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Cytological organization of the dorsal lateral geniculate nuclei in mutant anophthalmic and postnatally enucleated mice

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Summary

The dorsal lateral geniculate nuclei (dLGN) of anophthalmic and early postnatally enucleated mice were studied to determine the role retinal fibres play in the differentiation of postsynaptic target structures. Cell counts indicate that retinal fibres are necessary for the development and maintenance of the normal number of dLGN neurons and glia. This retinal fibre dependence is greater for animals enucleated on postnatal day 3 than for animals with a congenital absence of optic axons. Golgi analysis reveals, however, that the lack of retinal fibres does not preclude the development of the thalamo-cortical and intrinsic types of dLGN neuron.

Ultrastructural analysis reveals that in anophthalmic and early postnatally enucleated mice, dLGN synaptic sites normally occupied by optic axon terminals become innervated by large terminals containing round synaptic vesicles and mitochondria with an electron dense matrix. Significantly, the formation of these replacement terminals does not seem to depend upon either the previous existence of retinal fibres or the early postnatal stage at which retinal fibres are removed. The possibility that some of these large replacement terminals originate from cortical or recurrent collateral axons is considered.

Introduction

Fundamental to the design of the mammalian central nervous system is the interaction and possible interdependence of its various structural components during development. One such essential interaction involves primary afferents and their postsynaptic target neurons (see Cotman and Banker, 1974 for review).

The present investigation was initiated to determine what role retinal fibres play in the development and maintenance of the mammalian dorsal lateral geniculate nucleus (dLGN). While previous light microscope studies on enucleated rats, cats,

monkeys and mice indicate that the postnatal removal of retinal fibres does not result in the total elimination of the dLGN (see Cowan, 1970 for review), ultrastructural investigations on the dLGN of these enucleated mammals (Colonnier and Guillery, 1964; McMahan, 1967; Vaccarezza *et al.*, 1970; Lund and Cunningham, 1972; Wong-Riley, 1972; Famiglietti and Peters, 1972; Lieberman and Webster, 1974) have dealt with the sequence of degeneration which immediately follows eye removal and have not studied the possibility of synaptic reorganization within the deafferented nucleus. We have examined the dLGN of congenitally anophthalmic and postnatally enucleated mice to determine the compensatory response of neurons exposed to varying developmental periods of retinal afferent input.

Our use of the mutant anophthalmic mouse (Chase and Chase, 1941; Beck, 1963) allowed us to explore the degree to which the dLGN can differentiate in the complete absence of retinal fibres. In anophthalmic mice, lens induction is impaired and results in lens displacement and eventual resorption of the optic rudiment (Silver and Hughes, 1974). Consequently, retinal ganglion cells do not differentiate (Chase, 1945; Silver and Hughes, 1974), and the dLGN is never influenced by retinal afferent fibres. In contrast, by removing eyes from normal mice at different periods shortly after birth, we were able to produce animals whose dLGN received retinal afferent fibres for defined developmental intervals. Mice bilaterally enucleated before and after eye opening (day 13–14) were examined for differential synaptic associations. A summary of our results has been presented (Cullen and Kaiserman-Abramof, 1975).

Materials and methods

General

A total of 110 mice from CFW 1, ZRDCT-N, and ZRDCT-An strains were used in this study. The CFW 1 and ZRDCT-N animals have normal-sized albino eyes. The ZRDCT-An animals used in this investigation completely lacked eyes and optic nerves. All animals were adults (50 days or older) at sacrifice.

Enucleations

Animals were anaesthetized with either 5% chloral hydrate or Equinotic (W. A. Butler Co.) administered intraperitoneally. A total of 67 animals (ZRDCT-N and CFW 1) were bilaterally enucleated between 1 and 20 days of age. Following removal of the eyes, the orbits were packed with Gelfoam and the animals were maintained on a 12 h light: 12 h dark cycle until sacrifice.

Tissue Preparation

Four normal and three ZRDCT-An mice, along with two ZRDCT-N animals bilaterally enucleated on postnatal day 3, were perfused with 10% formalin at 50 days of age. The brains were stored for 1 week in formol-sucrose solution (30% sucrose w/v 10% formalin), and embedded in albumin-gelatin (Ebbesson, 1970). Frozen sections were cut at 25 μm. Every fourth coronal section was mounted on chrome-alum slides and stained with 0.25% cresyl violet (Ramon-Moliner, 1970). Adjacent sections were treated by a modified Bielschowsky method in which sections were impregnated overnight in a 2.2% silver nitrate-11% pyridine solution and subsequently treated for 3 min in a freshly prepared ammoniacal silver nitrate solution (2.5% sodium hydroxide, 7.2 ml;

concentrated ammonium hydroxide, 4.8 ml; 2.5% silver nitrate, 100 ml). The tissue was transferred to Nauta-Gygax reducer (Heimer, 1970) until uniformly brown and then rinsed in distilled water, fixed for 1 min in 5% sodium thiosulphate, and mounted on chrome-alum slides.

For Golgi-Kopsch preparations and electron microscopy, animals were perfused with the double aldehyde method of Brightman and Reese (1969). The fixed brains were left *in situ* overnight at 4° C.

Brains to be used for Golgi-Kopsch preparations (Colonnier, 1964) were stored in concentrated fixative for a minimum of one month before impregnation. Tissue from 52 adults (50–550 days) was impregnated, sectioned free hand in the coronal plane (100–150 micrometers thick), dehydrated in ethanol, and covered with Damar (Matheson, Coleman and Bell Co.).

Tissue from 40 operated mice and 9 normal and anophthalmic mice was processed for electron microscopy. Specimens were obtained by cutting 1 mm coronal slabs and washing them in 0.15 M cacodylate buffer. The slabs were osmicated with 2% osmium tetroxide in 0.08 M buffer, washed in 0.15 M buffer and dehydrated in a graded series of ethanols. Thick (1 μm) Araldite sections were stained with 1% toluidine blue and the posterior half of the dLGN identified for thin sectioning.

Quantitative Analysis

The entire rostrocaudal extent of the dLGN was determined in the Nissl stained sections. Composite photographs of sections through the dLGN were taken with a Leitz apochromatic 12.5 objective. Prints were made at a final magnification of 400 diameters and assembled into montages corresponding to each section. All the dLGN neurons with clearly visible nucleoli and glial cells with distinct nuclei were counted. Three independent cell counts were made for each section; the results were averaged and the means were used to estimate the number of neurons and glial cells in each dLGN. Means were analyzed by Student's *t*-test for small samples (Sokal and Rohlf, 1973) and a $P < 0.05$ value was the minimum acceptable level of significance.

Results

The dorsal lateral geniculate nuclei of normal-eyed CFW 1 and ZRDCT-N mice were similar in size, in histological and ultrastructural organization, and in their response to enucleation. Since animals of both strains were suitable controls for comparisons with eyeless mice, the observations on CFW 1 and ZRDCT-N mice were grouped together.

LIGHT MICROSCOPIC ANALYSIS

Control animals

The dLGN is situated on the lateral aspect of the diencephalon. It is limited laterally by the optic tract, ventrally by the ventral division of the lateral geniculate nucleus (vLGN), medially by the fibres of the radiatio thalamica superior (Gurdjian, 1927; Sidman *et al.*, 1971), and dorsally by the nucleus lateralis posterior thalami (Fig. 1; Bucher and Nauta, 1954; Shintani, 1959).

The neurons of the dLGN were randomly distributed throughout the nucleus while corticofugal and retinofugal fibres (Montero and Guillery, 1968) formed transverse bundles that crossed from the ventrolateral to the dorsomedial aspect of the nucleus (Fig. 2).

Anophthalmic animals

The dLGN of the anophthalmic mouse lacked an optic tract and was instead limited laterally by the pial surface of the diencephalon (Figs. 3 and 4). The other boundaries were similar to those seen in normal mice.

The anophthalmic mouse dLGN had approximately 76% of the neurons and 55% of the glial cells that occur in the normal animals (Figs. 7 and 8). As in controls, the cells appeared randomly distributed. Transverse bundles of corticofugal fibres ran through the medial portion of the dLGN, but fibre bundles were not present in the dorsolateral regions of the nucleus (Fig. 4).

Early postnatally enucleated animals

To compare the effects of anophthalmia with those of early postnatal deafferentation, control animals were enucleated bilaterally on the third postnatal day and sacrificed on postnatal day 50.

The dLGN of the enucleated animals was smaller in all dimensions when compared with control animals; however, when compared with ZRDCT-An animals, the only reduction occurred in the dorsoventral axis. Furthermore, the number of neurons and glial cells in the deafferented dLGN was significantly less than that produced by the anophthalmic condition (Figs. 7 and 8).

In enucleated mice, transverse bundles of fibres ran through the ventromedial portion of the dLGN while a few fibres of unknown origin coursed through the area normally occupied by the optic tract (Fig. 6).

Figs. 1–6. Coronal sections from 50 day old mice. Ventral is down and medial is to the right. x 45.

Fig. 1. Approximate middle of the dorsal lateral geniculate nucleus (*dLGN*) of a control mouse. The medium size cells of the dLGN are demarcated from the smaller cells of the ventral lateral geniculate nucleus (*vLGN*). The distribution between the cells of the dLGN and the cells of the nucleus lateralis posterior thalami (*LP*) is less clear. *DG*, dentate gyrus. Nissl stain.

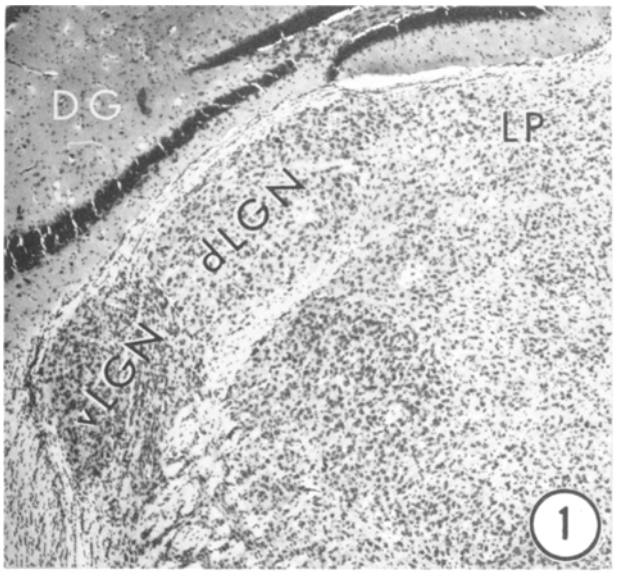
Fig. 2. Approximate middle of the dLGN of a control mouse. The fibres of the optic tract (*ot*) form the lateral border of the dLGN. The medial border is formed by the fibres of the radiatio thalamica superior (*rts*). Transverse bundles (*tb*) of corticofugal and retinofugal fibres cross through the dLGN. Modified Bielschowsky stain.

Fig. 3. Approximate middle of the dLGN of an anophthalmic mouse. The dLGN is reduced in size when compared with the control mouse dLGN (see Fig. 1). The dLGN neurons are still distinctly demarcated from the cells of the surrounding thalamic nuclei. Nissl stain.

Fig. 4. Approximate middle of the dLGN of an anophthalmic mouse. The dLGN of anophthalmic mice is limited medially by fibres of the radiatio thalamica superior. In contrast to controls (see Fig. 2), no optic tract fibres are present at the lateral edge of the nucleus. Modified Bielschowsky stain.

Fig. 5. Approximate middle of the dLGN of a control mouse bilaterally enucleated on postnatal day 3. The dLGN is reduced in cross sectional area to approximately half normal size (see Fig. 1). Localized areas of cell loss are not seen. Nissl stain.

Fig. 6. Approximate middle of the dLGN of a control mouse bilaterally enucleated on postnatal day 3. Transverse bundles of fibres are almost completely missing. Fibres of the radiatio thalamica superior form the medial border of the dLGN, while fibres of unknown origin (*x*) run in the area normally occupied by the optic tract (see Fig. 2). Modified Bielschowsky stain.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LP
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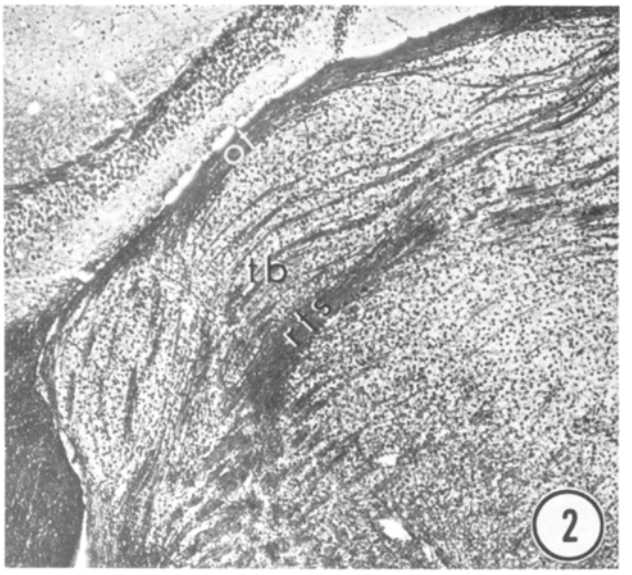
1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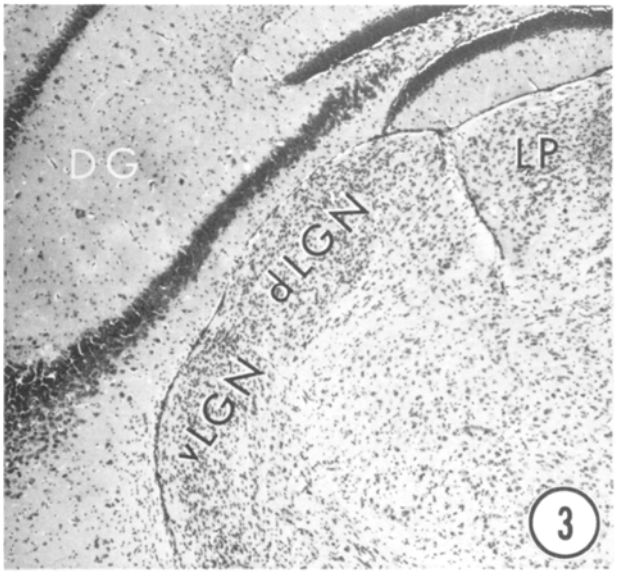
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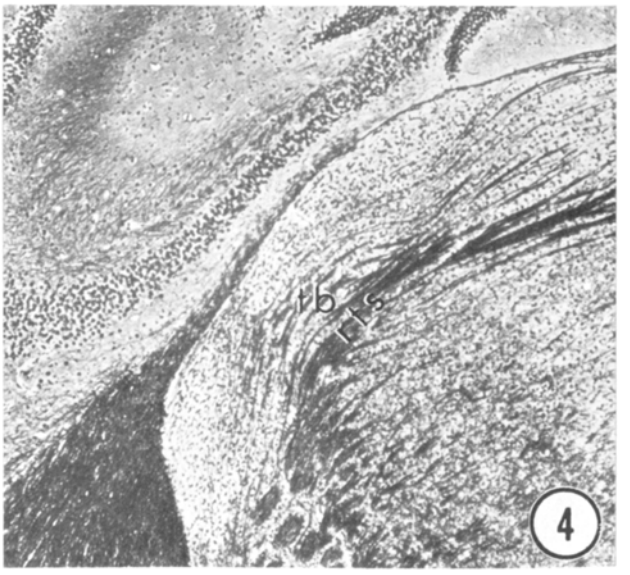
3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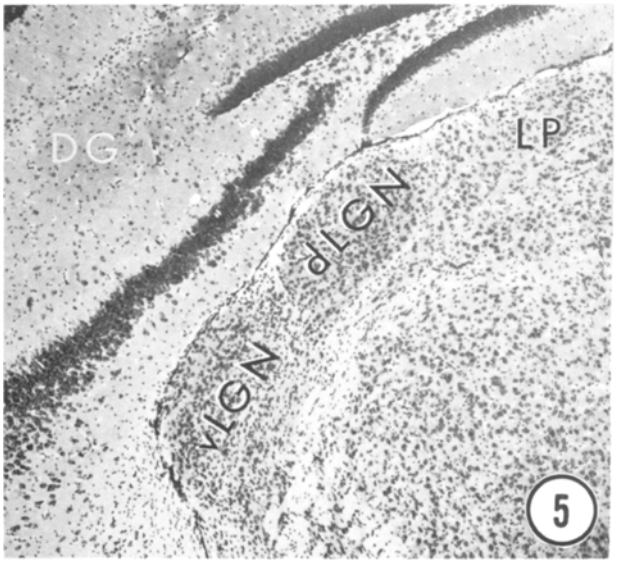
DG
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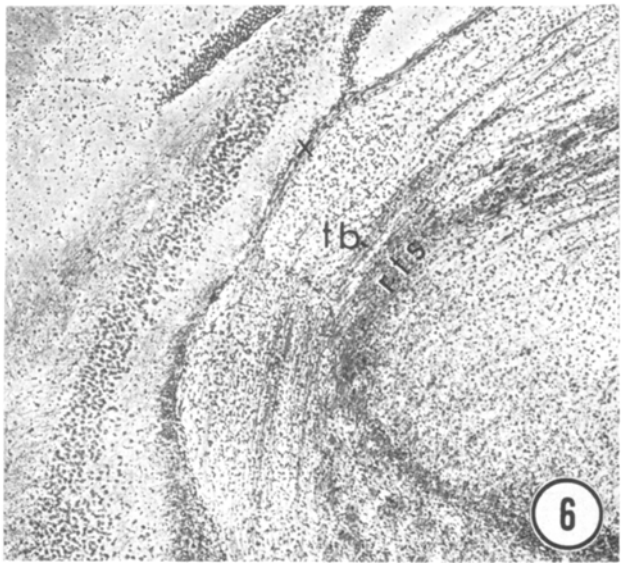
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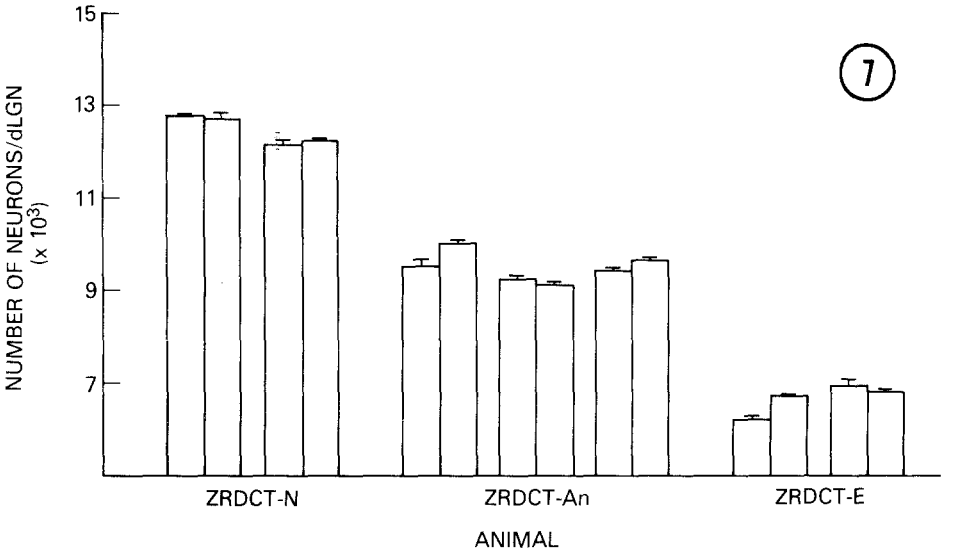

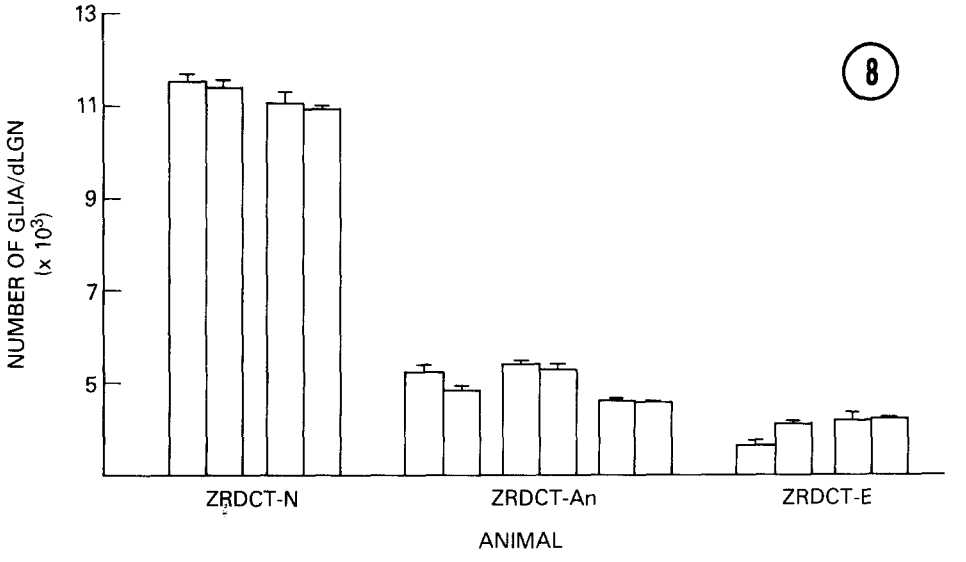

Figs. 7 and 8. Histograms of the estimated total number of cells per dLGN of 50 day old mice. Each bar represents the mean cell numbers and standard deviation for one dLGN. Abbreviations: ZRDCT-N, normal; ZRDCT-An, anophthalmic; ZRDCT-E, bilaterally enucleated on postnatal day 3.

Fig. 7. Total number of neurons per dLGN.

Fig. 8. Total number of glial cells per dLGN.

GOLGI ANALYSIS

Control animals

The mouse dLGN contains two general populations of neurons; the thalamo-cortical relay (TCR) and intrinsic neurons. Present observations were in close agreement with the detailed analysis of the dLGN of C57BL/6J mice (Rafols and Valverde, 1973) and will be discussed only briefly below.

The perikarya of the TCR neurons were round to oval in shape and measured approximately 13–22 μm in diameter. Usually 3–6 dendrites, approximately 2 μm in diameter, emerged from these perikarya and branched into secondary dendrites that radiated into all planes of the dLGN (Fig. 9a). Dendritic shafts were studded with a moderate number of peg-like protuberances (Fig. 9, upper inset), small individual spines, and an occasional cluster of spines at branching points. The initial segment of the axon was evident on some TCR neurons; however, axonal collaterals were not observed.

Intrinsic neurons had round, oval or spindle-shaped perikarya measuring 7–22 μm in diameter (Fig. 9d). Usually there were 2–3 main dendrites; however, as many as 5 were occasionally observed. In contrast to the dendritic pattern of TCR neurons, the dendritic arborization of most intrinsic neurons was oriented along the dorsoventral axis of the dLGN. Also characteristic of the intrinsic neurons was a unique type of dendritic appendages which has been termed a spheroidal body by Rafols and Valverde (1973). Spheroidal bodies have a large, bead-like knob (0.5–2 μm in diameter) attached by a slender stalk to the smooth portion of the dendrite. Many dendrites were densely covered with spheroidal bodies while some displayed relatively few. No definite axon could be identified.

Overall, the major morphological differences between TCR and intrinsic neurons were the general orientation of their dendrites and the characteristic dendritic protrusions. Although these differences could be used to classify most of the dLGN neurons, some cells were intermediate in appearance and could not be clearly assigned to either the TCR or intrinsic neuronal types.

Anophthalmic and early postnatally enucleated animals

Neurons characteristic of both the TCR and intrinsic types were present in anophthalmic and enucleated mice. TCR neurons were approximately 12–20 μm in diameter (Fig. 9b). The TCR cell body gave rise to 3–6 main dendrites, approximately 2 μm in diameter, that radiated branches into all planes of the dLGN. Although our observations suggested that TCR neurons had fewer dendritic protrusions than normal, quantitative comparisons between normal and eyeless animals were not possible because of the relatively small number of neurons impregnated, as well as the large variation in their morphology.

Most intrinsic neurons of anophthalmic and enucleated mice were normal with regard to size, shape and number of main dendrites. Dendrites were covered with typical spheroidal bodies. In addition, the majority of the intrinsic neurons aligned their dendrites along the dorsoventral axis of the dLGN.

Unique to anophthalmic and early postnatally enucleated animals were several large, complex cells with dendrites oriented along the dorsoventral axis of the dLGN. The dendrites of these cells were covered with appendages having a long slender stalk and large, bead-like knob (Fig. 9c). Because of the similarity of these appendages to spheroidal bodies, as well as the dorsoventral orientation of the dendrites, these complex cells are tentatively classified as intrinsic neurons.

In summary, TCR and intrinsic types of neuron are found in the dLGN of anophthalmic and early postnatally enucleated mice. Most of these cells are within the normal range of size, shape and number of main dendrites.

ELECTRON MICROSCOPIC ANALYSIS

Control animals

Our observations were in general agreement with previous ultrastructural investigations of the normal mammalian dLGN (Guillery, 1971; Famiglietti and Peters, 1972; Lund and Cunningham, 1972; Wong-Riley, 1972; Szentágothai, 1973; Rafols and Valverde, 1973; Lieberman, 1974; Lieberman and Webster, 1974). In the

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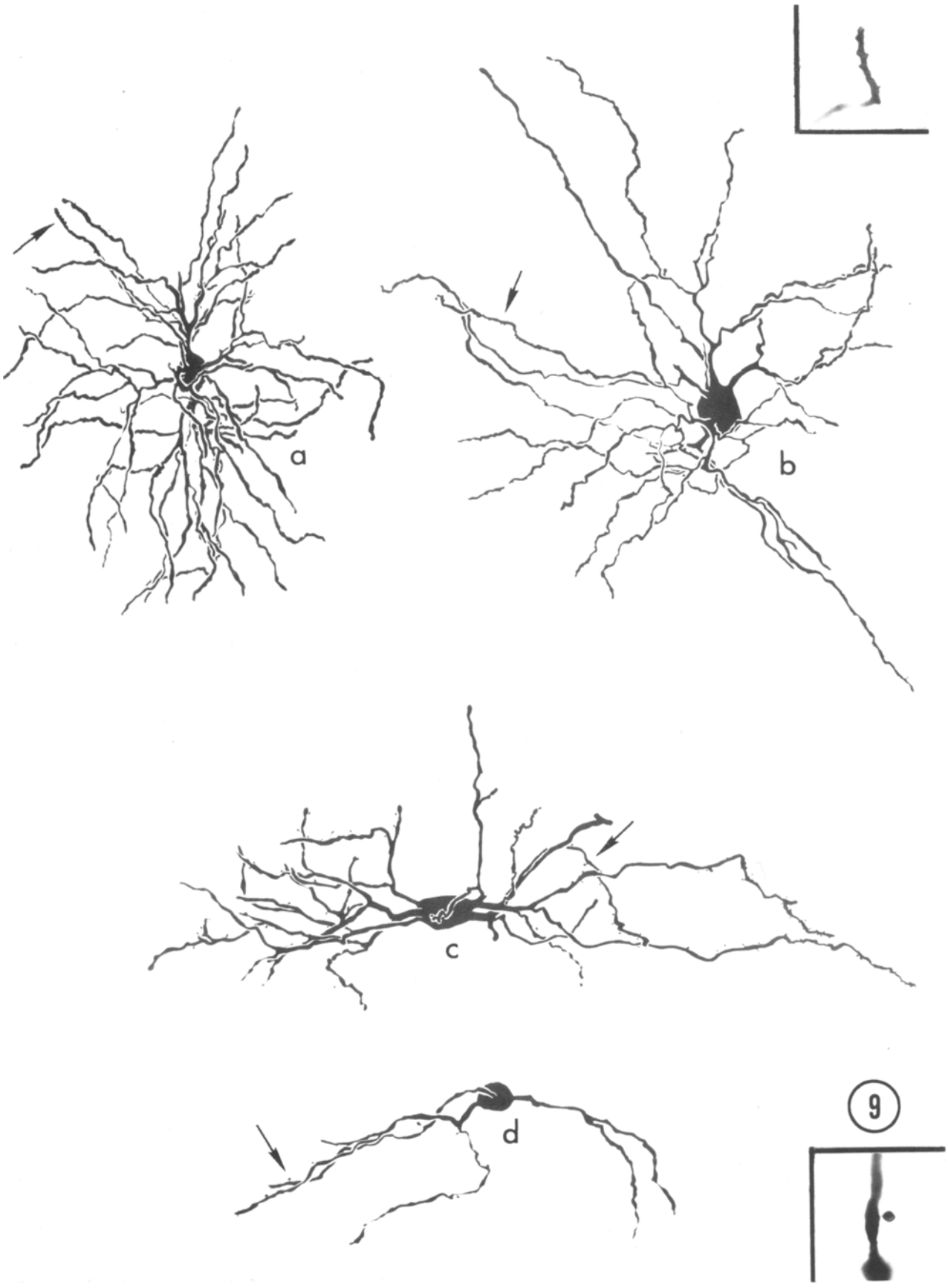
Fig. 9 and insets. Golgi-Kopsch preparations of dLGN neurons. Thalamo-cortical relay neurons (TCR) from a normal (a) and anophthalmic (b) mouse radiate dendrites into all planes of the dLGN. In addition to the radial dendritic arborization, TCR neurons are characterized by peg-like dendritic protrusions (upper inset). These peg-like protrusions are present on the dendrites of normal, anophthalmic and early postnatally enucleated mouse TCR neurons (arrows). The large anophthalmic mouse TCR neuron (b) is within the normal range of perikaryal size and number of main dendrites. The control mouse TCR neuron (a) is typical of small relay neurons. Characteristic of intrinsic neurons are a dorsoventral dendrite orientation and large dendritic spheroidal bodies (d, arrow). Although most intrinsic neurons found in anophthalmic and early postnatally enucleated mice resembled the normal mouse neuron shown in d, several eyeless animals displayed complex cells with dorsoventral dendritic arborizations and spheroidal bodies (c, arrow). These complex cells are tentatively classified as intrinsic neurons. Camera lucida drawings x 500. Photomicrograph insets x 1275.

Fig. 10. The dLGN neuropil of a control mouse. A probably F_2 terminal (F_2) is postsynaptic to an RLP terminal (*RLP*). A second RLP terminal profile associates with several dendritic spines (*sp*). An RSD terminal (*RSD*) with round vesicles and a mitochondrion with electron dense matrix makes asymmetric synaptic contact with a dendrite. The F_1 terminal (F_1) in the upper right corner contains flattened vesicles and makes a symmetric contact (arrowhead) with a dendrite. x 27 000.

Fig. 11. The dLGN neuropil of an anophthalmic mouse. The large terminal (6.2 μm in diameter) makes several asymmetric contacts with dendritic spines (*sp*). The large terminal contains round, 400–500 Å diameter vesicles and mitochondria with darkly stained, electron dense matrix. The terminal resembles an unusually large RSD terminal. These large terminals replace the RLP terminals of the normal mouse dLGN. x 27 000.

Fig. 12. The dLGN neuropil of an anophthalmic mouse. Two dendrites (D_1 and D_2) form an elaborate filamentous contact. Neurofilaments (*nf*) run almost parallel to the cytoplasmic face of the membrane. The intercellular cleft is filled with an electron dense material. An F_1 terminal (F_1) forms a symmetric contact with one of the dendrites (D_1). x 27 000.

Fig. 13. The dLGN neuropil of a 50 day old control mouse bilaterally enucleated on postnatal day 2. The large terminal (2.7 μm diameter) contains round vesicles and dark matrix mitochondria and makes asymmetric contact with a dendritic spine (*sp*). x 27 000.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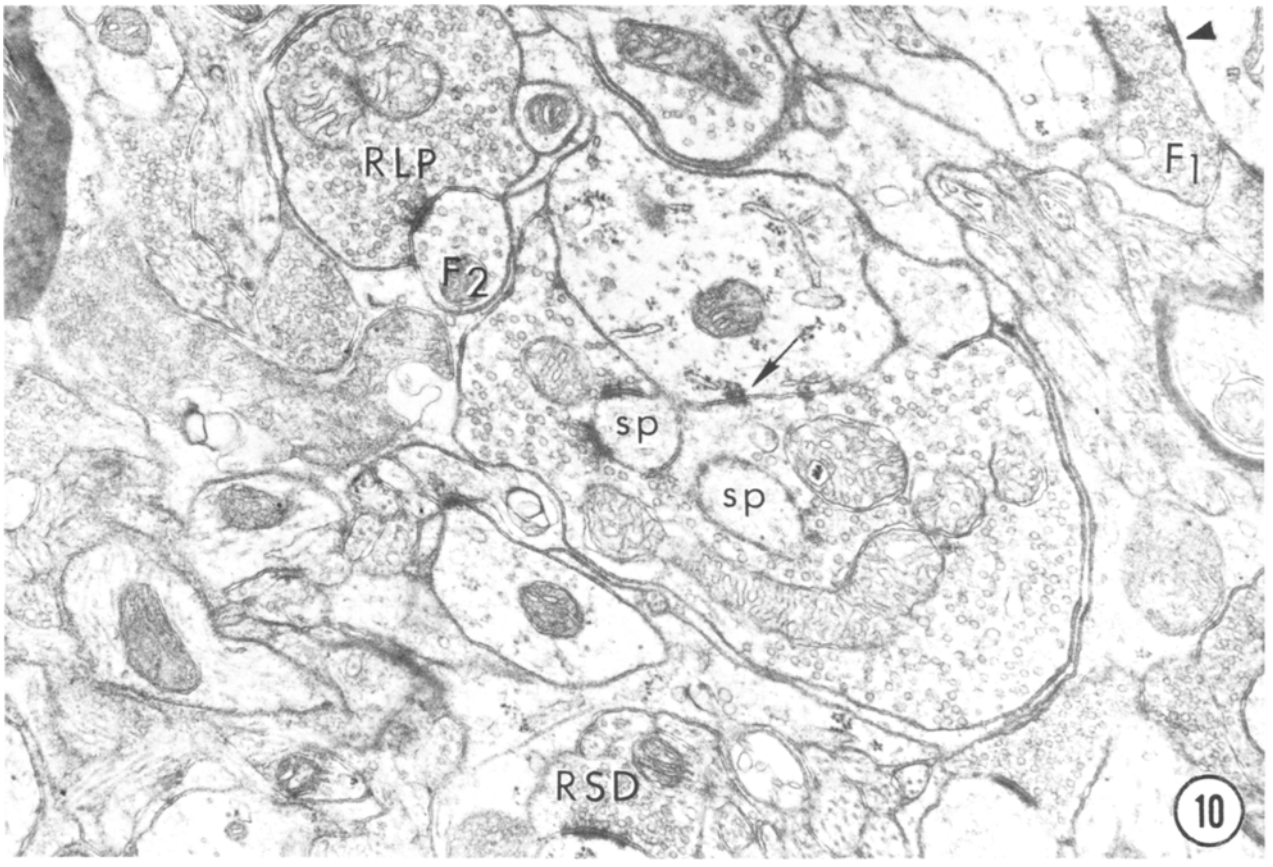
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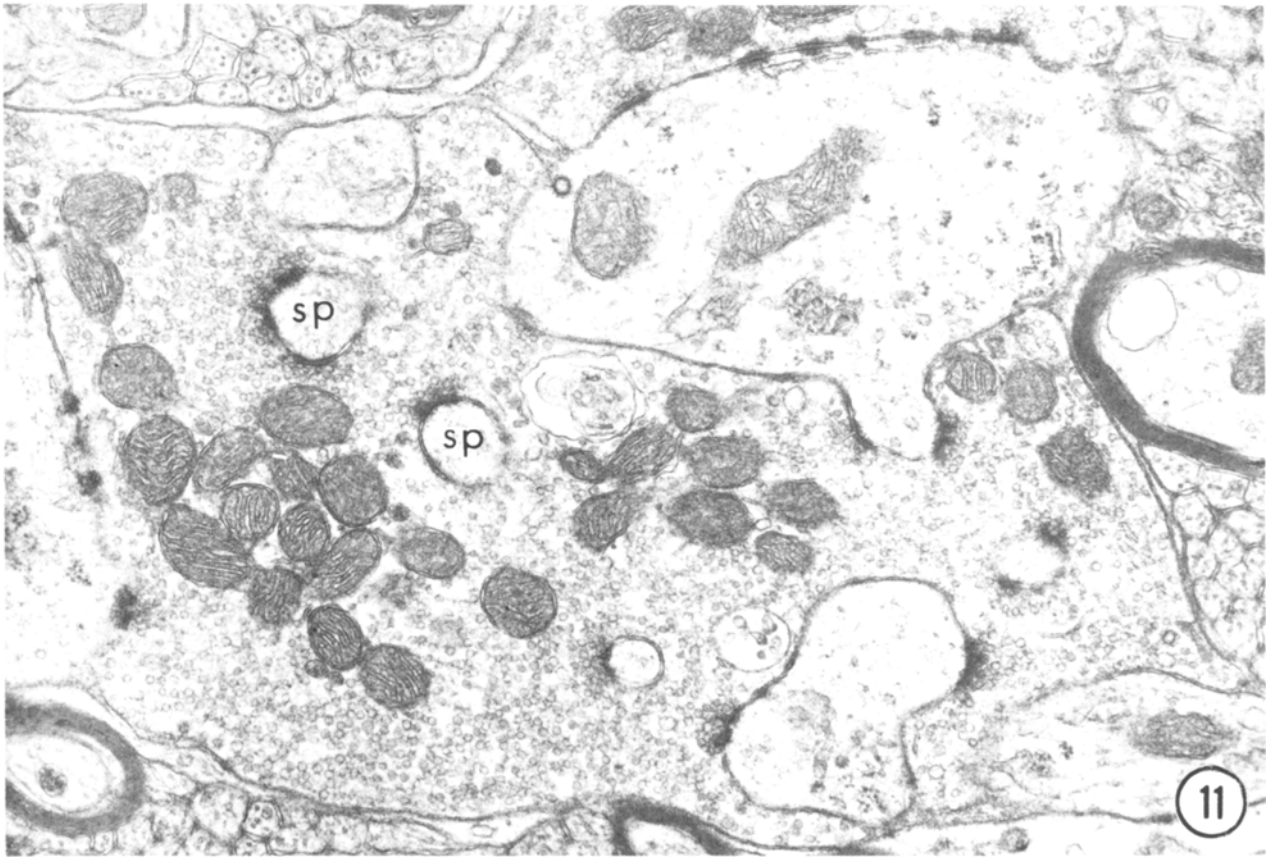
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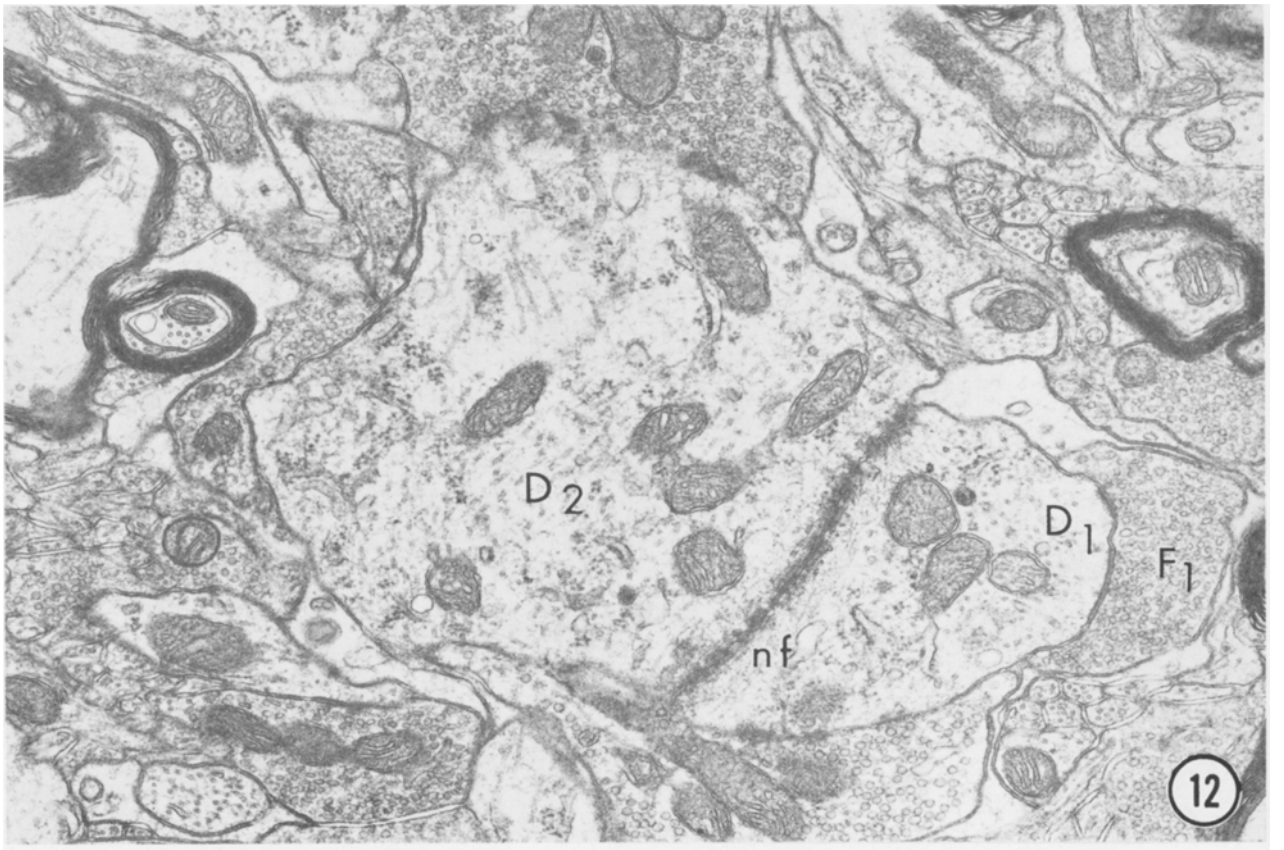
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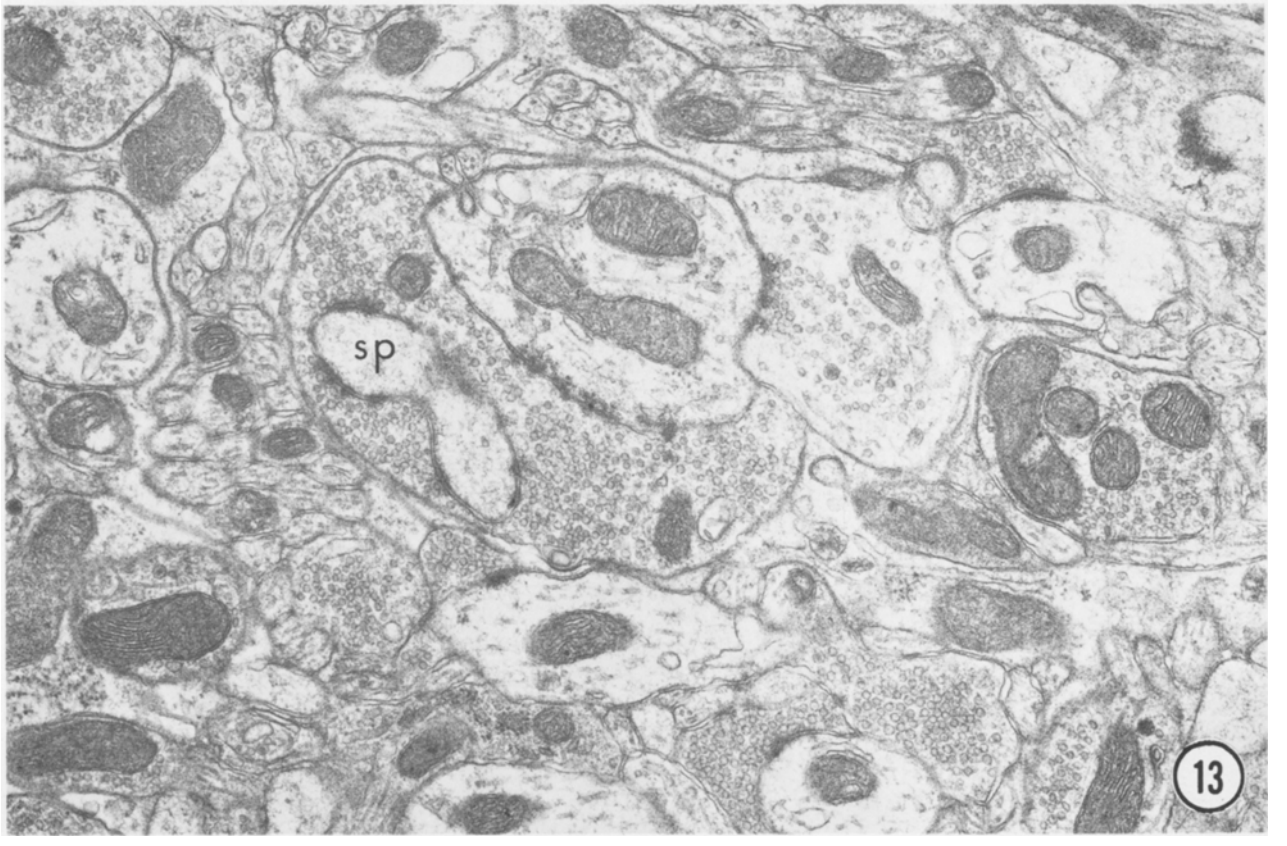
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13

present study, the dLGN terminal classification of Guillery (1969; 1971) has been used. Therefore, the synaptic terminals of the mouse dLGN were grouped into one of four categories; RLP, F_1, F_2, or RSD.

RLP (*R*ound vesicles, *L*arge size, *P*ale mitochondria) These were the largest terminals in the normal mouse dLGN. They were irregular in shape and usually between 1.3–3.3 μm in long diameter (range, 0.8–6.0 μm). The most distinguishing characteristic related to the mitochondria which have a pale staining, electron lucent matrix and few dilated tubular cristae (Fig. 10). RLP terminals formed asymmetric synapses with dendritic shafts 1.0–2.2 μm in diameter, dendritic protrusions (Fig. 10), and other terminals. RLP terminals were often associated with other neuronal processes in a glial ensheathed arrangement termed an encapsulated synaptic zone (Guillery, 1971; Lund and Cunningham, 1972) or glomerulus (see Szentágothai, 1973). Within the encapsulated zone RLP terminals were seen to synapse on both F_2 terminals (see below) and large, presumably TCR cell, dendrites. The F_2 terminals often synapsed in turn on the same dendrite. The development and possible physiological significance of these serial RLP → F_2 → dendrite synaptic arrangements have been described (Rafols and Valverde, 1973; Lieberman and Webster, 1974; Hámori *et al.*, 1974; Hámori *et al.*, 1975). Occasionally an RLP terminal will form a filamentous contact with a dendrite (Colonnier and Guillery, 1964). Enucleation experiments demonstrate RLP terminals to be of retinal fibre origin (see Szentágothai, 1973 for review).

F_1 and F_2 The flattened vesicle containing terminals can be separated into two types. F_2 terminals correspond in size and shape to the spheroidal bodies of light microscopy. F_2 terminals were typically postsynaptic to other terminals (Fig. 10) and contained round or slightly oval shaped synaptic vesicles. Occasionally microtubules were present; however, the relative lack of organelles gave a characteristic light appearance to the cytoplasm of these terminals. All the data suggest that F_2 terminals originate from the dendrites of intrinsic neurons (see Lieberman and Webster, 1974 for review). In contrast to the F_2 terminals which they closely resembled, F_1 terminals contained vesicles that were flatter and more densely packed (Figs. 10 and 12). Both F_1 and F_2 terminals made symmetric contacts with dendritic profiles, while only F_1 terminals synapsed with neuronal cell bodies. F_1 terminals appear to be of axonal origin (see Lieberman and Webster, 1974 for review).

RSD (*R*ound vesicles, *S*mall size, *D*ense mitochondria) The fourth type of terminal was 0.3–1.0 μm in diameter and densely packed with round vesicles. The synaptic complex was of the asymmetric type terminating on perikarya or on small (usually less than 1 μm in diameter) dendritic profiles. Many of the RSD terminals may be of cortical origin (Lund and Cunningham, 1972; Lieberman and Webster, 1974).

In summary, symmetric synaptic contacts were formed by 0.2–2.0 μm diameter

terminals of the F_1 and F_2 types; asymmetric contacts were made by terminals of the RLP and RSD types. Terminals larger than 2 μm that form asymmetric synapses with dendritic protrusions were of the RLP type. Filamentous axo-dendritic and neuronal soma-somatic contacts (Colonnier and Guillery, 1964) were observed.

Anophthalmic animals

The dLGN of adult anophthalmic mice appeared normal with respect to the perikarya, dendrites, axons, and F_1, F_2, and RSD terminals. F_2 terminals were postsynaptic to F_1 and RSD terminals. In contrast to the normal, however, RLP terminals were absent and instead large (range 1.0–6.2 μm) diameter terminals containing mitochondria with an electron dense matrix and 400–500 Å round vesicles occupied the dendritic protrusions normally covered by the RLP terminals (Fig. 11). These large terminals formed asymmetric synaptic contacts and, except for their size and location on dendritic protrusions, resembled RSD terminals. Like RLP terminals, these large terminals formed occasional axo-dendritic filamentous contacts.

A second feature of the anophthalmic mouse dLGN was the frequent occurrence of elaborate dendro-dendritic filamentous contacts (Fig. 12). We did not note such elaborate dendro-dendritic filamentous contacts in the CFW1 or ZRDCT-N mouse dLGN. It is of importance, however, that these contacts have been noted in both the C57BL/6J mouse dLGN (Lieberman, personal communication) and the monkey dLGN (Colonnier and Guillery, 1964). It may be that our failure to note these filamentous contacts in CFW 1 and ZRDCT-N mice reflects a normally low frequency of these contacts rather than their complete absence. The function of these elaborate contacts is unclear (see Colonnier and Guillery, 1964 for discussion).

Early postnatally enucleated animals

Control animals were bilaterally enucleated at either 1, 2, 3, 5 or 20 days after birth to determine whether postnatal deafferentation alters the dLGN in the same or in a different manner than the congenital absence of retinal fibres. All lesioned animals were 50 days or older at sacrifice.

Enucleation at different developmental stages before eye opening or after eye opening resulted in a similar synaptic rearrangement in the dLGN of all deafferented mice. RLP terminals were absent. Large terminals with 400–500 Å round vesicles and dark matrix mitochondria replaced retinal terminals on dendritic protrusions (Fig. 13). Type F_1, F_2 and RSD terminals, as well as filamentous contacts of the type described for anophthalmic mice, were present. With the exception of some distortions caused by swollen astrocytic processes, the dLGN of enucleated control mice appeared identical to the dLGN of anophthalmic mice.

In summary, neither the congenital absence of retinal fibres nor the postnatal removal of retinal fibres prevented the development and maintenance of characteristic F_1, F_2, and RSD types of dLGN terminals. In addition, large terminals containing round vesicles and mitochondria with a dark matrix occupied asymmetric

sites that were normally innervated by axon terminals of retinal ganglion cells. Although some of these large terminals were ensheathed by layers of astrocyte membrane, the serial type synaptic associations involving F_2 terminals were not seen.

Discussion

Comparison of anophthalmic and enucleated mice demonstrates that much of the differentiation of the mouse dLGN does not depend upon retinal afferent fibres. Developing dLGN neurons can mature to form characteristic TCR and intrinsic types of neurons in the complete absence of influences from retinal afferent fibres. Furthermore, in anophthalmic and early postnatally enucleated mice the dendritic sites that are normally occupied by retinal fibre terminals develop and form distinctive synaptic relationships. It appears, therefore, that dLGN neurons and their dendritic protrusions may develop independently, as a result of direct induction by afferent fibres other than retinal fibres, or by some indirect induction process (Hámori, 1973).

Although many dLGN neurons appear not to be exclusively dependent upon retinal fibres, a certain percentage of neurons do degenerate in both anophthalmic and enucleated animals (see below). Of particular interest is our finding that more neurons are lost when the dLGN is initially exposed to retinal afferents and subsequently deafferented by eye enucleation than are lost as a consequence of complete developmental absence of retinal input. Previous theories stressing the greater sensitivity or trophic dependence of immature versus fully developed neurons (Cowan, 1970) do not adequately explain our findings. It seems probable that the degree of cell loss is more directly related to the recruitment of sustaining influences from the remaining afferent fibres. Neurons that completely lack a normal source of afferent fibres, as in anophthalmia, have a longer developmental period in which to recruit sustaining influences than is available to neurons previously allowed and later abruptly denied this normal input, as in enucleation. In addition, postnatal removal of eyes would be expected to lead to cell death unless the remaining afferents exceeded some sustaining threshold. The ability of mature neurons to survive enucleation (see Cowan, 1970 for review) may well reflect a sustaining margin afforded by the RSD and F terminals that persist in the deafferented dLGN.

Integral to the previous discussion is the assumption that the subnormal neuron population in anophthalmic and enucleated mice is due to cell degeneration rather than decreased proliferation. In the chick, if the peripheral organ is removed prior to the development of the related central neurons, the neurons form normally and later degenerate (Levi-Montalcini, 1949). The C.N.S. neurons are generally unaffected until the stage at which synaptic connections are formed. Similarly, the neurons of the mouse dLGN form independently from the retinal afferent fibres. The dLGN neurons arise between embryonic days 11 and 13 (Angevine, 1970) while the first

retinal fibres do not reach the brain until day 14 (Chase, 1945). Consequently, retinal fibres appear to be necessary for the maintenance of the normal number of dLGN neurons but are not necessary for their initial formation.

As an extension of the previous discussion, retinal fibres may be directly or indirectly responsible for both the development and the maintenance of the dLGN glial cells. DeLong and Sidman (1962) demonstrated that enucleation at birth reduced the number of glial cells in the adult colliculus. These reductions resulted mainly from a decrease in glial proliferation and to a lesser extent from a degeneration of existing glial cells. A similar response may occur in the dLGN of enucleated and anophthalmic mice.

Our comparison of anophthalmic and enucleated mice suggests that the initial presence of retinal fibres does not uniquely change the general mechanisms that control early development of the dLGN. All the data indicate that there are no qualitative differences between anophthalmic and early postnatally enucleated mice with respect to either dLGN neuronal types or synaptic arrangements. It is significant that these same neuronal types and synaptic arrangements were found in animals enucleated at various times between 1 and 20 days after birth. Although this implies that the response mechanism to the lack of retinal fibres is the same over the entire developmental period, direct evidence for such a contention is lacking.

The decision to enucleate animals over the interval between 1–20 postnatal days is based on the assumption that synaptogenesis in the mouse dLGN occurs mainly over the period extending from just before birth until shortly after eye opening (day 13–14). Golgi analysis of young mice (unpublished observations) demonstrates that mature dLGN neurons are present as early as 10 days after birth. In addition, in the rat it appears that the growth of retinal and cortical fibres into the dLGN is completed by the time of eye opening (Cunningham, 1972; Lund *et al.*, 1973). Again, although direct evidence is lacking, the implications are that most, if not all, of the extrinsic afferent fibres form their connections in time for eye opening. Any developmental influences exerted by retinal fibres would be minor by 3 weeks after birth.

Synaptogenesis in the mammalian C.N.S. involves a complex, highly ordered set of interactions. Integral to these interactions is a system for selecting 'appropriate' synaptic connections while excluding 'incorrect' synaptic associations. Since specificity, timing, and competition have been implicated in the selection process (see Cotman and Banker, 1974 for review), the question arises whether the large terminals noted in eyeless animals are specific substitutes for optic axon terminals or whether they simply result from a generalized response.

We have noted that terminals containing flattened synaptic vesicles do not replace optic axon terminals on dendritic protrusions. This may be because terminals containing flattened vesicles are not compatible with the available synaptic sites. An alternative possibility is that the terminals containing flattened vesicles form synapses relatively late in development and are capable of only minor compensatory responses in immature animals. In the rat superior colliculus, many terminals

containing flattened vesicles develop after eye opening (Lund and Lund, 1972). This may explain why they find relatively greater numbers of these terminals in enucleated adult rats than enucleated newborn rats (Lund and Lund, 1971).

The morphology of the large replacement terminals, as well as the characteristic asymmetric nature of the synaptic complex on dendritic protrusions, suggests the sprouting or modification of RSD terminals in anophthalmic and early postnatally enucleated mice. Many RSD terminals in normal animals degenerate following cortical lesions (unpublished observations, also Lund and Cunningham, 1972; Lieberman and Webster, 1974). Similarly, some of the large terminals in anophthalmic and early postnatally enucleated mice are lost after heat lesions to the occipital region of the neocortex (unpublished observations). While these findings suggest a local sprouting or modification of ipsilateral cortical and/or recurrent collateral axon terminals in eyeless mice, the rapid retrograde degeneration and the ensuing rearrangement within the dLGN make it difficult to determine whether large terminals are lost as a direct consequence of the cortical lesion or as a secondary consequence of the elimination of postsynaptic structures through retrograde cell death and/or cell atrophy. An unequivocal answer as to the role of cortical and/or recurrent collateral fibres in the reorganization of the dLGN of anophthalmic and early postnatally enucleated mice requires further investigation.

In summary, our evidence indicates that dendritic protrusions on dLGN neurons can receive afferent terminals other than optic axon terminals. Further studies are necessary to decide whether the formation of these replacement terminals is a random or a specific response. This information may give insight into the mechanism that prevents the formation and/or maintenance of these large 'inappropriate' terminals in normal animals.

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