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Development of Cell Size in the Medial Interlaminar Nucleus of Normal and Monocularly Deprived Kittens

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Cross-sectional areas of somata in the medial interlaminar nucleus (MIN) and lamina A of the cat were measured at different ages to determine neuronal growth characteristics. Normal and monocularly lid-sutured kittens of ages 2 – 16 weeks plus several adults were studied. During development of normal kittens, we observed that MIN cells grew until about 8 weeks of age and were larger, on average, than cells in lamina A at all ages. The growth pattern was similar for cells in both MIN and lamina A. In monocularly deprived kittens, a deprived/nondeprived cell size difference began to appear between 3 and 5 weeks of age and continued to increase until about 8 weeks of age. We conclude that monocular lid-suturing affects the size of MIN neurons much as it does neurons in lamina A, and the effect is concurrent with the period of normal growth.

INTRODUCTION

The medial interlaminar nucleus (MIN) of the cat has received attention in recent years due in part to evidence indicating that most of its neurons can be electrophysiologically classified as Y-cells[2,9–12,16]; (however, see ref. 13). Because a homogeneous population of neurons has obvious advantages for developmental studies, we recently completed such a study of the electrophysiological properties of MIN neurons[16]. The present study, which complements our prior one, describes the postnatal growth of the cross-sectional area of MIN neurons. We examined and compared the growth curves of MIN neurons in normal and monocularly deprived kittens. From these data, we hoped to gain further insights concerning the time course and mechanisms of the development of these neurons.

MATERIALS AND METHODS

Subjects

Twenty-eight kittens were used in this study. Fifteen were monocularly lid-sutured (cf. ref. 15) at 7–10 days of age and maintained in this fashion until sacrificed for histological analysis. The remaining 13 developed under normal conditions. In addition, the brains of 14 adult cats (at least 6 months of age) provided data for comparison with those of the kittens. Eight of these adults were monocularly deprived from 7 to 10 days of age, and the other 6 were normally reared cats obtained as adults.

Thirteen of the monocularly deprived kittens, 4 of the 7 monocularly deprived adults, and 4 of the normal kittens had injections of tritiated proline into the vitreous of one eye 24 h prior to sacrifice in order to determine ocular dominance boundaries for MIN[9]. In 4 of the deprived kittens, the closed, deprived eye was injected, and the others had their open, nondeprived eye injected. The 4 adults with eye injections (2 in the deprived eye and 2 in the nondeprived eye) were used as part of a previous study[10]. The remaining 2 kittens and 3 adults reared with monocular deprivation had only the cells in lamina A measured and, therefore, required no eye injection.

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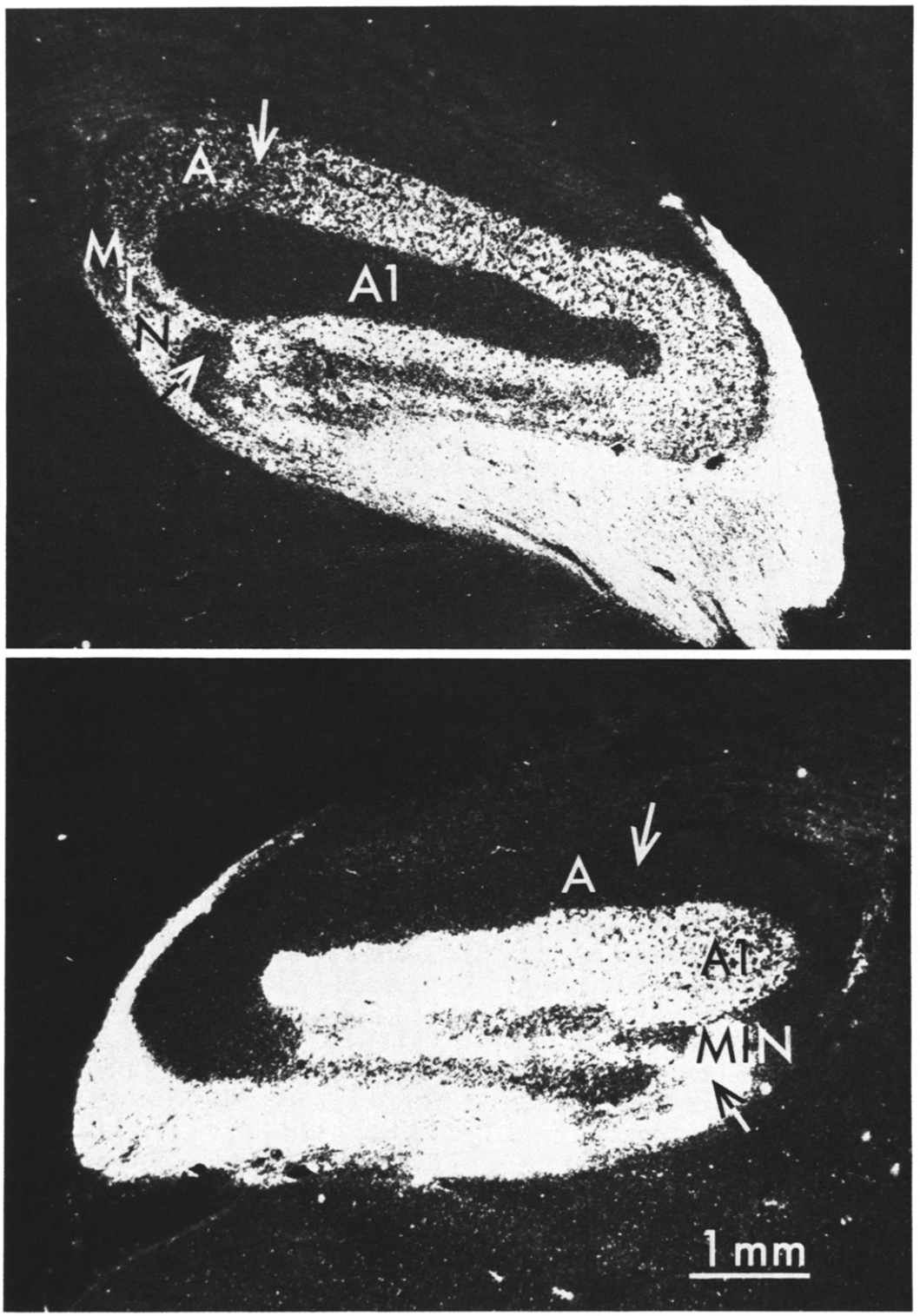

Fig. 1. Dark-field photomicrographs of the left (bottom) and right (top) dorsal lateral geniculate nuclei of a 10-week-old normal kitten. The left eye was injected with tritiated proline 24 h prior to sacrifice in order to identify clearly the termination areas in MIN of the right and left eyes. Arrows indicate the areas where cell size measurements were made for MIN and lamina A.

Histology

All animals were perfused transcardially with saline followed by 10% formalin. The brain of each animal was removed and immersed for several weeks in 10% formalin. The lateral geniculate nuclei were then blocked, embedded in egg yolk, and cut frozen in the coronal plane at 40 μm. Unfortunately, not all of the brains of the deprived kittens were processed by the same personnel and methods over the two years that these data were collected. This probably created variability between brains in terms of the absolute values of the cell sizes. Such variability would minimally affect intraanimal comparisons (e.g. between cells receiving input from the deprived or nondeprived eye), but it would significantly affect comparisons between animals (see Results).

One out of every 3 sections through the lateral geniculate nucleus was mounted on a slide. Sections which came from kittens with eye injections were dipped in photographic emulsion and exposed in the dark for 4–8 weeks prior to development[1]. All sections were stained with cresyl violet.

Soma size measurements

For cell size measurements, we chose sections that were located near the anterior/posterior center of the nucleus and had reasonably sharp borders between the separate eye terminal zones in MIN and between MIN and the adjacent C laminae (see Fig. 1). Only neurons with visible nucleoli were selected for measurement. We shall refer to the cross-sectional area of cells in this study as cell size. Once the area of MIN innervated by the injected eye was defined (by exposed silver grains over the tissue), the same zone in the MIN of the other hemisphere (defined by a lack of exposed silver grains over it) was located for the noninjected eye's terminal field. Fig. 1 illustrates typical areas in the dorsal lateral geniculate nucleus chosen for cell size measurements. The area selected for measuring cell sizes corresponds to layer 2 of the MIN as described by Guillery et al.[4]. Because of the restrictions in selecting a MIN area to be used for the measurements, we limited our sample size to 25 neurons. In several cases, two adjacent sections had to be used to obtain these 25 neurons. For the lamina A cell samples in kittens, 25 neurons were drawn near the mediolateral center of the lamina on each side of the brain using one of the same sections that was selected for the MIN sample (see arrows in Fig. 1). In the adult animals, however, 50 cells were measured in lamina A. Layer 2 of MIN has a temporal, uncrossed retinal input and lamina A has a nasal, crossed retinal input. Although it might have been more appropriate for us to measure cells in lamina A1 to compare to our MIN data, any difference due to this factor is likely to be small[5,7].

In the normal kittens and adults with eye injections, we measured MIN cells in both hemispheres to determine if there was any difference either between hemispheres or between the injected and noninjected eye's terminal zones of the same hemisphere. Except in one case (a 16-week-old kitten), the differences seen were not statistically significant, but did average 9%. This may reflect the variability to be expected for these measurements (see also ref. 5).

Once sections and zones were chosen for measurements, the outlines of the cell somata at the focal plane of the nucleoli were drawn at 1000× magnification using a microscope with an oil immersion lens (NA = 1.32) and a drawing tube attachment. The cross-sectional areas of the cells were then measured using a polar planimeter. Statistical comparisons based on *t*-tests were used to evaluate the data.

RESULTS

Normal development

Fig. 2 shows a comparison of the soma size distributions in lamina A and MIN of normal kittens at different ages. The distributions from both regions have essentially unimodal peaks of the same shape. Although the average size is larger for the MIN neurons, it is not due simply to more cells which are very large. Instead, the entire distribution is shifted towards larger cells by about 50 μm^2 at most ages. It is also evident that there is no consistent or substantial change in the overall shape of the distribution of either

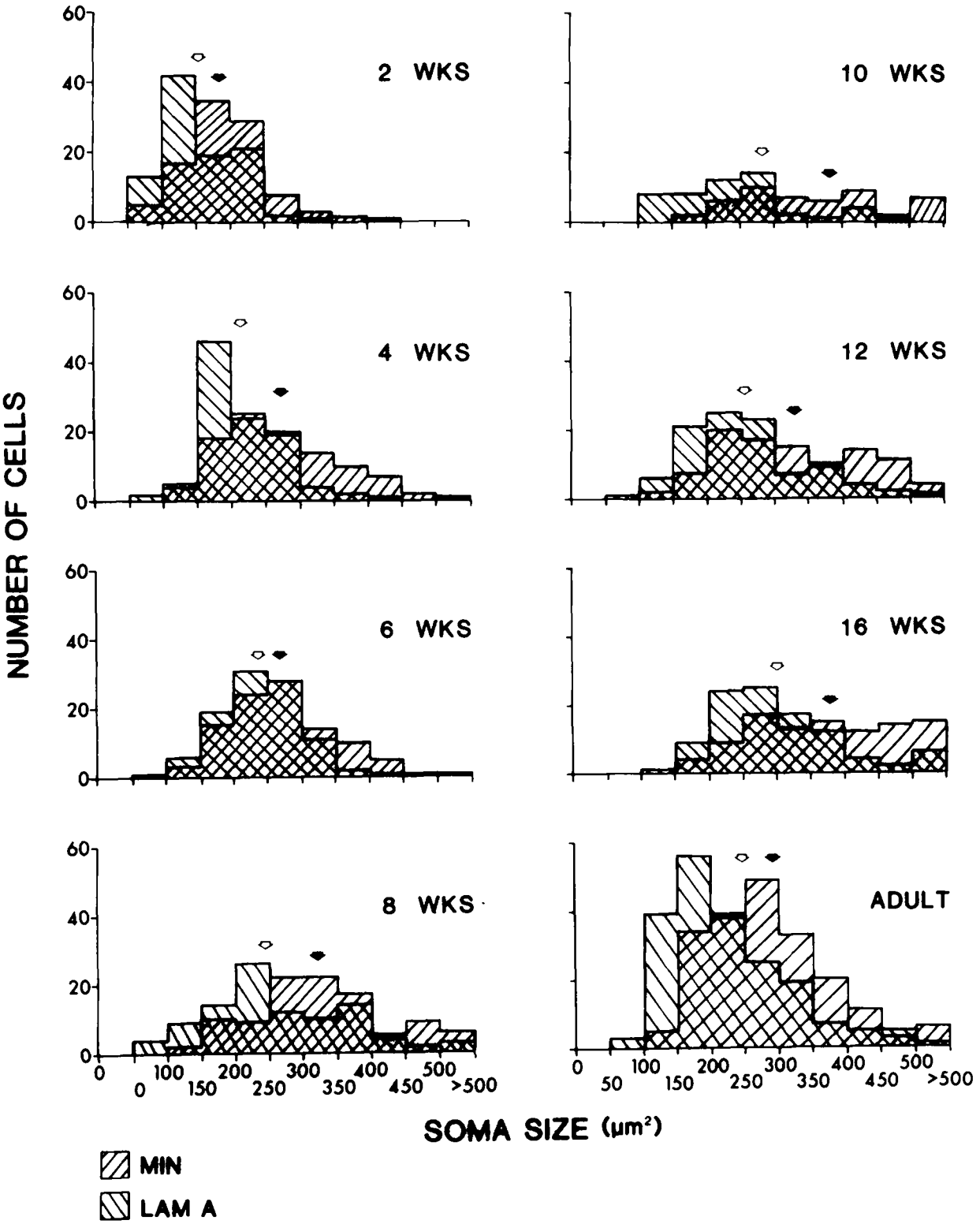

Fig. 2. Cell size distributions for MIN and lamina A for normal kittens at the ages indicated. The open arrows mark the mean value for cells in lamina A, and the solid arrows mark the mean value for cells in MIN. One kitten was used for the 10-week measurements, 6 cats used for the adult measurements, and 2 kittens were used for each of the other age groups.

lamina A or MIN cells during the developmental period.

We were particularly interested in the time-course of cell size development in MIN relative to that in lamina A. Fig. 3A plots as a function of age the average cell size in MIN divided by that in lamina A (i.e. the ratio: MIN/A). From the graph of these values, it can be seen that there is no dramatic change of this ratio with age, although the ratio may be slightly smaller at earlier and later ages than at intermediate ones. Thus, the neurons in MIN grow at a fairly constant rate compared to neurons in lamina A. In Fig. 3B, our raw MIN data (as depicted in Fig. 2) has been normalized to the lamina A data of Kalil[7]. That is, for each age we multiplied these average values of cell size from Kalil's work (dashed curve in Fig. 3B) by the ratio found in Fig. 3A to obtain the solid curve in Fig. 3B. The

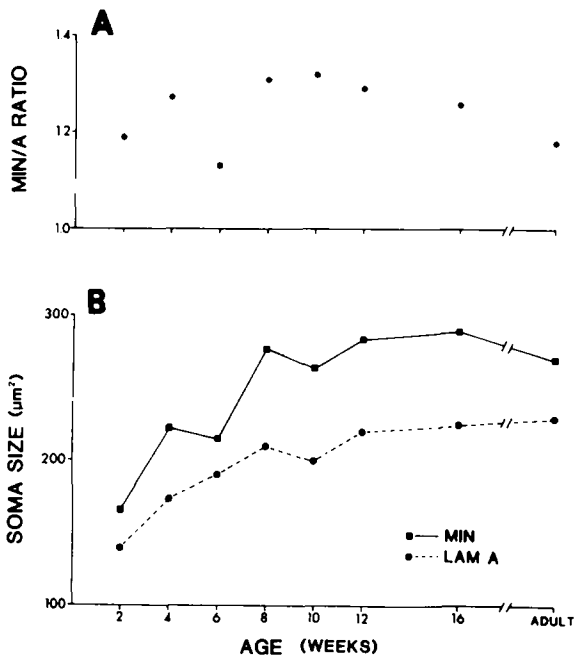

Fig. 3. Comparisons of average cell sizes between MIN and lamina A as a function of age. A: graph of points derived by taking the ratio of average cell sizes in MIN relative to those of lamina A (MIN/A) for each kitten at the ages given on the abscissa. Note that MIN cells are larger than cells in lamina A throughout development. B: growth curve of cells in MIN (solid line) normalized to the data from lamina A of Kalil[7] (dashed line). The normalization was accomplished for each age by multiplying the average cell size of Kalil's lamina A values (A_k) by the ratio of our values for MIN (MIN) and lamina A (A) of each kitten [i.e. (MIN/A) × (A_k)].

purpose of this procedure was to use the larger and less variable data base gathered by Kalil[7] in lamina A to obtain a growth curve for MIN that minimized the interanimal variability of our data; i.e. Kalil's processing techniques were designed to control for interanimal histological variability better than were ours (see Materials and Methods).

Two points can be made from Fig. 3. First, the growth of MIN cells continues to about 8 weeks postnatally, i.e. the adult value is reached at about 2 months of age. Second, at all ages MIN neurons are larger, on average, than cells in lamina A by approximately 25%. It is also clear that the growth of MIN neurons is greater than is that of lamina A neurons before 2 weeks of age, since even at this age MIN cells are larger.

Monocularly deprived kittens

The average size of neurons in MIN and lamina A for each monocularly deprived kitten is given in Table I both for cells innervated by the deprived eye and for those innervated by the nondeprived eye. These values fluctuate considerably across animals, again probably due to histological variables (see Materials and Methods and ref. 5). Since we were chiefly concerned with the changes due to deprivation, we determined the size difference between cells innervated by the deprived and nondeprived eyes for each separate animal, thereby eliminating artifactual intraanimal variability. These results for each animal are shown in Fig. 4. In one kitten at age 4 weeks, deprived MIN cells were 19% larger than nondeprived MIN cells, despite no such effect for lamina A. We cannot explain this result, particularly since the measurement was made by two of us to avoid sampling errors, and it derived from tissue studied autoradiographically so that we could be certain that our measurements were in layer 2. This data point is offset in Fig. 4, and is noted by the asterisk. Before 5 weeks of age, our MIN measurements do not reveal deprived cells that are significantly smaller than nondeprived cells, but from this time onward, such a difference in size exists. We therefore assume that the deprivation begins to affect the cell sizes in MIN between the ages of 3 and 5 weeks post-

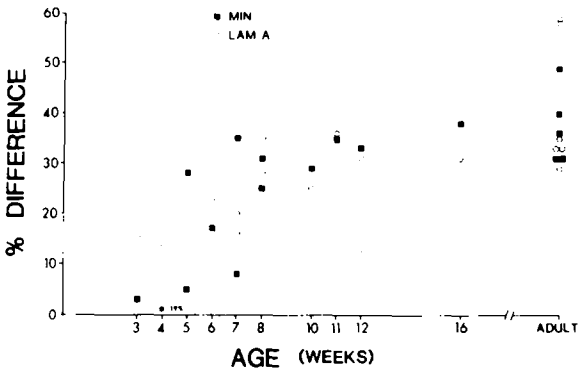

Fig. 4. Points depicting the difference between the average size of cells innervated by the deprived or nondeprived eye of monocularly deprived kittens for both MIN and lamina A at different ages. Each point represents the value for one animal. The ordinate scale is the percent difference between the deprived and nondeprived cells calculated by the formula: (nondeprived size -deprived size)/(nondeprived size). Asterisk at 4 weeks of age represents a value of (−19%) calculated as above for this kitten.

natally. The difference in size between the deprived and nondeprived cells in MIN continues to increase until about 8-12 weeks of age, at which time it approaches the adult average value of 34%. Finally, there is no major difference between lamina A and MIN regarding the time-course of these effects.

Comparison of normal and deprived kittens

Fig. 5 shows a comparison among the normal growth curves for MIN and lamina A as well as the curves depicting the development of the deprivation effects in these same areas. The end points have been obtained by taking the 2-week-old values (approximate time of eye opening) as

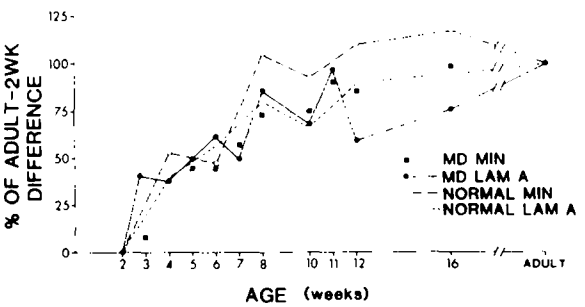

Fig. 5. Comparisons of development in cell sizes between MIN and lamina A neurons for normal and monocularly deprived (MD) kittens. See text for the derivation of these data points. The MIN data for the 4-week-old monocularly deprived kitten do not appear on this graph.

0% and the adult values as 100%. The value of each point for the normal kittens between 2 weeks and adult is derived by computing the difference in average cell size between the individual kittens (K_1) and the mean for the 2-week-old kittens (K_2), and dividing this by cell size difference between adults (K_a) and 2-week-old kittens: that is, $(K_1 - K_2)/(K_a - K_2)$. For the monocularly deprived (MD) kittens, each point was determined by dividing the mean size of nondeprived cells into the size difference between the nondeprived and deprived cells, (ND −DEP)/(ND), and expressing this as a percentage; this percentage was then normalized to the adult value by dividing it by 0.34 (cf. Fig. 4). The curves demonstrate that the difference between the deprived/nondeprived cell growth follows the same pattern as the period of normal cell growth and also that the maximum growth difference in the deprived animals also occurs at about the same time as the cessation of growth for the normal animals.

DISCUSSION

Normal MIN development

MIN cells grow monotonically until about 8 weeks of age, at which time the sizes of the cells are similar to those of adult animals. Throughout this developmental time period, the size of MIN cells is larger, on average, than the size of cells in lamina A, even though the MIN growth curve follows the same pattern as lamina A neurons. Since both groups of neurons reach their adult values at about 8 weeks of age in this study (see also ref. 7; however, see refs. 3, 5), it does not appear that MIN cells are larger due to a longer growth period. Instead, the absolute growth of MIN neurons must be more rapid than that of neurons in lamina A throughout the developmental period.

Monocularly deprived kittens

The major objective of this study was to examine the time-course of the deprivation effect upon cell sizes in MIN. Monocular deprivation was found to affect the size of neurons innervated by the deprived eye relative to sizes of non-

deprived MIN cells. This effect started between 3 and 5 weeks postnatally and reached its maximum value at about 8 weeks of age. Some small differential growth between the deprived and nondeprived neurons of MIN for another 2 4 weeks cannot be ruled out. Thus, monocular lid suture affects MIN cell growth in much the same way that it affects growth of cells in lamina A[5,8]. Since our findings show that a cumulative effect of deprivation upon MIN cell sizes continues for as long as growth normally occurs, the simplest explanation of the deprivation effect is that, between about 3 and 8 weeks of age, it retards the growth of MIN neurons innervated by the deprived eye. However, we have not ruled out the possibilities either that the cells innervated by the nondeprived eye may have hypertrophied[6] or that the deprived cells may have atrophied after some initial growth period[5]. We did observe that there was no change in the deprived/nondeprived cell size difference after 8 weeks of age.

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