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Synapses of Optic Nerve Afferents in the Rat Suprachiasmatic Nucleus

I. Identification, Qualitative Description, Development and Distribution

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Summary. Synapses of optic nerve afferents (optic synapses) in the rat suprachiasmatic nucleus (SCN) have been identified ultrastructurally. They are easily distinguished from other types of synapses. The optic boutons are characterized by the presence of large mitochondria with a swollen electron lucent matrix and an interconnected tubular system formed by their inner membrane. Other, more variable features include: 1) a scattered pattern of synaptic vesicles which are found throughout the entire presynaptic element with relatively little accumulation near the active zones; 2) the occurrence of dense core vesicles and glycogen granules; 3) the active zones, the majority of which is Gray-type I, but a minority can obviously be classified as Gray's type II; 4) the innervation of smaller peripheral dendrites and dendritic spines. Boutons of this kind are exclusively filled with anterogradely transported horseradish peroxidase injected into both eyes. Very few neuronal elements containing the typical mitochondria have been observed in the SCN on the 6th day *post partum*, increasingly more on the 9th and 12th day, but considerably higher numbers after opening of the eyes on the 17th and the following days. The location of normal and degenerating optic boutons was examined light- and electron microscopically. In the rostral third of the SCN there are relatively few optic synapses which are found close to the optic chiasma. In the middle portion of the SCN optic synapses increase in number; they are found not only in the ventral part of the nucleus but also in lateral regions. This becomes particularly obvious in the caudal third of the SCN.

Key words: Synapses – Optic nerve – Suprachiasmatic nucleus – Ultrastructure – Rat

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Terminology Used in Text:

1) Active zone = synaptic contact = presynaptic clear vesicle accumulation near dense projections upon presynaptic membrane, plus synaptic cleft, plus postsynaptic membrane with possible adhering postsynaptic density and possible subjacent specializations (subjunctional bodies).

2) Gray-type I or "asymmetrical" synapses or active zones = synapses with a relatively wide synaptic cleft and significant postsynaptic density (see Gray, 1959; Akert et al., 1972).

3) Gray-type II or "symmetrical" synapses or active zones = synapses with a relatively small synaptic cleft and insignificant or lack of a postsynaptic density (references as above).

4) Optic bouton = optic presynaptic element = axonal varicosity containing clear vesicles, characteristic mitochondrial profile(s); dense projections can be present or lacking.

5) Optic synapse(s) = synapse(s) of the afferents of the optic nerve = presynaptic element or bouton, plus active zone, plus postsynaptic element comprising the region of the dendrite, dendritic spine or soma in close vicinity of the postsynaptic density and subjacent specializations (subjunctional bodies).

Abbreviations Used in Text: dcv = dense core vesicle(s); GTI = Gray-type-I; GTII = Gray-type-II; HRP = horseradish peroxidase; SCN = suprachiasmatic nucleus.

Evidence exists for a projection of optic nerve afferents into the hypothalamic suprachiasmatic nucleus (SCN) from a number of studies in various species (Hendrickson et al., 1972; Moore and Lenn, 1972; Moore, 1973; Conrad and Stumpf, 1974, for review of the earlier literature; Hartwig, 1974; Tigges and O'Steen, 1974; Mason, 1975; Thorpe, 1975; Felong, 1976; Mai, 1976; Mason and Lincoln, 1976; Nishino et al., 1976; Wenisch, 1976; Mai and Junger, 1977; Mason et al., 1977; Millhouse, 1977).

The author has previously reported a tentative ultrastructural identification of the optic synapses in the SCN of the rat (Güldner, 1976). The present study confirms these results and provides more detailed data for recognizing optic synapses. The morphometrical results which reveal considerable structural variability of the optic synapses are presented in a subsequent paper (Güldner, 1978).

Materials and Methods

The observations of the present study were made on the suprachiasmatic nuclei (SCN) of 114 Sprague-Dawley rats. The animals were anesthetized with ether and perfused through the heart for 30 sec with 0.15 M sodium cacodylate buffer and for 10 min with a mixture of 3% glutaraldehyde, 3% paraformaldehyde and 0.005 M $CaCl_2$ in 0.05 or 0.1 M cacodylate buffer at room temperature. After storage at 4° C for several hours or overnight the skull was opened, the tissue containing the SCN was cut out of the brain and washed in 0.1 M cacodylate buffer for 20 min with two changes. Samples were then fixed with 1% OsO_4 in the same buffer, dehydrated in ethanol and embedded in Epon 812. The thin sections were poststained with 1% aqueous uranyl acetate and 2.7% lead citrate (Reynolds, 1963). The structure of the optic synapses was studied in single and in serial thin sections through the SCN in frontal and sagittal planes.

Eight adult rats received injections of horseradish peroxidase (Sigma, type VI) into both eyes (1 µl of a saturated solution in distilled water). After 6, 12, 18 and 22 h two animals were sacrificed by cardiac perfusion. Preparation and histochemical reaction followed the procedures given by Colman et al. (1976). Tissue blocks of seven animals were treated with hyperosmolar washing buffers (Güldner, 1976) to check the possible existence of flattened synaptic vesicles (Bodian, 1970; Valdivia, 1970).

The development of the optic synapses was studied in 6, 9, 12, 17, 24 and 28 day-old rats using two animals of each age. The distribution of optic synapses in the SCN was examined light and electron microscopically.

Light microscopically, the localization of degenerating optic boutons was shown with the aid of a newly developed method producing a silver impregnation of lysosomes (Gallyas, personal communication). For this purpose two rats were perfused with unbuffered 5% formaldehyde on the 3rd, 4th, 5th, 6th and 7th days after bilateral enucleation.

Electron microscopically, the location of optic synapses was studied in the rostral, medial and caudal portions of the SCN in eight male and female animals (250–300 g body weight). The thin sections through the resulting 24 planes were scanned in 50 to 80 micrographs (primary magnification 7000 times) being made in regular intervals over the whole area of the SCN. Afterwards the entire thin section through the SCN was photographed at low magnification (90 × mesh image). The position of every micrograph was defined by a bright spot, which was burnt into the section with the electron beam.

Results

In the neuropil of the ventral and ventro-lateral regions of the suprachiasmatic nucleus (SCN) a significant proportion of presynaptic elements and axons are characterized by strikingly light mitochondria, which contrast markedly with the dark mitochondria in other boutons, dendrites and glial elements (Fig. 1). After bilateral enucleation there was almost complete loss of axons and boutons containing light mitochondria (type L-mitochondria; Güldner, 1976), which suggested that they were of retinal origin. However, it could not be excluded that the loss of optic input into the SCN had changed the functional state of other synapses which was reflected in a structural change of the light mitochondrial type to a dark form. Therefore, an attempt was made to identify the optic synapses by using the anterograde transport of horseradish peroxidase.

I. Identification of Optic Boutons with the Aid of Horseradish Peroxidase (HRP) (Figs. 2, 3)

18 h after injection of HRP into both eyes, specific reaction product was detected in a small number of myelinated and unmyelinated fibers of the optic chiasma, and in unmyelinated fibers and presynaptic elements within the SCN. Mitochondria, when present in the observed profiles, were exclusively of the light type (Figs. 2, 3). However, only a small percentage of the synaptic profiles containing light mitochondria were labeled with HRP.

HRP is present in vesicles and in smaller or larger vacuoles, which may in some cases be irregular or sausage-shaped, especially in axons. In poststained sections (Fig. 3) dense core vesicles (dcv) may sometimes appear as electron dense as those containing HRP reaction product, but the latter usually do not show an electron lucent halo between the dense content and the membrane. In unstained sections, however, all dcv are pale, whereas HRP remains electron dense and clearly recognizable.

The fact that HRP was never found in boutons containing dark mitochondria, together with the previous observation of the author that light mitochondria disappear after enucleation, strongly suggests that the presence of light mitochondria is a diagnostic feature of optic boutons. It could still be argued that only a portion of these boutons are of optic origin, and that the others also change after enucleation, but the weight of localization and labeling experiments favor the idea that at least most, if not all, of these boutons are indeed of optic origin.

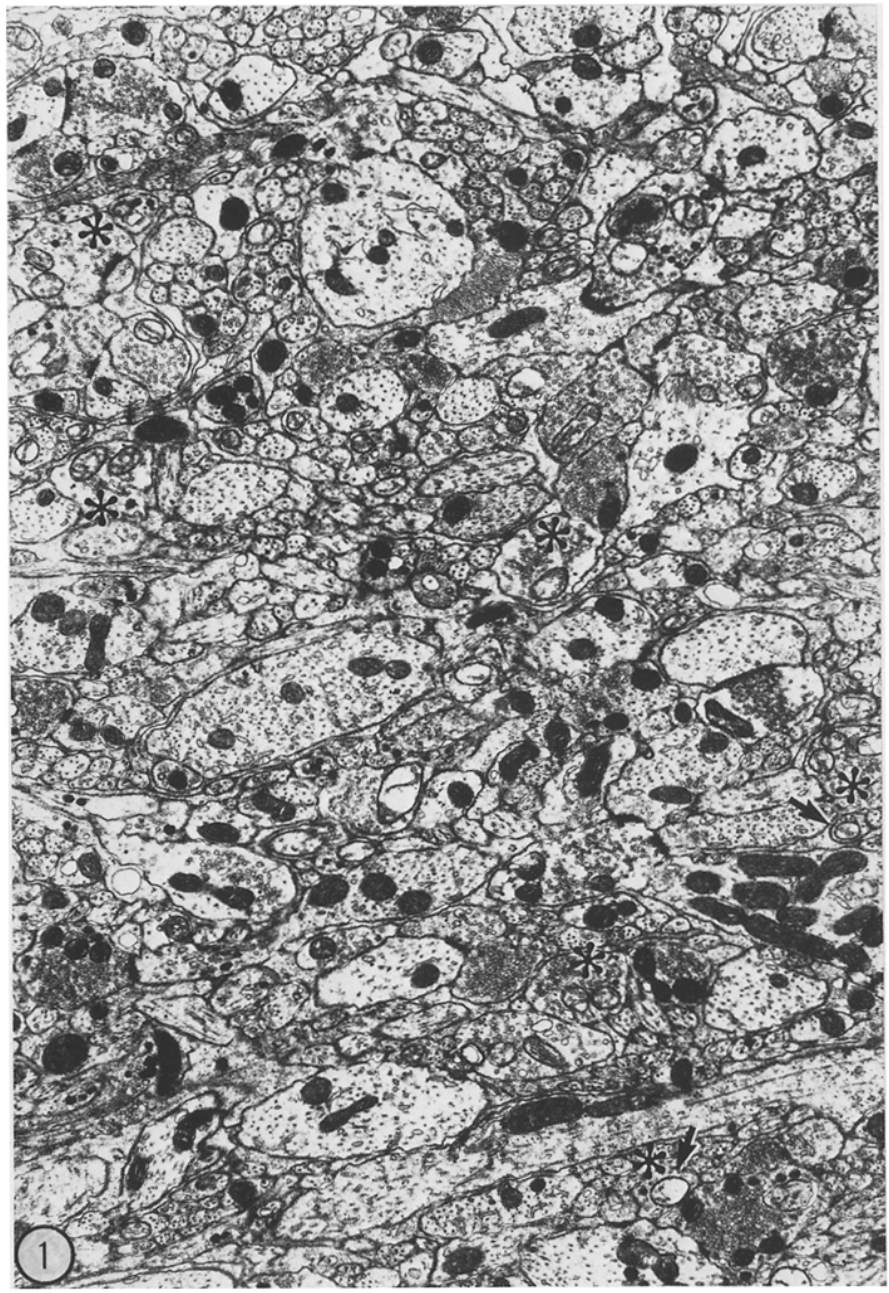


Fig. 1. Survey electron micrograph of the neuropil of the SCN close to the optic chiasma. In a number of axons and presynaptic elements, strikingly light mitochondria are visible (presynaptic elements: *asterisks*; axon: *asterisks* and *arrow*) in contrast to the dark mitochondria of other neuronal and glial elements. The axons and boutons containing the light mitochondria disappear after bilateral enucleation $\times 15{,}000$

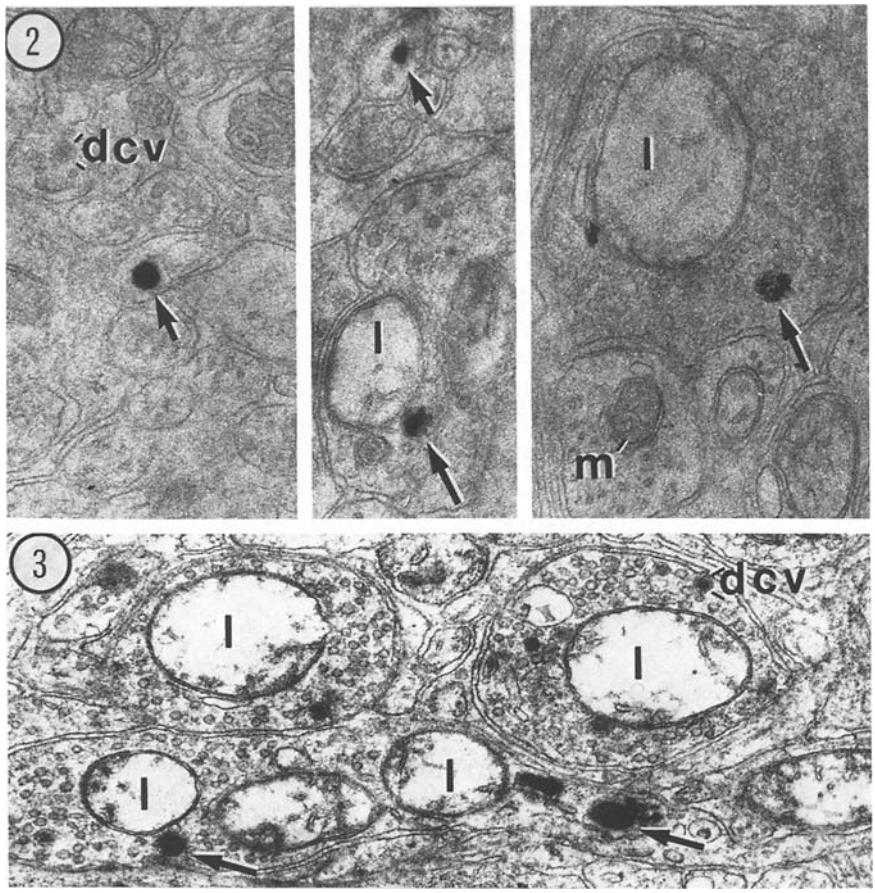


Fig. 2. Anterogradely transported horseradish peroxidase (HRP), unstained sections. After injection into the eyes, HRP reaction product is present in axons (*arrow*, left and middle) and in presynaptic elements (*long arrow*, middle and right), which contain the characteristic light mitochondria (*l*). Note a dark mitochondrion in the postsynaptic dendrite (*m*, right). Compare also the low electron density of dense core vesicles (*dcv*) with the high density of HRP reaction product. × 40,000

Fig. 3. Same as Fig. 2, but sections poststained with uranyl acetate and lead citrate. HRP reaction product is present in large vacuoles (*long arrows*). The core of some of the dense core vesicles (*dcv*) is nearly as electron dense as the specific reaction product. × 35,000

II. Optic Axons and Synapses

1. Optic Axons (Figs. 1, 2, 4, 6, 9, 11, 12). All optic nerve axons, as far as they could be recognized with certainty within the suprachiasmatic nucleus (SCN), are unmyelinated. They run in axon bundles of various size intermingled with axons of other origins. They contain several microtubules which are often seen to be connected by thin filamentous structures. Sometimes, dcv, clear vesicles, and elongated profiles of smooth endoplasmic reticulum, glycogen granules and, rarely,

lysosomal structures are found. Filaments are rare, in contrast to the myelinated axons in the optic nerve, where there are numerous filaments. Often, shreds of a flocculent material of medium electron density can be observed in the otherwise electron lucent axoplasm.

2. Optic Boutons (Figs. 1, 5, 9–12, 15; cf. Güldner, 1976, Fig. 1). The shape of the optic presynaptic elements in thin sections is quite variable: circular, oval and irregular profiles are found. Often optic boutons even show a concave adaptation to surrounding neuronal elements, whereas their outlines remain convex, when they are in contact with glial elements. Nevertheless, there are somewhat more circular or oval structures. Three-dimensional reconstructions have demonstrated the spherical or egg-like shape of most of the boutons, but in some cases they may be spindle shaped, or branched with all branches being completely filled with clear vesicles. The optic afferents form terminals and "en passant" synapses.

The *mitochondria* are usually sausage-shaped. They can be strongly bent like a horseshoe, and in some cases they are even branched. The light appearance of the mitochondria is due to a swollen, electron lucent matrix occupying a comparatively large volume (Figs. 4–6, 8, 11, 12, 16). The inner membrane mostly forms tubules or finger-like processes, which are connected to each other forming a three-dimensional tubular network (Figs. 4, 5, 8, 9). The number of tubules is usually low, but there are also examples with high tubular density. Fuzzy, sometimes granular material of medium electron density is often attached to be tubules. In rare cases, a small electron dense granule may be observed within the mitochondrial matrix. Such mitochondria are found in all unmyelinated optic axons within the SCN and in the optic chiasma (Fig. 6), but only in a few myelinated axons of the latter (Fig. 7A). However, most of the myelinated axons of the optic chiasma contain mitochondria showing tubular formations of the inner membrane, but a much less swollen matrix (Fig. 7B, C). Within the SCN, such mitochondria were not observed.

With very few exceptions the *clear vesicles* of the optic boutons are round or slightly oval (Figs. 4, 5, 10–12, 15). After treatment of the tissue with high osmolarity buffers (Bodian, 1970; Valdivia, 1970) the clear vesicles tend to remain spherical. Characteristically, the vesicles are loosely and nearly uniformly scattered throughout the whole bouton in most cases; but there may be a somewhat denser aggregation close to the active zone (Figs. 4, 5, 12 left, 15). Segregation of vesicles within the bouton is rare and a tight hexagonal crystalline packing has never been seen. Occasionally, the clear vesicles appear to be attached to filamentous material. They may also be connected with the presynaptic membrane by a filament or a membrane-like bridge. In other cases, two or more vesicles seem to be connected with each other by a membranous bridge (see Güldner, 1976; Fig. 1f).

Optic boutons usually contain spherical *dcv* (Figs. 3, 4, 5, 10, 12), but sometimes more elongated shapes can be observed. There is always a thin electron lucent halo between the membrane and the core. The density of the core may vary and in some cases the electron lucent halo is widened, due to an unusually small core with normal vesicle diameter or due to an unusually large vesicle diameter with normally sized core. *Coated vesicles* can be clearly recognized in about half the sections through an optic bouton (Fig. 5). Other vesicles may be only partially coated.

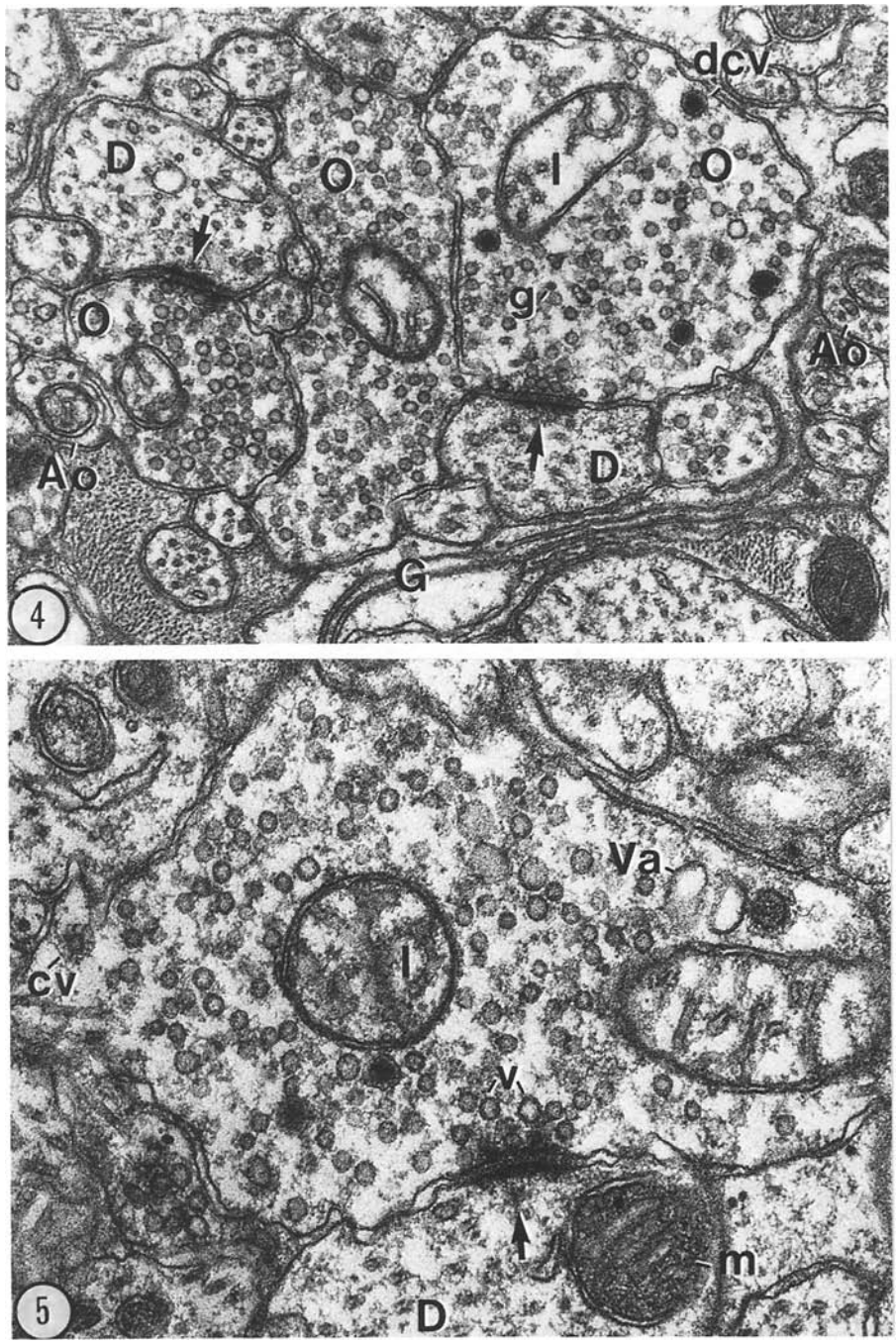
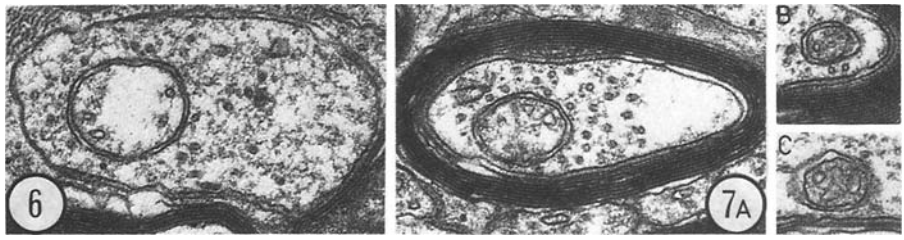


Fig. 4. Three optic boutons (*O*) and several axons, two of them of definite retinal origin (*Ao*). Two of the optic boutons form GT I or asymmetric active zones (*arrows*) with dendrites (*D*). The optic boutons contain the typical mitochondria (*I*) with an electron lucent, swollen matrix, and tubular formations of the inner membrane. Note scattered distribution of the synaptic clear vesicles, which only slightly accumulate near the active zone, and the presence of dense core vesicles (*dcv*) and glycogen (*g*) in the boutons; *G* astroglial processes. × 40,000

Fig. 5. Higher magnification of an optic synapse. The *arrow* points to the active zone with a subjunctional body and a distinct postsynaptic density on the dendritic side, and two dense projections surrounded by slightly accumulated clear vesicles (*v*) on the presynaptic side. Note branching of the tubules visible in one of the presynaptic mitochondria (*I*), the different appearance of the mitochondrion (*m*) in the postsynaptic dendrites (*D*) and the relatively round shape of the presynaptic dense projections; *cv* coated vesicle, *va* vacuole. × 60,000



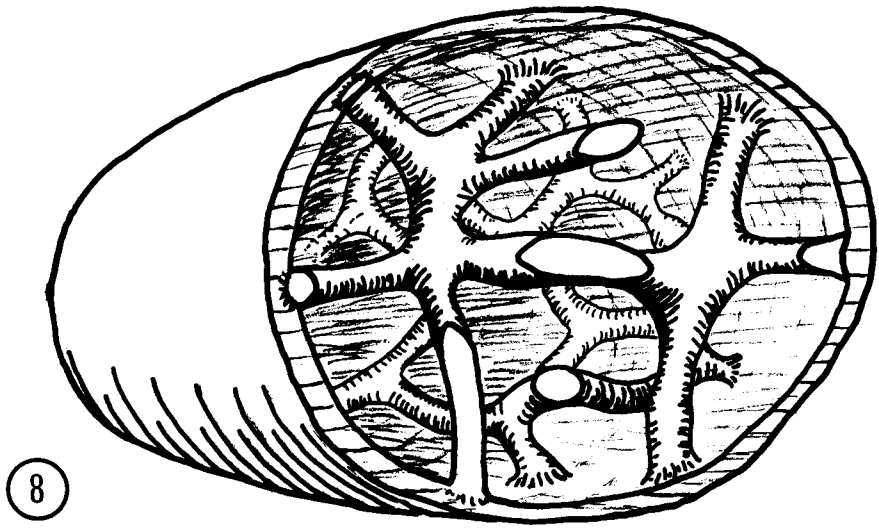
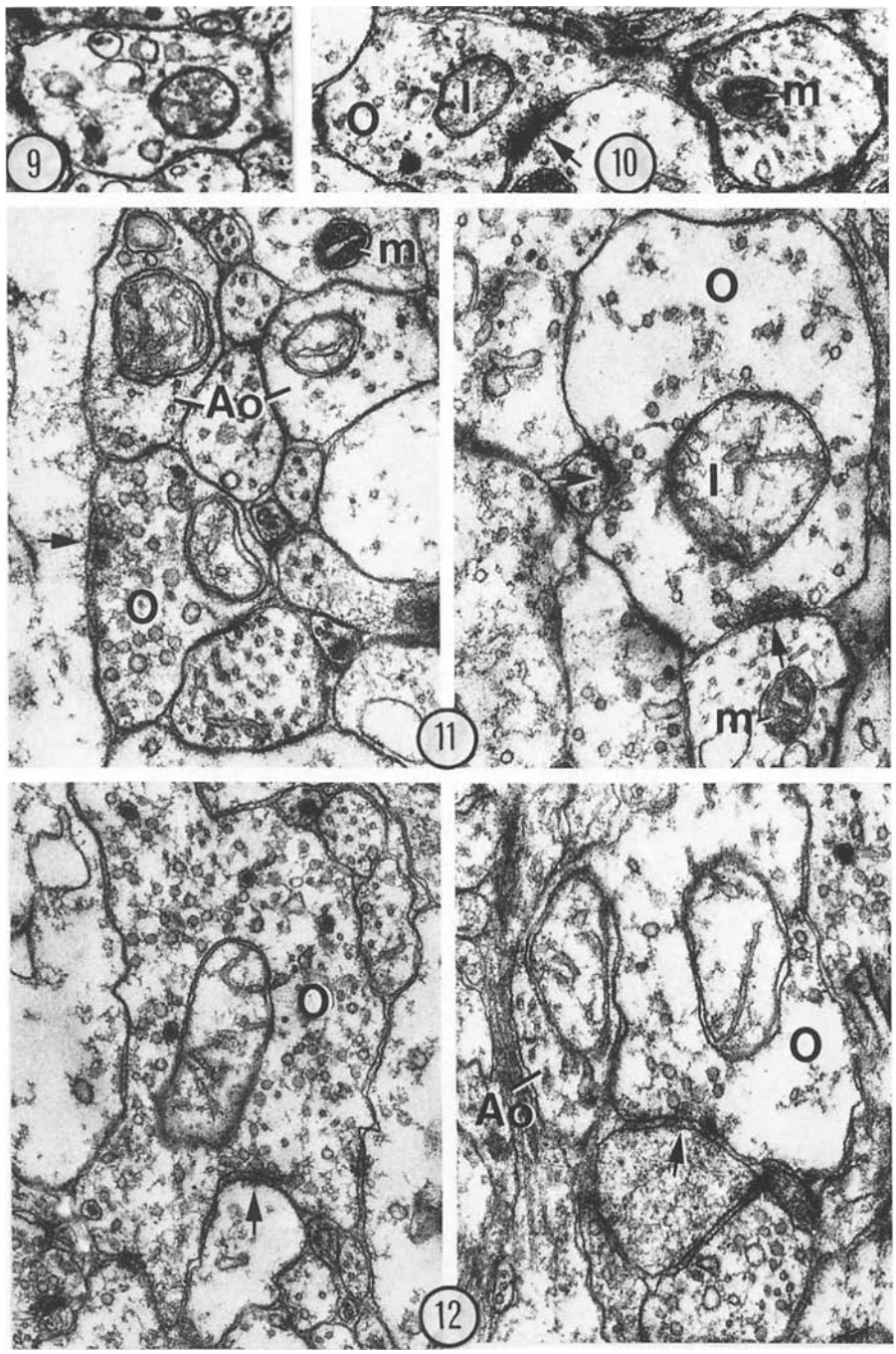


Fig. 6. Unmyelinated axon in the optic chiasma containing a light mitochondrion typical of optic afferents innervating the SCN. × 60,000

Fig. 7. A One of the few myelinated axons in the optic chiasma containing light mitochondria typical of optic afferents innervating the SCN. **B** and **C** Myelinated axons in the optic chiasma containing darker mitochondria with tubular inner membrane, but lacking the swelling of the matrix. × 60,000

Fig. 8. Schematic presentation of a mitochondrion as it regularly occurs within the retino-hypothothalamic afferents innervating the SCN, revealed by three-dimensional reconstructions. The interconnected tubules of the inner membrane form a three-dimensional network within the swollen matrix. The fuzzy coat of particles lying upon the tubules was omitted for sake of clarity

Vacuoles and *irregular profiles* of smooth surfaced endoplasmic reticulum are found in a considerable number of optic boutons (Fig. 5). Three-dimensional reconstructions revealed that the sacs of the endoplasmic reticulum are very often closely related to the mitochondria. Many of these sacs turned out be early stages in the development of *multivesicular bodies,* showing the typical plaques of coated membranes and sometimes a few vesicles within their lumen (see Quatacker, 1975, for references, cf. Birks et al., 1972; Weldon, 1975). More mature multivesicular bodies with electron dense content are also found, but less often.



Figs. 9–12. Optic axons and boutons during development. **Fig. 9.** Postnatal day 6. Presumptive optic axon containing a typical mitochondrion and several vacuoles. × 30,000

Fig. 10. Postnatal day 9. A presumptive optic bouton (*O*) forms a GT I active zone (*arrow*) with a dendritic element. Note here and in the following micrographs the structural difference between the light mitochondria in optic afferents (*I*) and dark mitochondria (*m*) in other neuronal elements. × 33,000

Fig. 11. Left and right: postnatal day 12. Optic axons (*Ao*) and two optic boutons (*O*) innervate dendrites with GT II or symmetric active zones (*arrows* in dendrites). At this stage, most of the optic boutons have a swollen appearance (see right micrograph). Left: × 42,000; right: × 37,000

Fig. 12. Left and right: Postnatal day 17. Optic axons (*Ao*) and two optic boutons (*O*) form GT II active zones (*arrows*) with dendritic elements (spines) lacking microtubules. Darker and more "mature"-appearing boutons as shown in the left micrograph become more numerous at this stage. Left: × 33,00; right: × 40,000

Glycogen granules are a further characteristic constituent of many boutons (Güldner, 1978, Fig. 4). The *microtubules* which have been observed in some optic boutons (Güldner, 1978; Fig. 11) lie close to the mitochondria. The electron lucent *cytoplasm* of the optic boutons contains a flocculent or filamentous material of medium electron density.

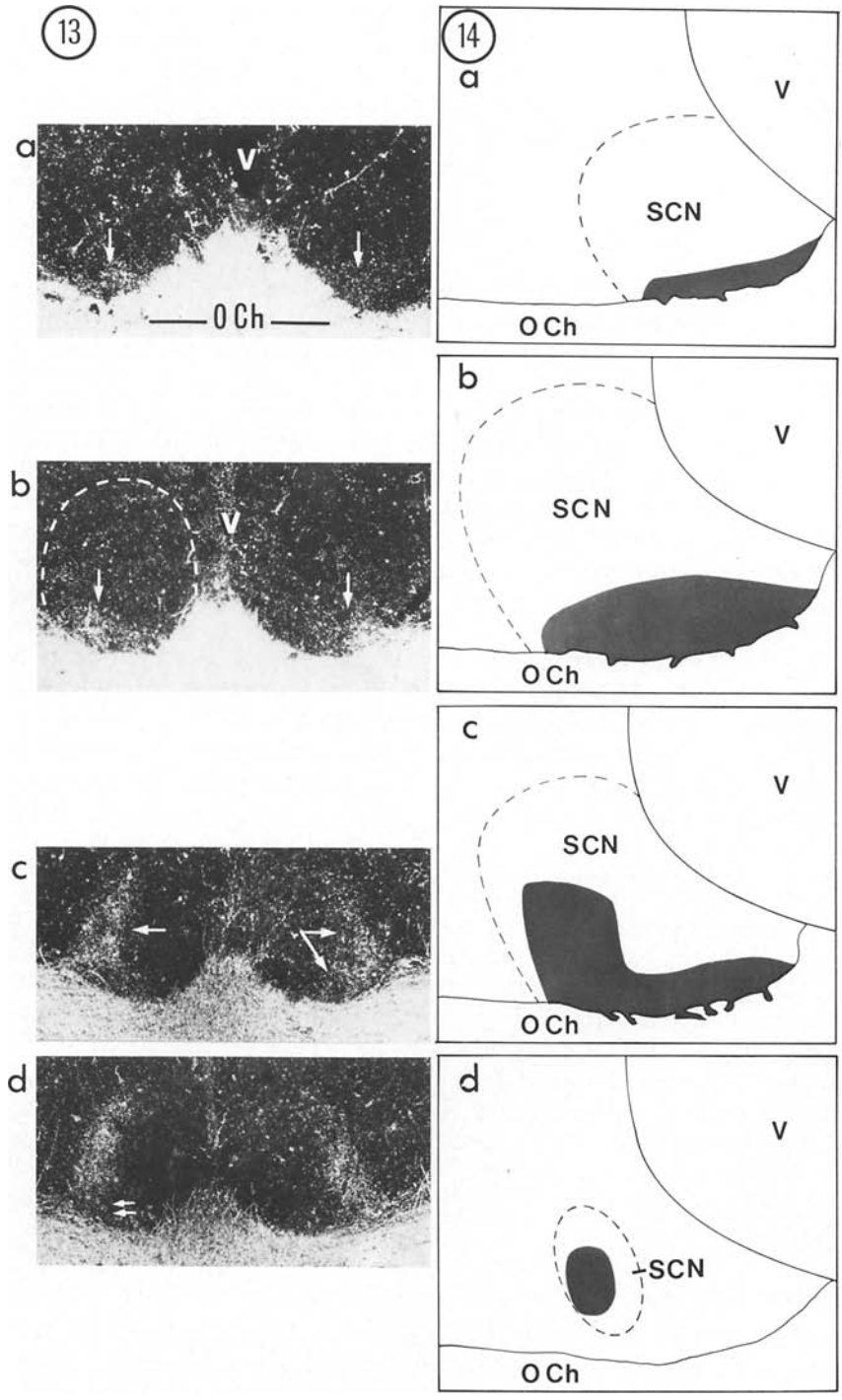
The *active zone* is marked by *dense projections* which mostly appear to be rounded up and surrounded by a thin halo and a membranous structure (Figs. 5, 15; Güldner, 1978, Figs. 7, 11); spiny protrusions can be occasionally seen radiating from them (cf. Jones, 1975). The active zones of optic synapses are macular with a disk-like or oval shape in most cases. Large active zones may sometimes be more irregular. One optic bouton usually forms several active zones, but nearly always only one with each dendritic element. Rarely, a bouton forms several active zones with one dendrite, which are then totally separated from each other. Sometimes, *attachment plaques* can be found lying separated from the active zone by a small gap of unspecialized membrane (Fig. 15), but fusions of both structures have also been observed.

3. Postsynaptic Elements. Most optic boutons contact the peripheral parts of the dendrites and dendritic spines. A few of them contact the larger main dendrites, and only very rarely have they been found to form synaptic contacts with neuronal somata. In a three-dimensional reconstruction, it appeared that an optic bouton directly contacted the neuronal soma without forming an active zone with the perikaryal membrane.

In most cases the dendrites form a distinct postsynaptic density opposite to the dense projections of the optic boutons (Figs. 4, 5). These synapses, which have a relatively wide synaptic cleft with an obvious electron dense band, belong therefore to Gray's type I. In a smaller portion of the optic synapses the postsynaptic density is inconspicuous or even completely lacking. The synaptic cleft is then generally smaller, so that these synapses have to be classified as GT II contacts (see Güldner and Wolff, 1978a; Güldner, 1978). Occasionally *subjunctional bodies* (Milhaud and Pappas, 1966) can be observed lying subjacent to the postsynaptic density (Fig. 5; Güldner, 1978, Figs. 7, 11; see also Güldner, 1976 and Jones, 1976 for

Figs. 13, 14. Location of the optic boutons in the SCN. Frontal planes. **Fig. 13.** SCN six days after enucleation of both eyes. Degenerating optic axons (*OCh*) and boutons (*arrows*) are shown with the aid of a silver impregnation for lysosomes (see Methods) in dark field. Both SCN are represented, the approximate outline of one SCN can be seen in b (dotted lines). Note ventral location of the optic synapses in the rostral parts of the SCN (**a, b**) and ventral plus lateral location in the more caudal parts (**c, d**). The left SCN is somewhat more caudally cut than the right SCN. The "optic area" begins to separate from the optic chiasma towards the caudal end of the SCN as shown in d, left (*double arrow*). *O Ch* optic chiasma; *V* optic recess of the third ventricle. ×1

Fig. 14. Schematic representation of the distribution of optic boutons (dark area) in the SCN from rostral (**a**) to caudal (**d**) as determined electron microscopically. Only the left SCN is shown (dotted lines). The sections shown in Fig. 13a and b correspond roughly to the level of a and b in this figure (frontal and middle third of the SCN). The sections of Figs. 13c and d lie in a level between those shown in c and d of this figure. After perfusion fixation for electron microscopy (see Methods) the third ventricle is considerably widened causing a distortion of the SCN in a lateral direction. The optic synapses, therefore, appear to be somewhat more ventrally situated compared to preparations with a narrow ventricle. The latter case occurs after perfusion fixation with unbuffered formalin (see Fig. 13)


13
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more detailed description). In rare cases, two opposite optic boutons can form so-called "double plug crest synapses" (cf. Milhaud and Pappas, 1966; Akert et al., 1972) with one dendritic spine or crest-like protrusion "sharing" one set of subjunctional bodies with each other (see Güldner, 1976, Fig. 1). Such "crest synapses" can also be formed by non-optic GT I synapses within the SCN, which is, however, equally rare.

III. Development (Figs. 9–12)

On the 6th day after birth a few profiles were found containing the mitochondria typical of optic afferents (Fig. 9). These profiles may contain vacuoles with small electron dense cores and/or some clear vesicles. No optic synapses could be found at this stage.

On the 9th day some optic synapses were detected after systematic examination of the thin sections (Fig. 10). Only a slight increase in number could be found on the 12th day. The structure of the optic boutons was already similar to that in adult animals, although the early boutons seem to be more swollen, and the active zones belong much more often to Gray-type II (Fig. 11).

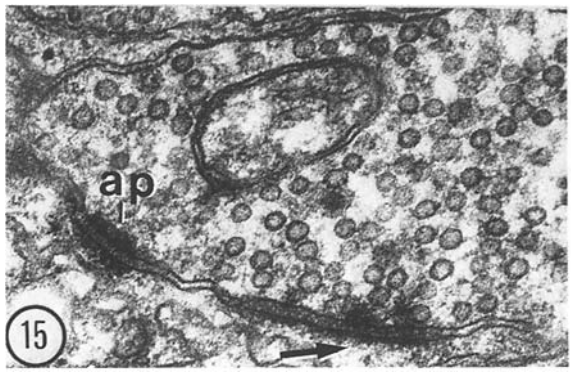
The number of optic synapses found per area of SCN had risen considerably by the 17th day. Swollen optic boutons with relatively few clear vesicles were found together with boutons containing higher numbers of vesicles and dcv, like those in adults. (Fig. 12 left). The proportion of the more mature appearing optic boutons increases from day 17 at least up to day 27 after birth. At this time an optic bouton was found which formed a GT I active zone with one dendrite and a GT II active zone with another dendritic element. Subjunctional bodies were not observed in optic synapses up to that time, although they were definitely present in synapses of non-retinal origin as early as the 21st day.

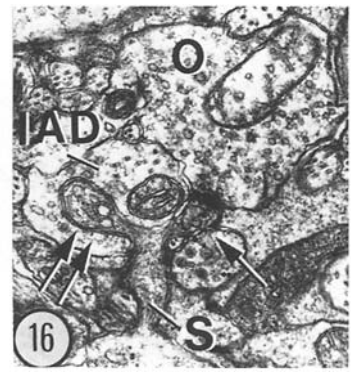
IV. Location of Optic Synapses in the SCN (Figs. 13, 14)

The most ventrally situated neuronal somata, or spurs of the neuropil, form indentations in the optic chiasma. Optic synapses were observed in frontal sections of the SCN cut from the rostral to the caudal poles as soon as these characteristic indentations appeared. They occur already in the rostral third of the SCN. Here, only a small number of optic synapses was found in close proximity to the optic chiasma (Figs. 13a, 14a). More caudally, their number increases steadily (Figs. 13b, 14b) and they begin to appear in the lateral parts of the SCN. This is particularly striking in the caudal third of the nucleus (Figs. 13c, d, 14c). At the caudal limit of the SCN, the optic synapses still occur, but in a circumscribed area somewhat dorsal to the optic chiasma (Figs. 13d, 14d). At this point no more indentations can be seen in the chiasma.

V. Relationships Between Optic Boutons and Other Types of Synapses (Figs. 16, 17)

The dendrites in contact with optic boutons are the target of at least four types of presynaptic elements: 1) boutons forming GT I active zones, 2) boutons forming GT II active zones (AD synapses) 3) boutons invaginated by a spine-like dendritic protrusion (IAD synapses, GT II), and 4) dendrites which form reciprocal dendro-





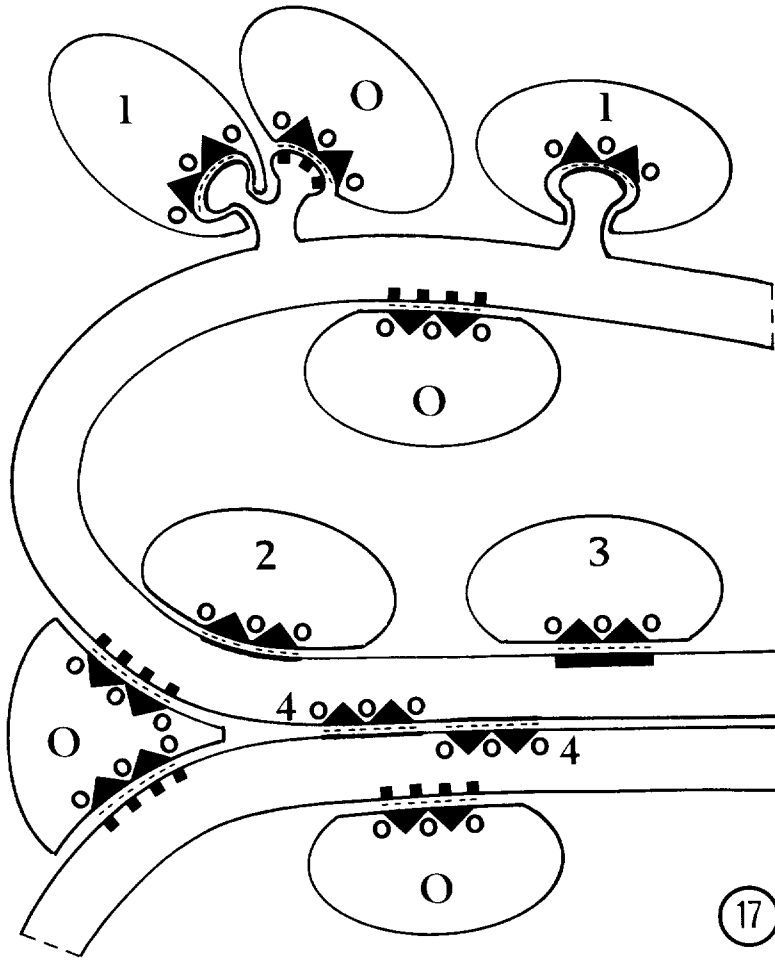


Fig. 15. An optic synapse showing an attachment plaque (*ap*) close to the active zone (*arrow*). × 60,000

Fig. 16. A dendritic spine (*S*) bifurcates into two branches. One of them (*arrow*) is innervated by an optic bouton (*O*), the other one (*double arrow*) is postsynaptic in an "invaginated axo-dendritic" synapse (*IAD*), which is presumably inhibitory. × 25,000

Fig. 17. Schematic drawing showing that at least four other types of synapses are found on the neurons which are innervated by optic afferents. *O* optic synapses forming Gray-type I and type II active zones, symbolized by the interrupted postsynaptic density; *1* invaginated axo-dendritic (and axosomatic) Gray-type II synapses (*IAD*); *2* axo-dendritic (and axo-somatic Gray-type II synapses (*AD*), possibly belonging to more than one type of synapse; *3* non-optic axo-dendritic Gray-type I synapses; *4* dendro-dendritic (dendro-somatic and somato-dendritic) Gray-type II synapses which may be reciprocal

dendritic synapses (DDS, GT II). These types of synapses are fully described in other papers (Güldner, 1974, 1976; Güldner and Wolff, 1978b). Together with the optic synapses, all the presynaptic elements mentioned above, except the IAD synapses, form so-called "complex synaptic arrangements" in that they are clustered together without being separated by glial processes (Güldner and Wolff, 1977). A single dendritic spine can be innervated by an optic bouton and an IAD synapse, the latter contacting a branch of the spine (Fig. 16).

Discussion

I. Identification and Location of Optic Boutons

There is now convincing evidence that the type of synapse described in the present paper is formed by optic nerve fibers projecting to the SCN due to the following reasons:

1) These synapses disappear after bilateral enucleation (Güldner, 1976).

2) Only the boutons of this type of synapse are filled with vesicles and vacuoles containing anterogradely transported horseradish peroxidase after injection of this enzyme into both eyes.

3) The ventral and ventrolateral location of these synapses in the SCN corresponds to those areas showing i) light- and electron microscopically detectable degeneration between the 2nd and 8th (up to 11th) day after bilateral enucleation (Güldner, unpublished results), ii) accumulation of ^{3}H-labeled amino acid injected into the eye (Hendrickson, 1972; Moore and Lenn, 1972; Moore, 1973; Conrad and Stumpf, 1976; Mail, 1976), iii) fibers filled with cobalt salt which diffused from the cut end of the optic nerve (Mason, 1975; Mason and Lincoln, 1976; Wenisch, 1976; Mason et al., 1977), and iiii) fibers filled with anterogradely transported horseradish peroxidase (Nishino et al., 1976).

4) This type of synapse is very similar to those synapses described as or assumed to be of retinal origin in other regions of the vertebrate nervous system (see Szentágothai et al., 1966; McMahan, 1967; Le Vay, 1971; Cullen and Kaiserman-Abramof, 1976: lateral geniculate nucleus of rat, mouse and monkey; Lund, 1969; Lund, 1972; Lund and Lund, 1972; Sterling, 1973; Valverde, 1973; Vrensen and De Groot, 1977: superior colliculus of mouse, rat, rabbit, cat and monkey; Yamada, 1974: medial terminal nucleus of the accessory optic system of the mouse; Répérant and Angaut, 1977; tectum of pigeon). Other authors, however, describe mitochondria in optic boutons of the fish tectum (Laufer and Vanegas, 1974) and pigeon tectum (Hayes and Webster, 1975) with somewhat different features. In these mitochondria, the space between the outer and inner membrane is widened and the matrix appears less swollen.

With the exception of the autoradiographic studies of Mai (1976) and Mai and Junger (1977), the light microscopic methods mentioned above were not able to detect optic afferents in the frontal third of the SCN, since they are relatively few in number, and are masked by the highly-labeled dorsal fibers of the optic chiasma, with which they are in close contact. Anterograde diffusion of cobalt salt only demonstrates the innervation of the caudal fifth of the SCN (Mason and Lincoln,

1976; Mason et al., 1977), although this method is very useful for delineating further essential characteristics of the afferent retinal fibers. Only the ventral and lateral regions of the SCN are innervated by optic afferents. This "pars optica" is quite distinct from the dorsal and dorso-medial regions of the SCN which are occupied by specialized neurons containing vasopressin and neurophysin (Vandesande et al., 1975; Weindl, personal communication; Krisch, 1976).

II. Mitochondria in Optic Boutons

The light mitochondria were suggested as characteristic of optic boutons 12 years ago by Szentágothai et al. (1966). The tubular nature of their inner membrane was first described by LeVay (1971) and later by Yamada (1974). The present study gives a still more detailed description of their structure showing that the tubules are at least partly branched and interconnected, forming a three-dimensional network. In the optic chiasma, these mitochondria occur in all unmyelinated retinal fibers, but only in a very few myelinated axons. The bulk of the myelinated axons contain mitochondria without a swollen matrix, although the tubular formations of the inner membrane are still obvious. The functional difference between the two modifications is unknown. Perhaps, mitochondria without a swollen matrix belong to the large retinal ganglion cells (Y cells), and those with a swollen matrix to the smaller ganglion cells (X and W cells, see Cleland and Levick, 1974a, b; Fukuda and Stone, 1974; Kelly and Gilbert, 1975). According to the Golgi studies of Brauer and Winkelmann (1974) and Winkelmann et al. (1976), there are two types of optic boutons in the lateral geniculate body, differing in diameter (2a terminals = 3 μm; 2b terminals = 1.3 μm) and location. The optic boutons found within the SCN correspond to 2b terminals in size (see Güldner, 1978). It would be worthwhile investigating whether the smaller 2b terminals in the lateral geniculate body contain the same mitochondria as those in the SCN, and whether the 2a terminals show mitochondria similar to those found in most of the myelinated axons of the optic chiasma.

It could be argued that the swollen aspect and the tubular inner membrane of mitochondria in optic boutons were artificially produced by poor fixation and prolonged hypoxia. In this study, however, only those preparations were used where perfusion was complete within the first minute after opening of the thorax, and where all blood vessels were flushed. Sometimes fixation may produce swelling in portions of mitochondria in all neuronal and glial elements. However, such mitochondria cannot be mistaken for mitochondria of optic boutons, which have a uniformly swollen matrix and occur regularly in a special type of bouton in the SCN. Nevertheless, the striking aspect of mitochondria in optic boutons might be a specific fixation artefact, and it remains to be seen whether freeze-dried preparations (Van Harreveld et al., 1974) show a different appearance. A swollen matrix and tubular inner membrane might characterize mitochondria in special functional states, quite different from those with cristae and a dense matrix. Indeed, configurational changes of the inner membrane from cristae to tubules have been reported in mitochondria in different energy states (Green et al., 1968). If this is the explanation of the peculiar morphology of these mitochondria, the functional state

of the mitochondria must be fairly constant, as significant changes in their morphology have not been observed to date. This is in contrast to the very variable structure of the mitochondria in IAD synapses (Güldner, 1976).

From the foregoing discussion, it can be stated that optic boutons can be recognized in most cases by virtue of their mitochondrial content. The only confusion that might ensue is that sometimes mitochondria in other neuronal elements of non-retinal origin may have an electron lucent matrix, however, they usually contain cristae which are often arranged in parallel. Under certain conditions, as yet undefined, the mitochondria of the IAD synapses in the SCN can become quite similar to those in optic boutons. However, this type has very different structural features in other respects: (i) the pre-synaptic elements are invaginated by club-like protrusions of the postsynaptic dendritic element; (ii) their few scattered clear vesicles are usually much more variable in size; and (iii) they always form GT II active zones with the dendritic invaginations,. On the other hand, optic boutons form in more than 70% of the cases GT I active zones (Güldner, 1978; Güldner and Wolff, 1978a, b), and their synaptic contacts were never found to be invaginated by the postsynaptic element. Thus, confusion is avoidable in most cases. Errors are probably only made in boutons not showing an active zone when 1) the clear vesicles of IAD boutons are exceptionally uniform and numerous, or 2) optic boutons show a more swollen appearance and possess vesicles with more variable size (Güldner, 1978).

Optic boutons lacking mitochondrial profiles in the plane of section may still be tentatively identified, if they show the other characteristics described above, i.e., loosely scattered clear vesicles occurring in all portions of the bouton, one or a few dcv and glycogen granules, and a concave adaptation to the surrounding neuronal elements. Nevertheless, such boutons have not been used for morphometric examination due to the higher diagnostic uncertainty.

III. Development

A recent autoradiographic study by Felong (1976) has shown that the ingrowth of optic afferents into the SCN begins at about postnatal day 4. Mason et al. (1977) observed the first few cobalt-filled fibers even earlier on day 3. In the present studies up to postnatal day 6, the number of optic synapses in the SCN seems to be so small that their detection at the electron microscopic level is largely a matter of chance or excessive search. At least from the 9th postnatal day onwards, optic synapses can be quite easily traced. This agrees with the observations of Lenn et al. (1977), who studied the development of the synapses in the SCN. They found that the two types of mitochondria (light and dark) were not clearly defined after one week; however, after two weeks, the authors observed several examples of presynaptic elements containing typical electron lucent mitochondria with tubules. While it is possible that the mitochondria of optic boutons pass through a stage with different structural features, the close coincidence between the appearance of the afferent fibres and the initial appearance of the typical light mitochondria suggests that such a stage would be very brief, if it exists at all. However, as during development similar mitochondria may occur in some neuronal somata, dendrites and IAD-synapses, diagnostic errors might be more often made in young than in adult rats.

In comparison with other types of synapses in the SCN, i.e., the axo-dendritic (AD) and invaginated axo-dendritic (IAD) GT II synapses, the bulk of optic synapses develops relatively late. This correlates with the finding that optic boutons are mainly found on small peripheral dendrites and spines. It would be tempting, but premature, to speculate that all optic boutons abutting on the thick proximal parts of dendrites or even on somata represent the oldest synapses; synaptic remodelling and formation of new synaptic sites might occur in both distal and proximal regions of the neurons even in later stages.

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