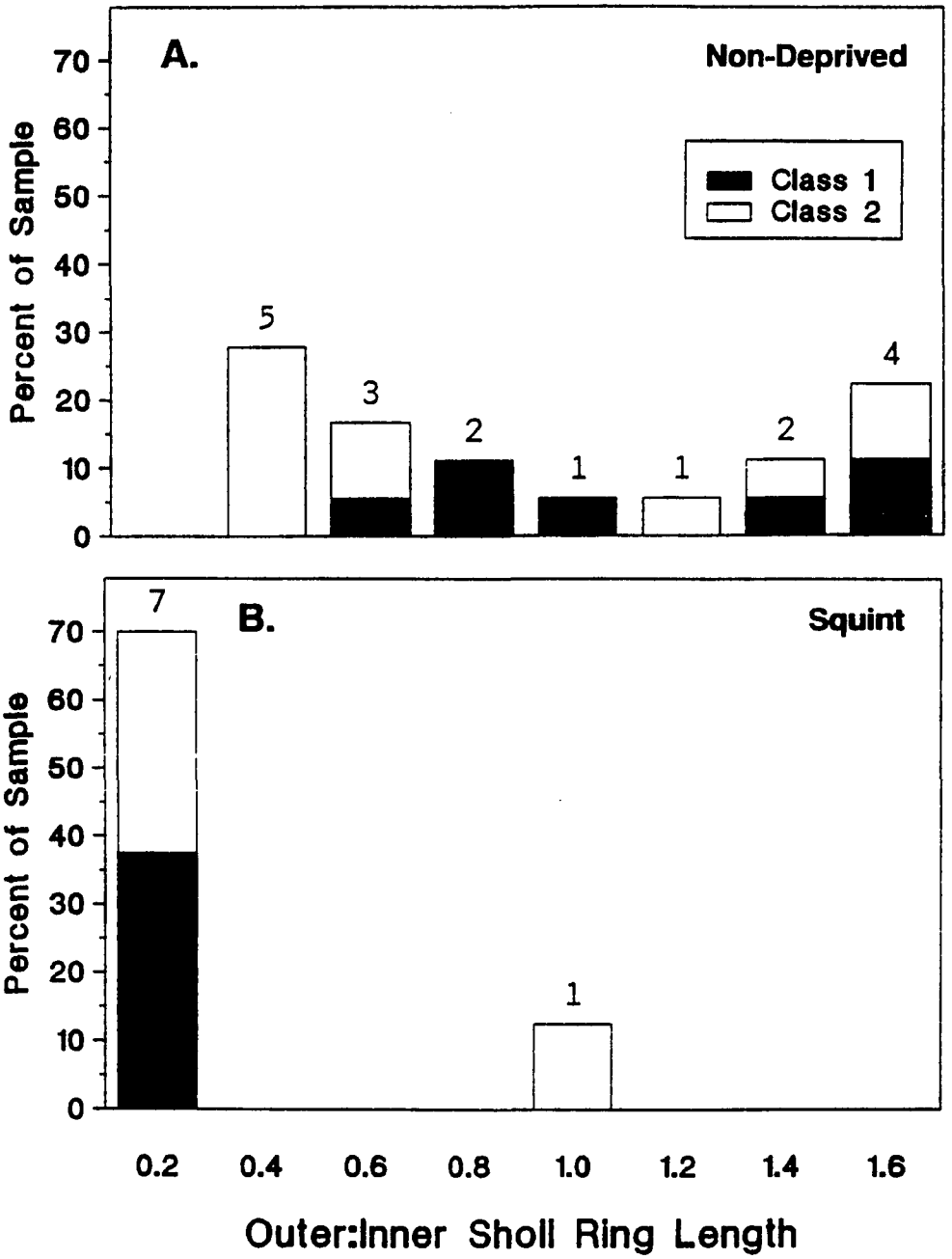
**Effect of Deprivation on the Ratio of Outer:Inner Sholl Ring Length in Class 1 and Class 2 Cells From Cats With an Intact Optic Chiasm**



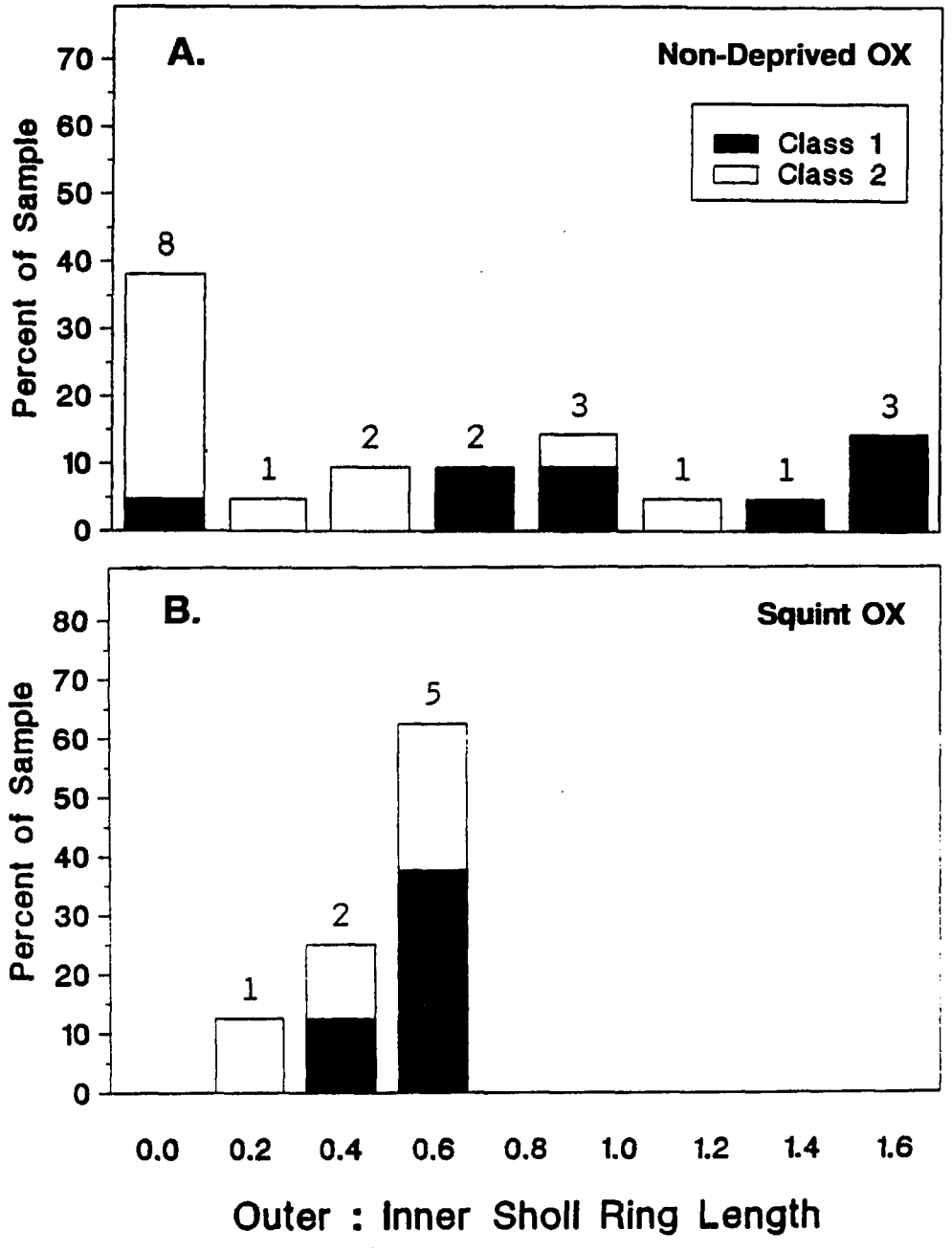
**Effect of Deprivation and Chiasm Section Combined On the Ratio of Outer:Inner Sholl Ring Length in Class 1 and Class 2 LGN Cells**



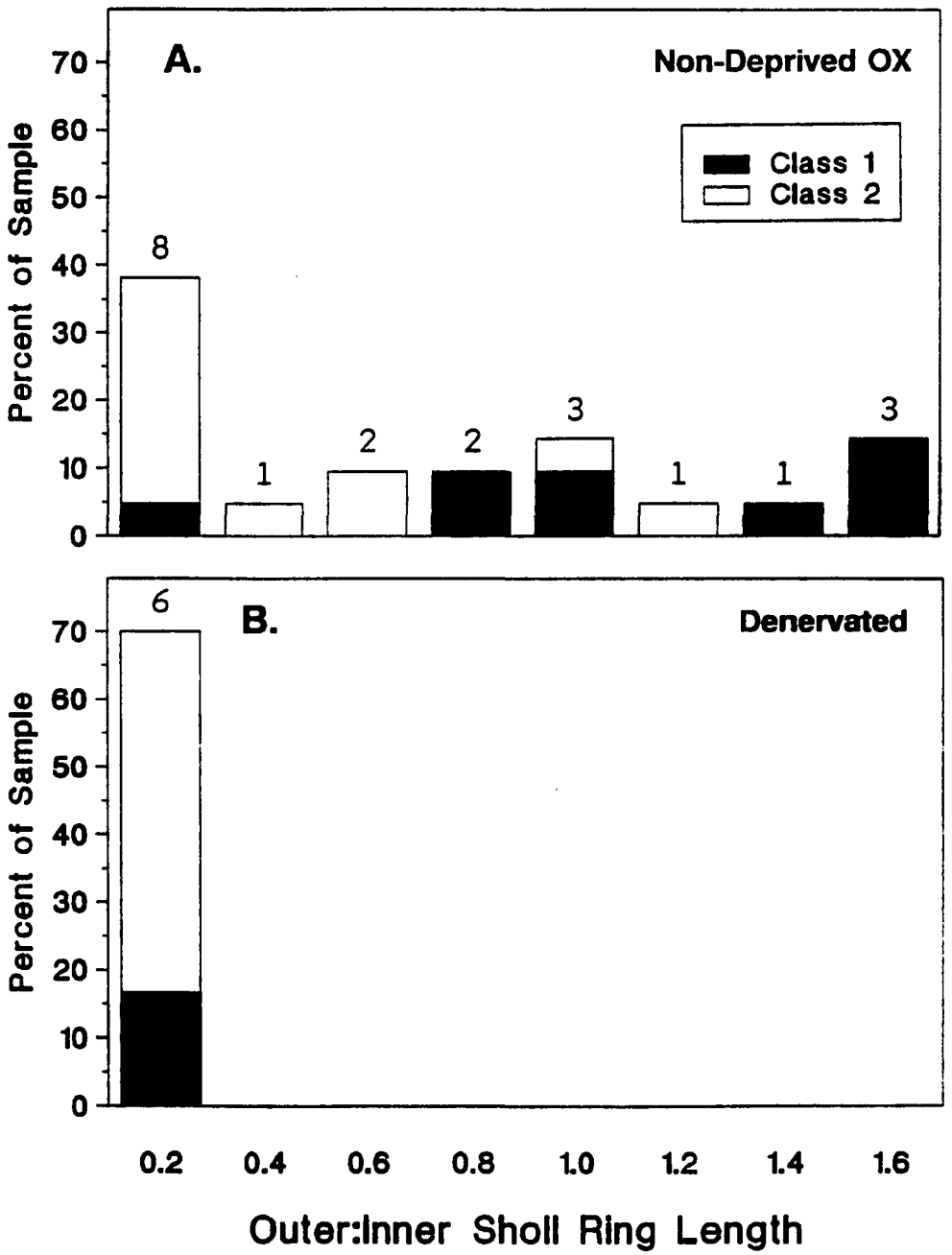
**Effect of Squint on the Ratio of Outer:Inner Sholl Ring Length in Class 1 and Class 2 Cells From Cats With an Intact Optic Chiasm**



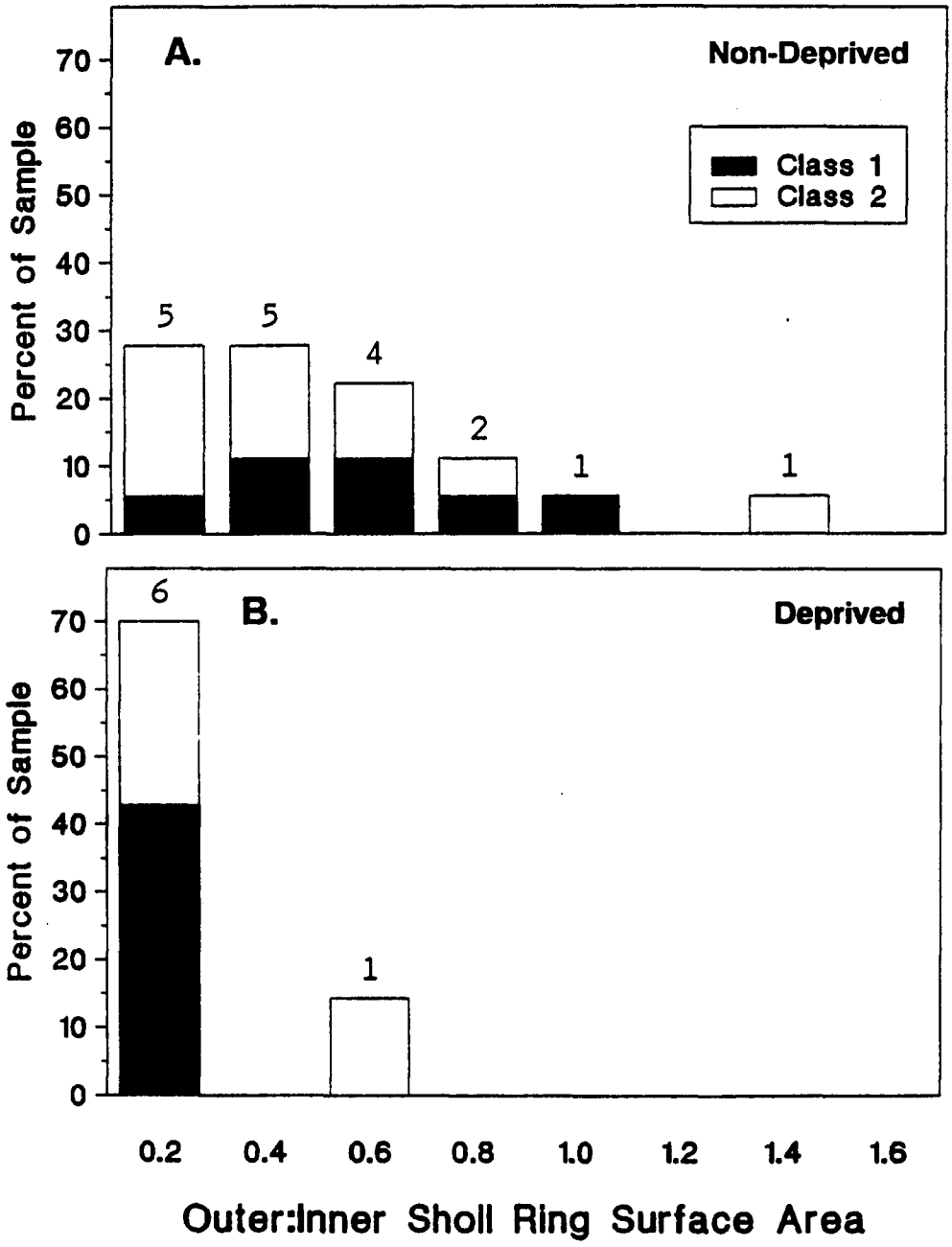
**Effect of Squint and Chiasm Section Combined On the Ratio of Outer:Inner Sholl Ring Length in Class 1 and Class 2 LGN Cells**



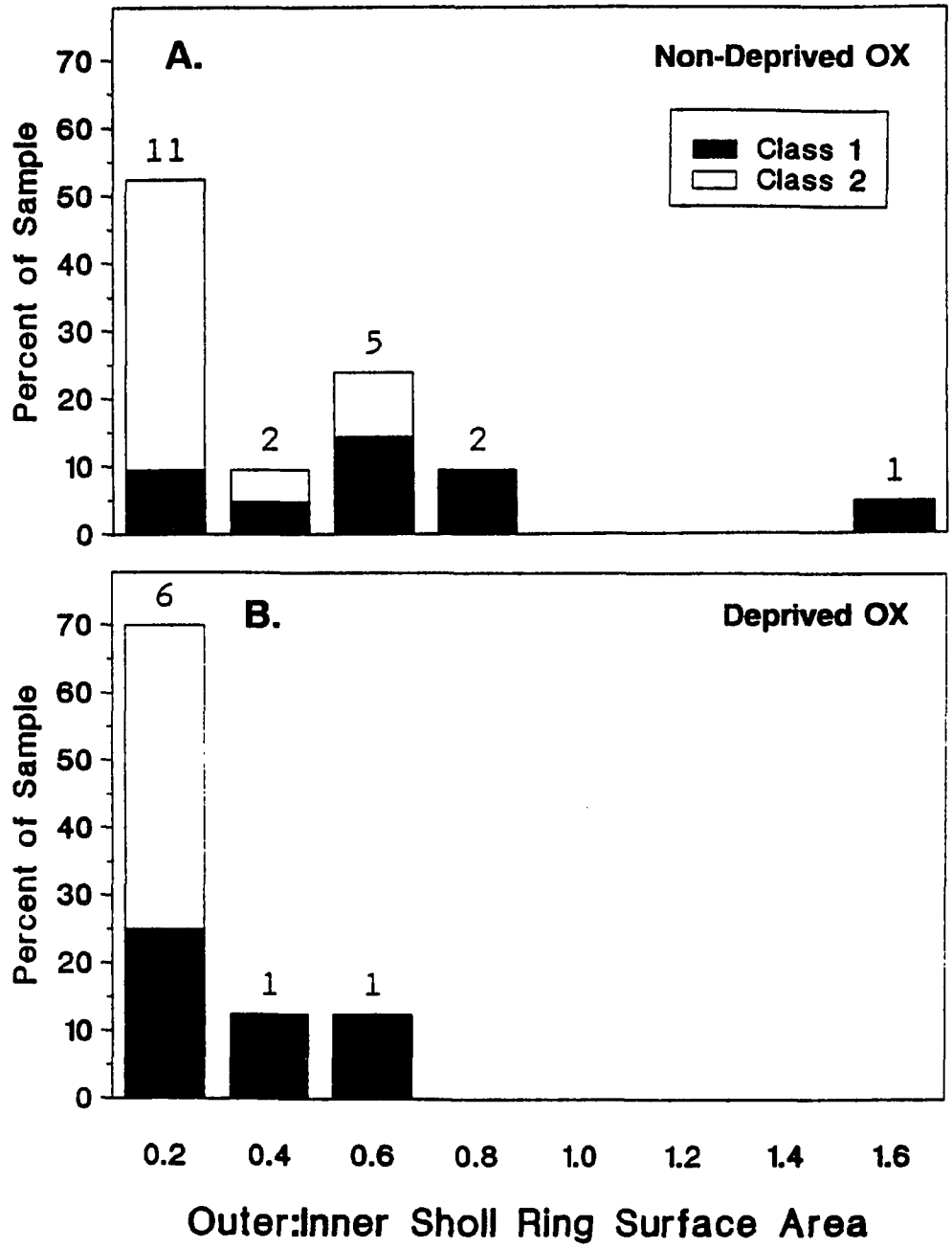
## Effect of Denervation on the Ratio of Outer:Inner Sholl Ring Length in Class 1 and Class 2 Cells



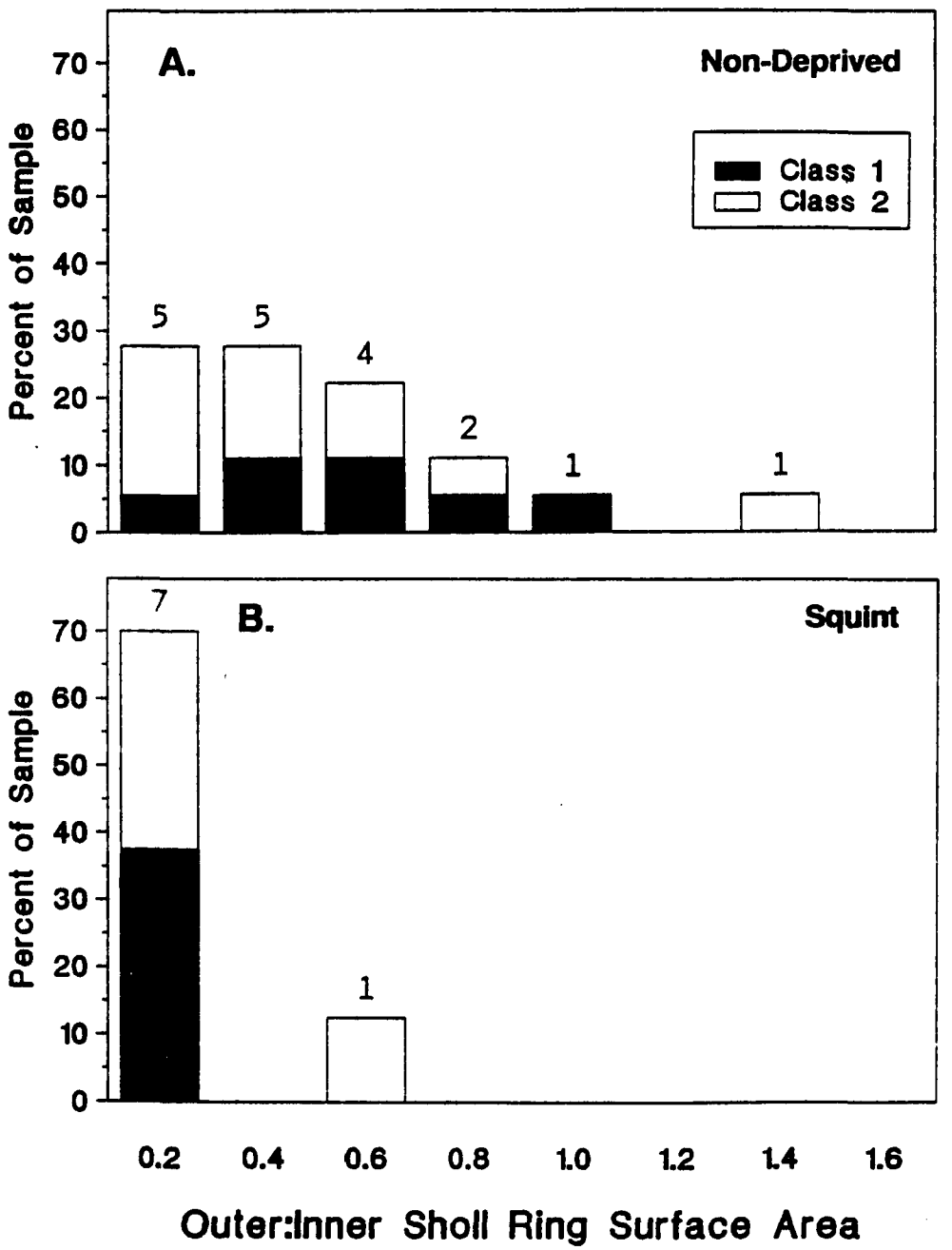
**Effect of Deprivation on the Ratio of Outer:Inner Sholl Ring Surface Area in Class 1 and Class 2 LGN Cells From Cats With an Intact Optic Chiasm**



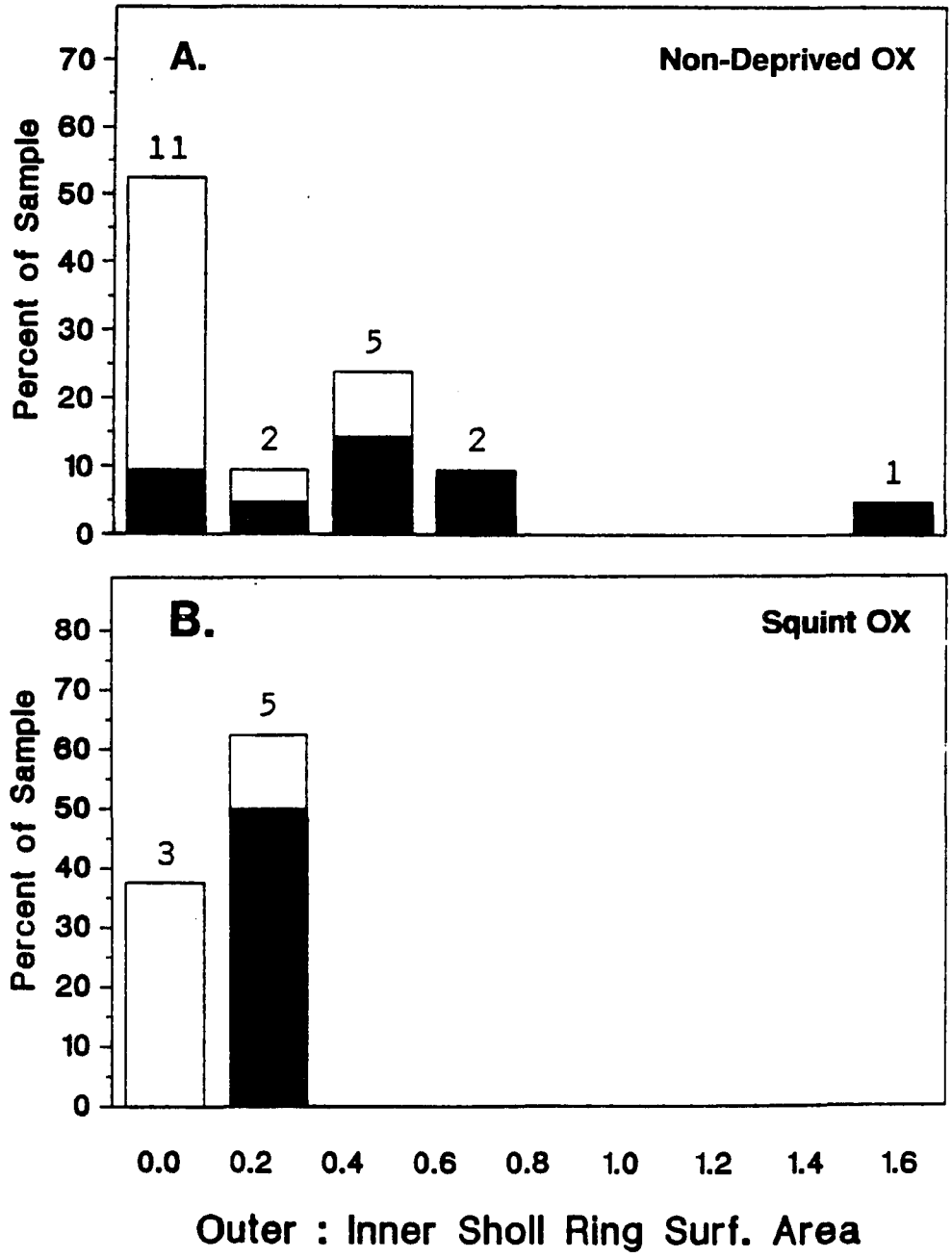
**Effect of Deprivation and Chiasm Section Combined On the Ratio of Outer:Inner Sholl Ring Surface Area In Class 1 and Class 2 LGN Cells**



**Effect of Squint on the Ratio of Outer:Inner Sholl Ring Length in Class 1 and Class 2 Cells From Cats With an Intact Optic Chiasm**



**Effect of Squint and Chiasm Section Combined On the Ratio of Outer:Inner Sholl Ring Surface Area In Class 1 and Class 2 LGN Cells**



**Effect of Denervation on the Ratio of Outer:Inner Sholl Ring Surface Area in Class 1 and Class 2 LGN Cells**