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EFFECTS OF SLEEP DEPRIVATION ON THE POSTNATAL DEVELOPMENT OF VISUAL-DEPRIVED CELLS IN THE CAT'S LATERAL GENICULATE NUCLEUS

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INTRODUCTION

In spite of the numerous electrophysiological investigations directed to elucidate the mechanisms involved in sleep, little is known about its physiological significance. The amount of sleep, particularly of REM (rapid eye movements) sleep, is extremely high at birth, but progressively decreases with maturation (34). During this phase of sleep, pontine structures become active and send rhythmic and synchronous discharges to motor and sensory areas of the brain (ref. in 30, 59, 79). It has been hypothesized that ascending and descending impulses originating from the brainstem serve as an endogenous source of stimulation before appreciable exogenous stimulation is available to the central nervous system (CNS), thus contributing to structural maturation and differentiation of key sensory and motor areas during the ontogenesis (33, 59, 66).

Normal sensory stimulation plays an important role in the development and maintenance of the CNS. In particular, the developing visual system is exquisitely sensitive to sensory deprivation. Kittens submitted to monocular visual deprivation (MD) by suturing the lids of one eye show a period of high susceptibility, when even a short period of eye closure leads to profound functional reorganization of visual cortical areas (31, 74; cf. 88). This period, called the critical period, begins between the third and the fourth week when kittens start to use their eyes, and lasts till the sixth-eighth week. Sensitivity then decreases with age (31). At variance with their visual cortex, whose gross morphology appear normal (see, however, 43), kittens submitted to MD show notable cell hypotrophy in the layers of the lateral geniculate nucleus (LGN) receiving inputs from the occluded eye, namely the dorsal (A) and the magnocellular ventral (C) layer of the contralateral LGN and the middle (A1) layer of the ipsilateral LGN (87). The effects of MD are greatest in the critical period, during the early life (second month after birth), and then decrease with time, causing no appreciable change after three months of age. Although closely related to the critical period, a limited recovery in the cortical physiology is seen following opening of the eye, while no recovery of the geniculate shrinkage is observed (31).

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In addition to retinal inputs, the LGN also receives extraretinal inputs which are particularly active during REM sleep. These inputs actually contribute to the rhythmic ponto-geniculate-occipital or PGO activity, which is typical of this phase of sleep (ref. in 30, 79). In 1970, Pompeiano (59) postulated that the rhythmic discharges of the pontine structures, acting on the LGN and the visual cortex during REM sleep, could exert a protective influence against the severe deficits in the visual function following MD (see 87, 88). Indeed, there is evidence that the PGO activity is present 15 days after birth (1, 9, 15), at a time close to the beginning of the critical period. Moreover, ponto-mesencephalic electrolytic lesions performed bilaterally in two-weeks old kittens and suppressing PGO activity resulted in significant deficits in the maturation of the LGN, with reduction of LGN volume and cell size (15, 16).

To test the hypothesis of a role of sleep in brain maturation, experiments were performed in kittens to find out whether total sleep deprivation (SD) interferes with the neuronal maturation in the LGN and modifies the well-known susceptibility of LGN neurons to visual deprivation (87). In particular, pairs of twin kittens of different age were submitted to MD. One kitten in each pair was in addition submitted to SD during the last period of MD. The cell sizes in the different layers of the geniculate nucleus were then measured and compared with those obtained in the mate, which was allowed to sleep. Preliminary accounts of this research have been reported previously (60, 61).

METHODS

Ten pairs of twin kittens were used to study the morphological effects of MD on LGN neurons under different animal states. The method used for visual deprivation was to suture together the lids of the right eye end to end. This operation was performed under aseptic conditions and light ether anesthesia. It is assumed that under this condition the lid-sutured eye was completely deprived of form stimulation, and also, to a large extent, of stimulation by diffuse light (87). Table 1 summarizes the procedures carried out in the experimental animals. In particular, twin kittens were operated on the same day (12 to 42 days after birth) and were also sacrificed at the same time. MD was maintained for 2 to 23 days in different groups. Throughout this period the kittens were reared under light artificial illumination. However, while one kitten in each pair was allowed to sleep, the other was submitted to total SD during the last period of MD (2-6 days, corresponding to 20-100% of the total period of visual deprivation). SD was induced continuously by gentle handling in order to minimize stress.

At the end of the experiments the animals were deeply anesthetized with ether and then perfused with normal saline followed by 10% formaline. Brains were embedded in paraffin, and sectioned coronally at 25 μm. One every five serial sections were mounted and stained with cresyl violet (Nissl method). The extent of the morphological changes of LGN neurons was first assessed qualitatively in each pair of experiments. Quantitative observations were usually made in those cases where clear changes in cell size could be seen at the light microscope (Exps. 5, 6, 15-20) and, among the remaining cases in which no detectable changes were observed, in Exps.13 and 14. Measurements were performed bilaterally in the two dorsal layers (A and A1) which are clearly defined, relatively thick and rich in large size cells, as well as more ventrally in the magnocellular layer C, corresponding to layer B of the old terminology (81; see also 87), but not in the parvicellular layers C1-C3, as defined by Guillery (26) and others (cf.19).

It is known, from experiments performed in kittens (28), that cells of the medial and intermediate parts of the LGN, where most of the adjacent layers A, A1 and C are located, receive afferents from opposite eyes (i.e, layers A and C receiving input from the contralateral retina and the interposed layer A1 from the ipsilateral retina). These cells give a binocular input to the visual cortical area. This large *binocular segment* of the LGN can be distinguished from the small lateral part of the nucleus, which receives only a monocular input from the nasal margin of the retina (i.e., from the temporal part of the visual field) and the corresponding cells project to a cortical area receiving only this monocular input. This *monocular segment* of the nucleus lies in those parts of layers A and C which extend beyond the lateral edge of layer A1, and are no longer separated from each other by layer A1. Our observations were performed exclusively on the binocular segment of the LGN at the junction between the posterior and middle third of this structure, where the three main layers A, A1 and C are circumscribed and distinct. All comparisons of normal with visual-deprived cells were thus made at corresponding sites and at the same antero-posterior level of the two geniculate nuclei in the same brain.

A quantitative method for measuring cross-sectional areas of cell bodies in the LGN layers of normally sleeping and sleep-deprived kittens was adopted and slightly modified after the method of Matthews et al. (46). In particular, camera lucida outlines of the cell body for 200 neurons from each visual-deprived and nondeprived layer were drawn on a mm graph paper, the linear magnification being adjusted to exactly 1000 times. Only cells sectioned at the level of the nucleus and nucleolus were drawn, the presence of the nucleolus being the only criterion for their selection. Direct measurement (in μm^2) of the projected areas of the cell bodies was evaluated by using a planimeter (Salmoiraghi, mod. 236, Milan, Italy), but control measurements were also obtained by counting the number of mm^2 within a particular outline. Attempts were also made to estimate qualitatively the cell density in different layers of the LGN, both in normally sleeping and in sleep-deprived kittens.

The results of the qualitative observations will be described first, followed by the quantitative observations on the cell areas which, for the sake of clarity, will be presented both in a tabular form and graphically. In the assessment of cell shrinkage, mean sizes of cells for visual-deprived and nondeprived geniculate layers were compared by the Student t-test, and percentage changes with their 95% confidence limits were calculated.

RESULTS

Age of the animals, duration of the experiments and procedures applied to the ten pairs of twin kittens are shown in Figure 1 and Table 1.

As reported in the Methods, the cell sizes in the present experiments were evaluated by examining neurons located in the binocular segment of the LGN, which includes the most prominent part of the magnocellular layers A, A1 and C, but not in the monocular segment, which lies in those parts of layers A and C located laterally to the edge of layer A1. In both segments, the most dorsal (A) and the magnocellular ventral (C) layers receive input exclusively from the contralateral retina, while the interposed layer A1 receives input from the ipsilateral retina.

Kittens submitted at the age of 12 days to MD for 6 and 10 days (Exps. 1 and 3, respectively) did not show any reduction in the mean cell areas in each of the deprived with respect to the nondeprived layers of the LGN. In the corresponding mates, submitted to SD during the last 2 days of the total period of MD (Exps. 2 and 4, respectively), no clear morphological change in any layer of the LGN was detected.

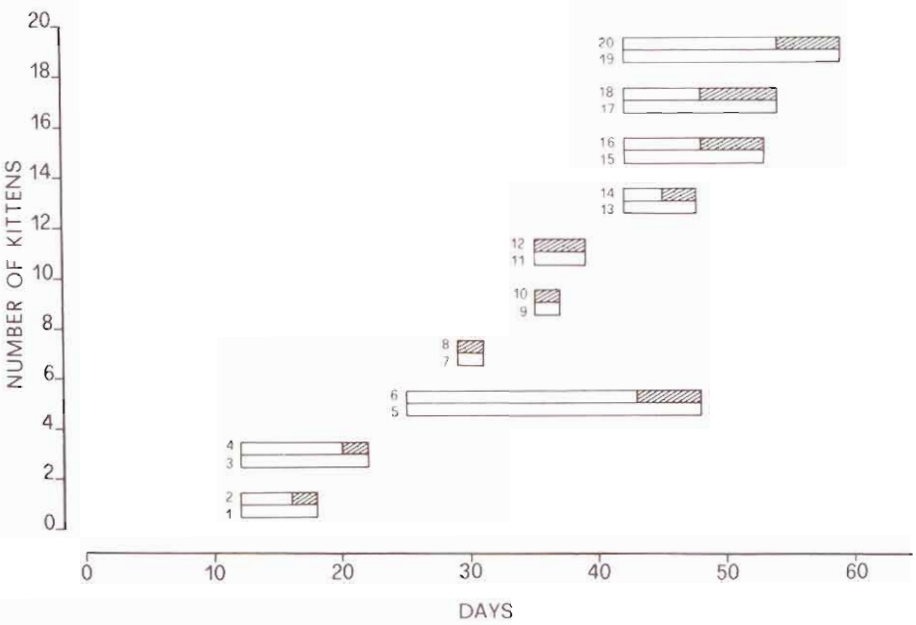


Fig. 1. - *Population of operated kittens.*

Ten groups of twin kittens were submitted to eyelid suture of the right side, each group being operated between 12 and 42 days after birth. MD was maintained for 2 to 23 days in different groups. One of the twins in each group was allowed to sleep (white columns), while the other was submitted to total SD for 2 to 6 days during the last period of MD (striped columns).

MD started in kittens at the age of 25 to 42 days, and continued for 11 to 23 days, produced a slight reduction of the mean cell area in visual-deprived LGN layers, namely layers A and C of the LGN contralateral to the occluded eye, and in one instance (Exp. 5) also in layer A1 of the ipsilateral LGN. This reduction in cell size was, however, most severe and could also involve the deprived layer which was unaffected by MD (layer A1), if the corresponding mates were also submitted to 5-6 days of SD. In these experiments the mean cell areas of the visual-deprived layers were compared with those of the corresponding nondeprived layers and expressed in percentage changes relative to the last values. Data from Exps. 15-16 and 17-18, where the effects were most representative, are reported in Table 2. The animals were submitted to MD at the age of 42 days. Visual deprivation was continued for 11 days in kittens 15 and 16, and for 12 days in kittens 17 and 18, while SD was performed during the last 5 or 6 days of MD in kittens 16 and 18, respectively. In Exp.15, in which the kitten was allowed to sleep, there was only a moderate decrease in the mean cell area by 5.9% in the contralateral layer A. No reduction was observed in the contralateral layer C and in the ipsilateral layer A1. However, in the corresponding mate which was also sleep-deprived (Exp. 16), the mean cell area significantly decreased by 14.7% and 16.7% in the contralateral

Table 1. - *Experimental procedures applied to ten pairs of twin kittens.*

No. of pair	No. of experiments	Age at onset of MD days	Normal sleep		Sleep-deprived			
			Duration of MD days		Duration of MD alone days		Duration of MD with SD days	%
I	Exps. 1,2	12	Exp. 1	6	Exp. 2	4	2	(33)
II	" 3,4	12	" 3	10	" 4	8	2	(20)
III	* " 5,6	25	" 5	23	" 6	18	5	(28)
IV	" 7,8	29	" 7	2	" 8	2	2	(100)
V	" 9,10	35	" 9	2	" 10	2	2	(100)
VI	" 11,12	35	" 11	4	" 12	4	4	(100)
VII	* " 13,14	42	" 13	5.6	" 14	3	2.6	(46)
VIII	* " 15,16	42	" 15	11	" 16	6	5	(45)
IX	* " 17,18	42	" 17	12	" 18	6	6	(50)
X	* " 19,20	42	" 19	17	" 20	12	5	(29)

MD, monocular visual deprivation; SD, total sleep deprivation. Values in parentheses represent the duration of SD expressed in % of the total period of MD. Asterisks indicate kittens in which the cross-sectional areas of cell bodies in different layers of the LGN has been quantitatively evaluated.

layers A and C, respectively, and by 38.3% in the ipsilateral layer A1. In these instances, the decrease in the mean cell areas observed in the visual-deprived layers was quite prominent in the sleep-deprived kitten with respect to the normally sleeping kitten, the difference being statistically significant (see Table 2). In Exp. 17, the observed decrease in cell size in the visual-deprived layers A and C was of 13.1% and 9.2%, respectively. The effects became more severe in the corresponding mate submitted to 6 days of SD (Exp. 18). In this case the reduction of the mean cell area was of 31.3% and 14.6% for the contralateral layers A and C, respectively. There was also a slight, although not significant difference in cell size between the ipsilateral and the contralateral layer A1 in the sleep-deprived with respect to the normally sleeping animal (see Table 2).

The absolute data on cross sectional areas (in μm^2) of the cell bodies located in each of the layers A and A1 of the binocular segment of the LGN, ipsilateral or contralateral to the occluded eye, were also considered and the differential-layers effect of MD was assessed by computing for each animal the mean cell size ratio A1:A for the LGN ipsilateral to the occluded eye and the mean ratio A:A1 for the LGN contralateral to the occluded eye. Since the most dorsal layer A receives input exclusively from the contralateral retina, while the next ventral layer A1 receives input from the ipsilateral retina, the above mentioned ratios can give an indication

Table 2. - *Effects of MD on cross-sectional areas of cell bodies (mean ±S.D.) in different layers of the LGN, both in normal and sleep-deprived kittens.*

No. of experiments	Layer	Left LGN	Right LGN	Left vs Right	Shrinkage
Exp. 15	A	**94.1±29.6*	100±29.4	p<0.025	+
	A1	100±34.7	***107.1±38.6*	p>0.05	NO
	C	***111.0±36.5*	100±35.7	p<0.005	NO
Exp. 16	A	**85.3±28.3*	100±38.1	p<0.0005	++
	A1	100±32.0	***61.7±24.4*	p<0.0005	+++
	C	***83.3±29.8*	100±35.9	p<0.0005	+++
Exp. 17	A	***86.9±27.4*	100±36.1	p<0.0005	++
	A1	100±30.5	*103.4±34.2†*	p>0.05	NO
	C	*90.8±25.6†*	100±27.4	p<0.0005	+
Exp. 18	A	***68.7±25.6*	100±37.1	p<0.0005	+++
	A1	100±36.3	*98.3±32.7†*	p>0.05	NO
	C	*85.4±38.6†*	100±37.7	p<0.0005	++

Pairs of twin kittens operated of eyelid suture of the right side at the age of 42 days after birth, and visual-deprived for 11 days (Exps. 15 and 16) or 12 days (Exps. 17 and 18). For each pair, one kitten (Exp. 15 or 17) was normally sleeping, while the other was submitted to 5 days (Exp. 16) or 6 days (Exp. 18) of SD during the last period of MD. Student *t*-test for differences of means are given. The mean cell areas, evaluated over 200 cells in each sample, were expressed in percent changes in visual-deprived layers (in italics) relative to the nondeprived layers (taken as 100). +, ++, +++: significant shrinkage <10%, between 10-15% and >15%, respectively, in visual-deprived layers with respect to nondeprived layers in the same kitten; NO: lack of shrinkage. Significant (*p <0.01, **p <0.001) and non significant (†p >0.05) increases in shrinkage induced in different layers of the LGN by MD applied in sleep-deprived kittens with respect to normally sleeping kittens.

of the potentiating effect of SD on the shrinkage induced by MD. In fact, a comparison of the effects of MD obtained in the four experiments illustrated in Table 2 indicates that the ratio A1:A evaluated in the LGN ipsilateral to the occluded eye corresponded to 101.2 in the normally sleeping kittens (Exps. 15 and 17), but decreased to 85.6% after SD (Exps. 16 and 18). Moreover, the ratio A:A1 evaluated in the LGN contralateral to the occluded eye corresponded to 94.5 in the normally sleeping kittens, but decreased to 70.7 after SD. Considering that under normal conditions the mean cell sizes for layers A and A1 are almost comparable (cf. also ref. 45), it appeared that on the ipsilateral side SD decreased the mean cell size in the visual-deprived layer A1 by 15.6 (i.e., 101.2 minus 85.6) with respect to the nondeprived layer A, while on the contralateral side SD decreased the mean cell size in the visual-deprived layer A by 23.8 (i.e., 94.5 minus 70.7) with respect to the normal layer A1.

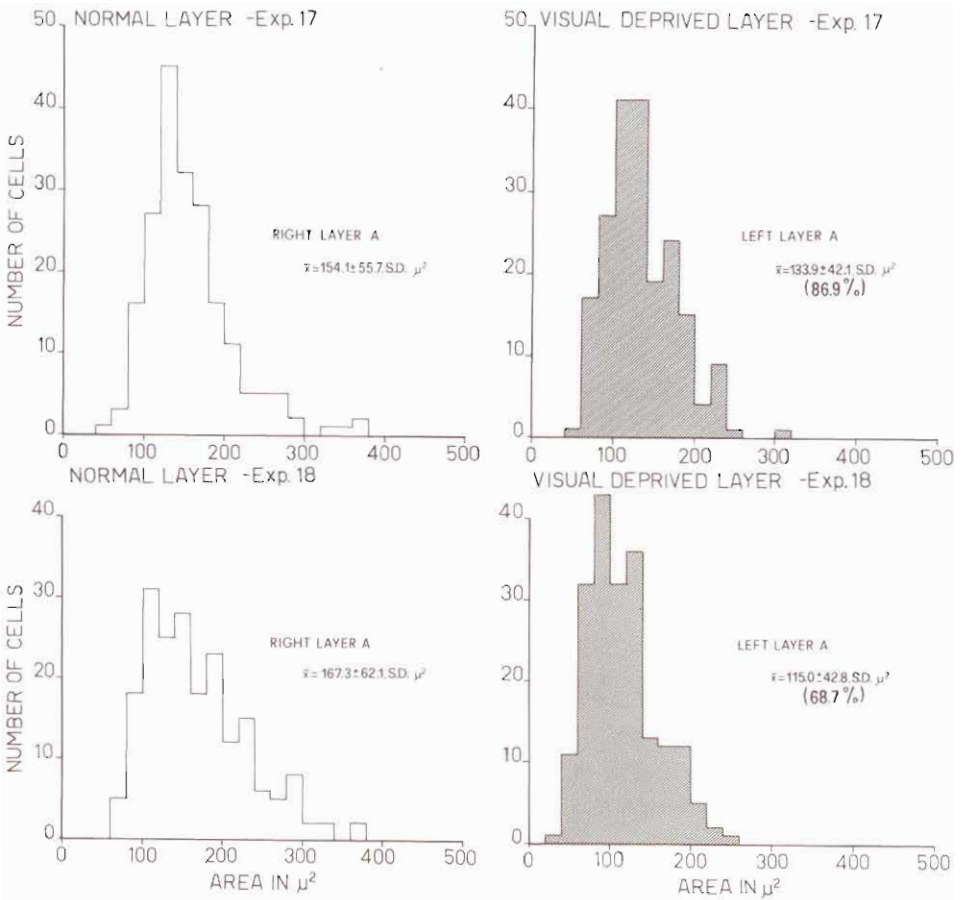
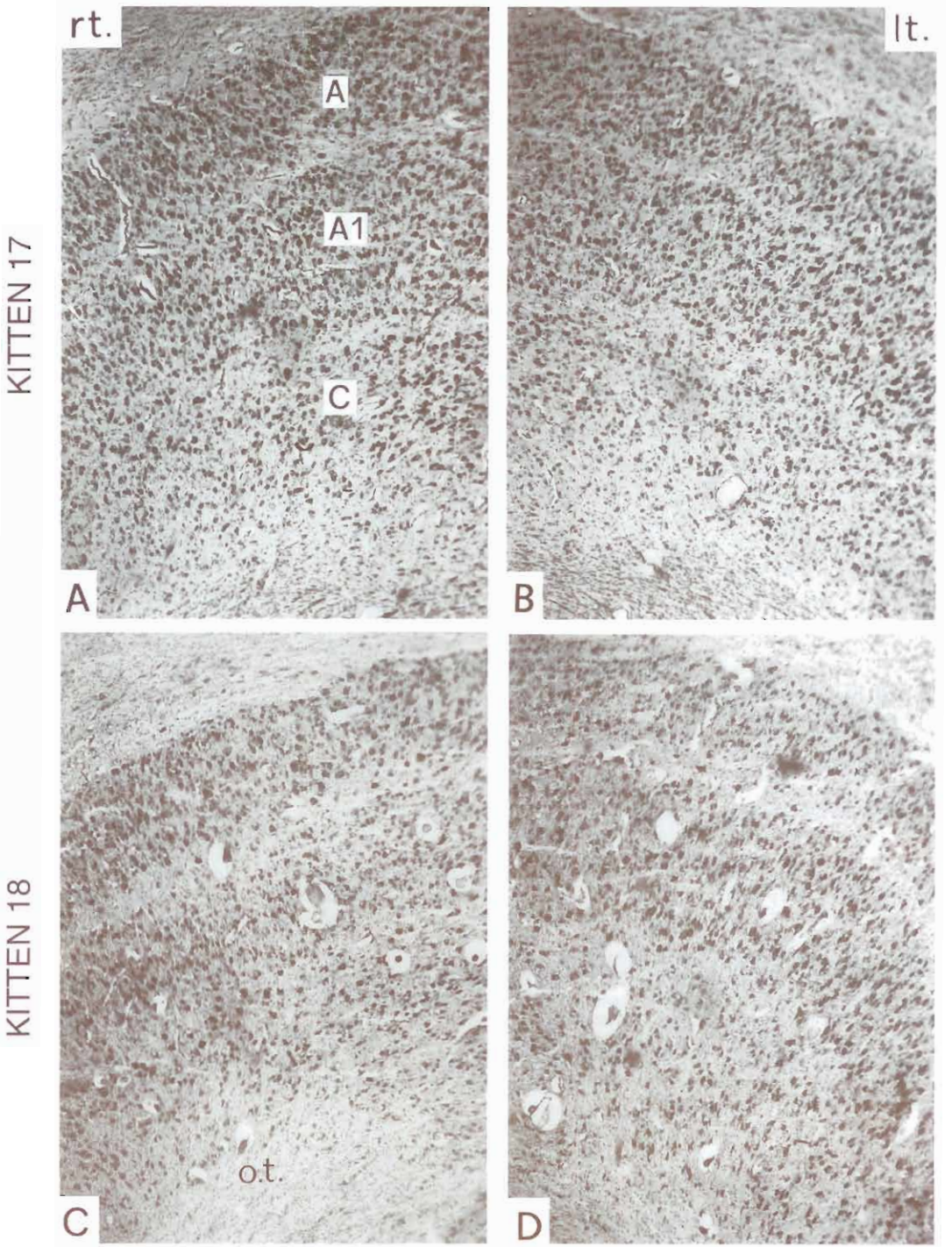


Fig. 2. - *Distribution of cross-sectional areas of cell bodies in layer A of the LGN in a pair of twin kittens submitted to MD, associated or not with SD.*

Kitten 17 was visual-deprived by right eye closure at the age of 42 days after birth for 12 days and was allowed to sleep as usually. Kitten 18 was also operated of eyelid suture on the right side at the same age of 42 days for 12 days; this animal, however, was submitted to total SD for 6 days during the last period of MD. The left histograms (white columns) illustrate the distribution of the cell areas in the nondeprived layer A of the right LGN, while the right histograms (striped columns) illustrate the distribution of the cell areas in the visual-deprived layer A of the left LGN.

The mean cell area in the visual-deprived layer A corresponded in kitten 17 to 86.9% of the control value obtained in the nondeprived layer, but further decreased to 68.7% in kitten 18 after SD. Note that the mean cell area in the non-deprived layer A was only slightly higher in kitten 18 (167.3 μm^2) than in kitten 17 (154.1 μm^2) (t-test, $p<0.05$), while the mean cellular area in the visual-deprived layer A was much more depressed in the sleep-deprived kitten 18 (115.0 μm^2) than in the normally sleeping kitten 17 (133.9 μm^2) (t-test, $p<0.001$).

In addition to these findings, we tried to find out whether the increase of cell shrinkage in the visual-deprived layers of the developing LGN, following SD, was also associated with an increase of cell size in the nondeprived layers. The results obtained indicated that the mean cell areas of these normal layers were slightly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larger in the sleep-deprived kittens than in the normally sleeping animals. This change could be attributed to individual variabilities of the cell size of the geniculate neurons in twin kittens. The alternative possibility, however, was that the increased visual experience resulting from an increased wake time in the nondeprived layers exerted a positive contribution on the neuronal development. In this case, the increased maturation of the nondeprived layers might have enhanced the morphological abnormalities in the visual-deprived layers. An appropriate answer to this question is given by the results of Exps.17 and 18, which show the distribution of cross-sectional areas in the geniculate layer A of both sides in a pair of twin kittens operated 42 days after birth and visual-deprived for 12 days (Fig. 2). In these experiments, the mean cell area in the ipsilateral nondeprived layer A was only 8.6% higher after SD (167.3±62.1,S.D. μm^2) than in the normally sleeping mate (154.1±55.7,S.D. μm^2) (Fig. 2, left histograms). In contrast, the mean cell area in the contralateral visual-deprived layer A, which was slightly reduced to 133.9±42.1,S.D. μm^2 in the normally sleeping kitten (i.e., by 13.1% with respect to the nondeprived layer A), prominently decreased to 115.0±42.8, S.D.μm^2 after SD (i.e. by 31.3% with respect to the normal layer) (Fig. 2, right histograms). It appears, therefore, that the increase by 18.2% (i.e. 31.3 minus 13.1%) of the cell shrinkage in the contralateral visual-deprived layer A induced by SD with respect to the value obtained in the normally sleeping kitten (t-test, $P<0.001$), was on the average much higher than the increase by 8.6% in the mean cell area which occurred in the ipsilateral nondeprived layer A after SD with respect to the normally sleeping mate (t-test, $P<0.05$). These effects were observed throughout the binocular segment of the LGN, as illustrated in Fig. 3. Note the moderate hypotrophy (or failure to grow) in the visual-deprived geniculate layers of the normally sleeping kitten (Fig. 3, A and B), and the prominent hypotrophy which appeared in the corresponding layers of the sleep-deprived mate (Fig. 3, C and D). These layers appeared also tinner than normally. Fig.4 illustrates at higher magnifications the shrinkage in Exp. 18 of the LGN neurons located in layers A and C, but not in layer A1 of the contralateral side. In most instances, the reduction in cell size was associated with a reduction of Nissl substance, giving the cells a pale appearance. Normally stained cells were, however, interspersed among the hypotrophic cells.

Fig. 3. - *Morphological changes in the three main layers of the LGN observed in a pair of twin kittens submitted to MD associated or not with SD.*

Coronal sections through the LGN of two kittens operated of eyelid suture of the right side at the age of 42 days after birth, and visual-deprived for 12 days. As reported in the legend of Figure 2, kitten 17 was allowed to sleep, while kitten 18 was submitted to total SD for 6 days during the last period of MD. A and C are from the right (rt.) LGN, ipsilateral to the occluded eye. B and D are from the left (lt.) LGN, contralateral to the occluded eye. Sections were stained with cresyl violet (magnification: 55x). A, A1 and C: layers A, A1 and C of the LGN; ot: optic tract. Note the striking hypotrophy in the geniculate layers receiving input from the covered eye, i.e. in the dorsal (A) and ventral (C) layers of the left side and in the middle (A1) layer of the right side. This finding was much more prominent in the sleep-deprived kitten 18 than in the normally sleeping kitten 17.

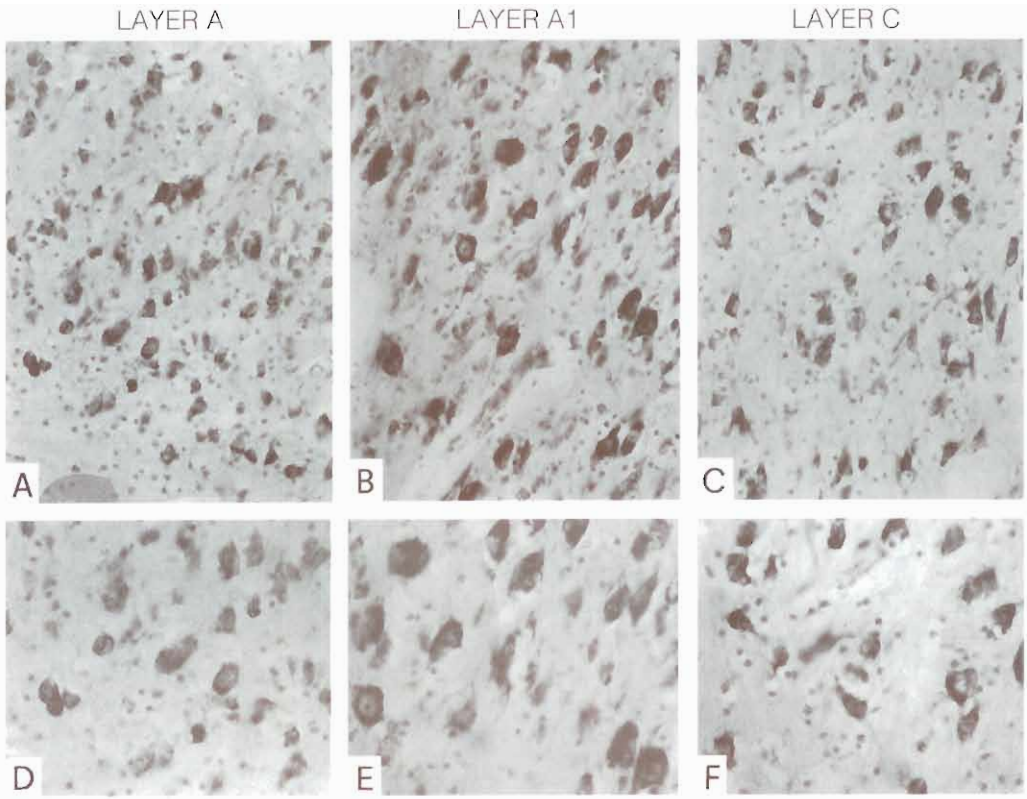

Fig. 4. - *Morphological changes of LGN neurons in a kitten submitted to both MD and SD.*

Coronal sections through the left LGN of kitten 18 (same experiment as in Fig. 3, D), showing neurons from layers A, A1 and C. Photomicrographs were taken at different magnifications: 180 x for A-C, and 270 x for D-F. Note the small size (shrinkage) of the visual-deprived LGN neurons in layers A and C, and the large size of the non-deprived LGN neurons in layer A1. In this experiment the mean areas (±S.D.) of the cell bodies in the visual-deprived layers A, A1 and C, evaluated in μm^2, corresponded to 115.0±42.8, 166.7±55.5 and 139.6±63.2, respectively, while the mean areas of the cell bodies in the nondeprived layers A, A1 and C corresponded to 167.3±62.1, 169.5±61.5 and 163.5±61.6, respectively. These values indicate how prominent was, in kitten 18, the shrinkage which occurred in the visual-deprived layers A and C.

Qualitative evaluation of the cell density in the visual deprived layers A and A1 indicated that the cell density was either unmodified or only slightly higher in the sleep-deprived kittens than in the normally sleeping mates. No obvious glial infiltration was found in these preparations.

DISCUSSION

It is known that during the critical period (31), the developing visual system of mammals depends upon activity driven by visual experience (ref. in 74). In the

present experiments, we have observed that MD determines a cell shrinkage in the visual-deprived layers of the LGN in kittens. This is a well-known phenomenon originally described by Wiesel and Hubel (87; see also 27). Our observations were performed on the binocular segment of the adjacent layers A, A1 and C, but not on the more laterally located monocular segment of layers A and C, since there is evidence that the former rather than the latter segment undergoes a cell size reduction following MD (28; ref. in 74). Moreover, unilateral eyelid closure was most effective when performed in kittens at the age of 42 days, probably because in younger kittens tested, MD was either started too early with respect to the critical period or done for a too short period. The cell size, however, represents a quite gross parameter to examine. New molecular techniques may allow to explore in greater detail the structural and functional organization of the brain during development. For example, subtractive hybridization techniques have permitted the identification of at least 200 different molecules selectively expressed in the kitten visual cortex during the critical period (63), which represent candidates for important roles in functional plasticity in response to early experience.

In addition to the findings reported above, we have shown that, shortly after birth, total SD greatly enhanced the structural abnormalities produced by MD in the visual-deprived geniculate layers, while it slightly modified the growth of LGN neurons receiving normal visual input, at least in our conditions. In particular, the nondeprived layers showed a slight tendency towards increased size after SD. This effect could in part at least be attributed to increased monocular visual experience of the sleep-deprived animal accompanying increased wake time in the light. However, the increase in shrinkage of the visual-deprived layers after SD was more prominent than the increase in cell size seen in the nondeprived layers, indicating that the enhanced influence of MD on the visual-deprived layers following SD could have resulted from removing an influence of sleep. These findings have been recently confirmed and extended by Roffwarg et al. (45, 72), who found that an exacerbation of the effects of MD in kittens appeared particularly after selective deprivation of REM sleep.

A brief analysis of the mechanisms responsible for the structural abnormalities which affect the visual system following MD is essential in order to understand the role of sleep in maturation of the visual system. While the first two steps (pathways selection and target region selection), the earliest of a three-step developmental program for synapse formation, seem to depend principally on molecular recognition, the last stage (cellular selection) relies on activity dependent mechanism (35). This is particularly true for the primary visual cortex, whose late developmental events, occurring also before eye-opening, require neural activity (ref. in 14). MD during the critical period determines a shift in the distribution of ocular dominance in the visual cortex in favour of the opened eye, accompanied by a profound reorganization of LGN axons in the cerebral cortex due to a disruption of the geniculocortical pathways for the occluded eye (22, 82; ref. in 73). This ocular dominance plasticity has been attributed to the possibility that the geniculocortical afferents for the two eyes compete for cortical synaptic space during development (89; ref. in 7). The shrinkage observed in the deprived layers of the LGN would

thus be secondary to the use-dependent competition between axons for layer IV cortical neurons and then to a loss of synaptic arbor (as discussed in ref. 7). The geniculocortical terminals of the deprived pathway, in fact, project to smaller ocular dominance patches in layer IV of the visual cortex, where they make fewer and abnormal synapses (7). On the other hand, the non-deprived neurons expand their synaptic space (22, 82) and correspondingly increase in size (cf. 74). However, this increase in cell size following MD is less pronounced that the cell-size reduction (cf. 74). As a result of these findings, a direct relationship between synaptic activity and neuronal morphology has been proposed (57). The possibility, however, that the hypotrophy in the LGN results in part at least from the deprivation itself cannot be excluded. In fact, alterations in the morphology of retinogeniculate axon arbors have also been described following MD in the postnatal kitten (see 14 for review).

The conclusion that neural activity is required for axon segregation in the visual cortex can also be extended to the LGN. However, while the formation of the ocular dominance columns in the visual cortex occurs in the cat entirely postnatally, that of LGN layers occurs largely prenatally (ref. in 73). It is known that during the initial development of retinogeniculate connections in mammals there is a total overlap of the projections from the two eyes followed by a segregation phase, during which fibers originating from the left and right eyes become restricted to separate layers of the dorsal LGN (cf. 65, 86). It has been postulated that at this stage competitive interactions, similar to those described for the visual cortex, are driven by the spontaneous patterned activity which occurs in retinal ganglion cells during prenatal development (23, 52). However, at a still later stage, visually driven activity plays a major role in the development also of the LGN.

The fact that SD enhances the structural abnormalities produced in the LGN by MD can be understood only if we consider that in addition to the retinal input, extraretinal inputs impinge on the visual system. These inputs may in part at least utilize noradrenergic and cholinergic systems, which emerge early during development (18, 20), thus having the potential of playing a role during postnatal plasticity. Indeed, there is evidence that activation in the visual cortex of noradrenergic (4, 36-38, 58) and to a lesser extent also of the cholinergic system (4, 24), facilitates the shift in ocular dominance following MD. These systems could also act on the LGN. There is in fact evidence that both the noradrenergic neurons located in the locus coeruleus, LC (ref. in 20) and the cholinergic neurons located in the dorsolateral mesopontine tegmentum, namely in the pedunculopontine tegmental nucleus, PPT, and the laterodorsal tegmental nucleus, LDT (3, 39, 53, 54) send afferents to the LGN (2, 40, 47, 78). In the cat, these two populations of neurons appear to be coextensive and intermingled (cf. 32).

A comparison between the cholinergic and noradrenergic projections to the LGN has also been performed (17, 19; see 79 for ref.). Both in the PPT and in the LC the retrogradely labeled cells immunostained for choline acetyltransferase, ChAT (the enzyme which provides the synthesis of acetylcholine, ACh) were much more numerous than the retrogradely labeled cells immunostained for tyrosine hydroxylase (TH) and dopamine ß-hydroxylase (enzymes which are involved in

the synthesis of noradrenaline, NA). However, unlike the retinal afferents to the LGN, which form specific point-to-point projections (ref. in 74), the cholinergic and the noradrenergic afferents to the LGN were diffuse (ref. in 79). In particular, the ChAT-positive fibers formed a dense network within the A layers and the magnocellular C layer (cf. also 80), where they gave rise to clusters of boutons on proximal dendrites of relay cells and in specialized synaptic glomeruli (cf. 64), with the parvicellular C layers (C1-C3) containing fewer fibers (19). On the other hand, TH-immunoreactive fibers were evenly distributed in the LGN and showed a slightly higher density in the parvicellular C layers than in the A layers.

As reported previously, the plastic changes which affect the visual system after MD depend on the fact that glutamatergic (cf. 55,73) retino-geniculate and geniculo-cortical afferents from the open eye activate a competitive process that leads to weakening and eventually a disruption of the connections conveying signals from the deprived eye (65, 86; cf. 14). For these competitive interactions to occur, signals from the open eye need to activate cortical and probably also geniculate neurons above a critical threshold, which is reached only if NMDA receptors participate in the use-dependent developmental plasticity (12, 13, 21, 77; cf. 25), and if neuromodulators like NA (37, 58) and ACh (4) are present in sufficient concentration to facilitate the activation of NMDA receptors (10, 24). In the visual cortex these effects are apparently mediated through β1-adrenoceptors (75) and muscarinic M1-receptors (24), respectively. The story, however, is more complicated, since at cellular level the effects of NA and ACh are not coincident. Observations made in slice preparations have, in fact, shown that microiontophoretic application of NA produced a *slow* depolarization of thalamic neurons with a delay to onset of 1-2 sec (51), while ACh produced a *rapid* depolarization with a delay of less than 10 msec, followed by rapid hyperpolarizing and slow depolarizing effects (49, 50).

In order to understand the effect of sleep (60, 61 and the present study), and in particular of REM sleep deprivation (45, 72), on the maturation of LGN neurons we should consider that the noradrenergic and NA-sensitive neurons in the LC, which are particularly active during waking, cease firing during REM sleep, while the presumably cholinergic (and/or cholinoceptive) neurons in the dorsolateral pontine tegmentum, which actually show tonic activity during waking, increase firing during REM sleep (cf. 30, 79). It is conceivable that the prominent activation of the *noradrenergic* LC neurons, which is likely to occur during forced waking, associated with some involvement of the cholinergic system contributes to the enhancement of the effects through which specific visual activity in the LGN is thought to cause weakening of deprived afferents, just as shown for the development of the ocular dominance changes which occurs in the visual cortex following MD (ref. in 4, 24, 36). Although the crossed and uncrossed optic fibers terminate predominantly in alternate layers of the dorsal LGN, electrophysiological experiments performed in cats have shown that an inhibitory binocular interaction occurs in units located in all geniculate layers (84). In fact, most geniculate neurons receive an excitatory input from one eye (the "dominant" eye) and an inhibitory input from the other ("non-dominant" eye), this convergence being mediated in

part at least through the perigeniculate nucleus (ref. in 84). We postulate that, during forced waking, NA and to some extent also ACh could potentiate not only the excitatory responses of the geniculate units in the nondeprived layers, but also the inhibitory responses of the units in the visual-deprived layers by acting on the LGN either directly or through the perigeniculate structures. Indeed, there is evidence that NA (85) as well as ACh (70, 71, 76) increase the magnitude of both the excitatory and the inhibitory components of unit responses to visual inputs, as shown in the visual cortex of rats and cats. These findings, if confirmed at the level of the geniculate neurons, would contribute to enhance the interlaminar cell-disparities in the LGN following MD.

In conclusion, it is likely that the increase in the use-dependent modification of synaptic connections in the developing visual system during forced waking results from the synergistic influence that both the noradrenergic and the cholinergic modulatory systems may exert by acting simultaneously (cf. 25, 77).

As to the cholinergic pontine neurons, whose activity increases during REM sleep (ref. in 30, 79), they could act on all layers of the LGN by preventing the occurrence of the abnormalities produced by MD. Opposite effects would obviously occur during SD. This conclusion is supported by the fact that during the episodes of REM sleep, pontine neurons show prominent and rhythmic discharges synchronous with the PGO waves (48, 56, 67; see also 30, 79). This extraretinal pontine input, which leads to phasic activation of the LGN neurons during the PGO waves (6, 44, 45, 69), could then compensate for the absence of the retinal input induced by monocular eyelid closure, thus exerting a protective influence against the severe geniculate deficits following MD. Supporting this hypothesis are the observations that, in kittens, the PGO waves appear during the third postnatal week, in correspondence with the beginning of the critical period (1, 9, 15) and that suppression of PGO activity after a bilateral ponto-mesencepalic lesion results in amplification of the effects of MD (15, 16). The demonstration, however, that PGO waves can be traced from the region of the brachium conjunctivum to the LGN (11, 41, 42, 56, 68; cf. also 30, 79) and that peribrachial (PPT) neurons discharge phasically 10-25 msec prior to the LGN PGO waves (48, 56, 67), does not allow to attribute the source of this ascending pathway solely to the cholinergic PPT neurons projecting to the LGN (cf. 30, 79). Excitatory pontine neurons, probably glutamatergic in nature, could also contribute to the PGO waves. These neurons, acting on the LGN either directly or through the medial vestibular nuclei (62; ref. in 59), would lead to some activity-dependent refinement of the synaptic connections between the retinogeniculate and geniculocortical neurons, which also are glutamatergic in nature (see 55, 73 for rev.). Coincident activation within the LGN of cholinergic and noncholinergic (probably glutamatergic) afferents at the time of the PGO may trigger the specific mechanisms which would lead to strengthening of coactivated synapses, thus counteracting the developmental response of the LGN to the asymmetrical input produced by MD.

It should be finally mentioned that the cholinergic system may act on the LGN not only by modifying the spontaneous and/or the induced discharge of the target neurons (in which case ACh would act as a neuromodulator), but also through

second messengers by modifying the expression of immediate early genes, which may in turn trigger specific changes in the expression of late genes involved in synaptic plasticity in the LGN (cf. 83). Other agents, such as nitric oxide, can also be co-released with ACh, thus playing a specific role in regulating gene expression. This hypothesis is supported by the fact that the cholinergic pathway from the peribrachial region of the brainstem to the LGN of the cat, ending within the layers A and C, stains positively for NADPH-diaphorase, a synthesizing enzyme of nitric oxide (5), and that this pattern of staining clearly changes following rearing of the cats with monocular lid suture (29).

In conclusion, it appears that the facilitatory influence that sleep (60, 61 and the present study), and in particular REM sleep (45, 72), exerts on the maturation of LGN neurons, can be attributed, in part at last, to endogenous activation of the cholinergic system which contributes to the PGO activity not only directly, but also through non-cholinergic afferents projecting to the dorsal LGN. These findings support the ontogenetic hypothesis of REM sleep function (66), as well as the proposal that individual components of active sleep, which display different ontogenetic and phylogenetic histories, may subserve independent functions during CNS development (8).

SUMMARY

1. Observations made by Wiesel and Hubel (87) in kittens during the critical period have shown that monocular visual deprivation (MD) produces hypotrophic changes in the deprived layers of the LGN. Since, in addition to retinal inputs, the LGN also receives extraretinal inputs which are particularly active during REM sleep (a phase which is highly represented at birth), we performed experiments to find out whether total sleep deprivation (SD) interferes with the neuronal maturation in the LGN, thus modifying the susceptibility of LGN neurons to MD.

2. Ten groups of twin kittens were submitted to eyelid suture of one side at the age of 12 to 42 days after birth, and maintained into MD for periods of time which produced only slight or negligible changes in the deprived LGN layers. However, one of the twins in each group was allowed to sleep, while the other was submitted to 2 to 6 days of SD obtained by gentle handling during the last period of MD. At the end of the experiments cross-sectional areas of cell bodies were measured in the binocular segment of different layers of the LGN of both sides, at comparable levels.

3. MD, started 25 to 42 days after birth and continued for 11 to 23 days produced a slight but significant reduction of the mean cell area in the visual-deprived magnocellular ventral (C) and/or dorsal (A) layers of the contralateral LGN, but not in the middle (A1) layer of the ipsilateral LGN. This shrinkage, however, was most severe and involved also the layer A1 if kittens were also submitted to 5-6 days of SD during the last period of MD. There was also a tendency towards increased size in the nondeprived geniculate layers, probably due to an increased monocular visual experience resulting from an increased wake time in the light. However, the slight increase in cell size seen in these layers contrasted with the

prominent increase in shrinkage of the visual-deprived layers after SD, indicating that this finding might have resulted from removing an influence of sleep. The effects of SD appeared to depend on the age of kittens (critical period) and the duration of MD.

4. In conclusion, shortly after birth, SD enhanced the structural abnormalities produced by monocular eyelid closure in the visual-deprived LGN layers. Since rhythmic discharges of pontine structures impinge on the LGN neurons during REM sleep, it is postulated that they could represent an endogenous source of stimulation leading to periodic read out of the synaptic connections between primary optic fibers and LGN neurons. This extraretinal input may thus collaborate with the retinal input to facilitate neuronal maturation of the LGN. The possibility that specific noradrenergic and cholinergic neurons, normally acting on the visual system during the sleep-waking cycle, intervene in the postnatal development of the LGN neurons has been discussed.

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