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Synaptology of the Rat Suprachiasmatic Nucleus *

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Summary. Within the suprachiasmatic nucleus (SCN) of the rat the fine structure of the synapses and some features of their topological arrangement were studied. Five types of synapses could be distinguished with certainty:

A. Two types of Gray-type-I (GTI) or asymmetrical synapses (~33%). The presynaptic elements contain strikingly different types of mitochondria. Size of clear vesicles: ~450 Å. Synapses with subjunctional bodies often occur, among these also "crest synapses". Localization: dendritic shafts and spines, rarely somata.

B. Three types of Gray-type-2 (GTII) or symmetrical synapses (~66%): 1) Axo-dendritic and -somatic (=AD) synapses. Size of clear vesicles: ~500 Å. 2) Invaginated axo-dendritic and -somatic (=IAD) synapses with club-like postsynaptic protrusions within the presynaptic elements (PreEl). Size of clear vesicles is very variable: ~400–1,000 Å. 3) Dendro-dendritic, -somatic and somato-dendritic (=DD) synapses occurring at least partly in reciprocal arrangements. They represent an intrinsic system.

Shape of clear vesicles: often oval; sucrose treatment partly produces flattening.

Dense core-vesicles (dcv) are found in all GTII- and most of the GTI-synapses after three-dimensional reconstruction. All types of synapses (mostly

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* Dedicated to Prof. Dr. Drs. h.c. Wolfgang Bargmann on the occasion of his 70th birthday.

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List of Abbreviations in Text: AD synapse=axo-dendritic (+somatic) GTII synapse; dcv=dense core vesicle; DD synapse=dendro-dendritic/dendro-somatic/somato-dendritic synapse; GTI synapse=Gray-Type-I synapse; GTII synapse=Gray-Type-II synapse; IAD synapse=invaginated axo-dendritic (+somatic) GTII synapse; PreEl(s)=presynaptic element(s); PostEl(s)=postsynaptic element(s); sb=subjunctional body(ies); SCN=suprachiasmatic nucleus.

GTII-synapses) can be enclosed by multilamellar astroglial formations. The synapses often occur in complex synaptic arrangements.

Dendrites and somata of females show significantly more multivesiculated bodies than those of males. Further pecularities of presynaptic (PreEls) and postsynaptic elements (PostEls) within the SCN are described and discussed.

Key words: Hypothalamus – Suprachiasmatic nucleus – Synapses – Mitochondria – Endoplasmic reticulum.

Introduction

Various experimental approaches suggest that the suprachiasmatic nucleus (SCN) plays an important role within the neuroendocrinological feedback system. For instance, a total destruction of this nucleus leads to a cessation of spontaneous ovulation (Raisman, personal communication, see also Critchlow, 1963; Wurtman, 1967). The SCN is the target of optic fiber terminals, as established in various species by the studies of Moore and coworkers (1972/1973), Hendrickson *et al.* (1972) and Hartwig (1974) (see also Conrad and Stumpf, 1974; Tigges and O'Steen, 1974; Mason 1975; Thorpe, 1975). This implies that there are light-induced regulatory influences of SCN neurons on the production and/or release of gonadotrophic hormones, since continuous illumination induces persistent estrous (for lit., see Hendrickson *et al.*, 1972; Discussion). However, other forms of circadian cyclic behaviour also seem to be under the control of the SCN. This nucleus is essential in the rat for 1) the cyclic behaviour of drinking and locomotor activity (Stephan and Zucker, 1972), 2) the maintenance of the adrenal corticosterone rhythm (Moore and Eichler, 1972) and 3) the maintenance of the circadian rhythm of pineal serotonin N-acetyltransferase activity (Moore and Klein, 1974). Recent results on the efferent connections of the SCN (Moore and Klein, 1974; Makara and Hodács 1975; Swanson and Cowan, 1975; Raisman, personal communication; cf. Szentágothai *et al.*, 1968; Záborszky *et al.*, 1973) give some preliminary indication of the sites of these regulatory influences; the studies of these authors show that the axons of SCN-neurons pass caudally and dorsally through the periventricular area coming into close contact with neurons of the periventricular nucleus and dendrites of neurons of the ventromedial, dorsomedial and arcuate nuclei. Other SCN-axons run to the lateral hypothalamic area and into the internal zone of the median eminence.

Since the detection and naming of the SCN by Spiegel and Zweig (1917) the morphology of the SCN has been the subject of several light microscopical studies (see Krieg, 1932; Suburo and Pellegrino de Iraldi, 1968; Szentágothai *et al.*, 1968). Preliminary results concerning the normal fine structural components have only been obtained in the last seven years. These studies were mainly confined to the ultrastructure of neuronal somata (Suburo and Pellegrino de Iraldi, 1968, rat; Clattenburg *et al.*, 1972, rabbit), neurono-glial relationships (Güldner and Wolff, 1973, rat) and the dendro-dendritic synapses which have so far only been recognized in the hypothalamus in this nucleus (Güldner and Wolff, 1974).

As knowledge of the general synaptology of the SCN is still incomplete, the aim of the present work is to describe and distinguish the various types of synapses within this nucleus.

Materials and Methods

Twenty male and 25 female Sprague-Dawley rats were perfused with a mixture of 3% glutaraldehyde and 3% paraformaldehyde in 0.05 M sodium cacodylate buffer. The tissue blocks were washed in 0.1 M buffer and postfixed in 1% OsO_4 solution (conventional treatment). To cause flattening of synaptic vesicles, tissue blocks of seven animals were rinsed for 1 hr in the same buffer containing 12% sucrose (~600 mosm) or 24% sucrose (~1,000 mosm) and postfixed in 1% OsO_4 solution with similar osmolarity. (sucrose treatment, see Bodian, 1970; Valdivia, 1970; Lieberman, 1973).

Four pairs of animals were unilaterally enucleated (left eye) and perfused on day 3, 4, 5 and 6 after operation. Two animals were bilaterally enucleated and perfused on day 12, 20 and 50 after operation (sucrose treatment). The tissue was dehydrated in ethanol and embedded in Epon. Frontal and sagittal sections were cut through the suprachiasmatic nucleus (SCN), post-stained with an aqueous solution of uranyl acetate (1%) and lead citrate, and examined in JEOL electron microscopes T8 and 100B.

The relative proportion of the different types of synapses, as well as the frequency of subjunctional dense bodies (sb) and of multilamellar astroglial coverings of synapses were examined by "scanning" through 21 thin sections each containing a section of the whole SCN. Only those synapses with perpendicularly cut active zones were taken into account. The area examined could be controlled in micrographs with a primary magnification of 90:1 ("mesh image"), where the trace of the electron beam is clearly set off against the darker background. As only the medial and ventral boundaries of the SCN can be well defined in the electron microscope, no results from the lateral, dorsal, frontal and caudal periphery of the SCN are included in this study. The size of the presynaptic elements (PreEls), clear and dense core vesicles (dcv), mitochondria and active zones and the width of the synaptic cleft in 47 GTI- and 80 GTII-synapses (48 AD-, 20 IAD- and 12 dendro-dendritic synapses) were measured in micrographs with magnifications of 41,000 and 63,000. Additionally, the number of dcv, interruptions of the active zones (in 132 GTI- and 130 GTII- =90 AD+40 IAD synapses) and the distance from the approximate center of the sb to the postsynaptic membrane (in 25 GTI-synapses) were determined. In 12 GTI- and 12 AD-, 6 IAD- and 5 DD-synapses in two areas of the SCN near the optic chiasma—totally reconstructed with the aid of 50 serial thin sections (thickness about 800–1,500 Å estimated from interference colours)—the size of the PreEls, the number of clear vesicles and dcv and the size of the active zone were measured. In two postsynaptic elements (PostEls) the mitochondrial aggregations were three-dimensionally reconstructed.

Results

I. General Remarks on the Suprachiasmatic Nucleus (SCN)

Localization: The two ovoid SCN lie directly upon the optic chiasma, making a slight indentation. The nuclei are separated from each other only by the median tubero-infundibular tract and the floor of the third ventricle (optic recess). Dorsally the SCN is bounded by the anterior portion of the periventricular nucleus, laterally and dorsally by the anterior hypothalamic nucleus. The rostral and caudal boundaries are the preoptic area and the retrochiasmatic area (see Krieg, 1932).

Size: The transverse axis of the SCN measures about 300 μm, the vertical 350 μm and the longitudinal axis about 600 μm. Thus, the total volume of the SCN is within the range of 50,000,000 μm^3, *i.e.*, 0.05 mm^3.

Neuronal Population: On each side there are about 10,000 closely packed neurons (Raisman, personal communication), which are among the smallest nerve cells in the CNS (between 6 and 15 μm, compare with Clattenburg *et al.*, 1972). According to preliminary Golgi-Cox studies, two to five dendrites arise from the cell bodies, which are only slightly branched, often showing a moderate number of club-like protrusions. The axons have been reported to run in a postero-dorsal direction (Krieg, 1939).

II. Types of Synapses

The two main forms of synapses originally described by Gray (1959) as type I and type II synapses can also be found in the SCN. According to the classical study of Gray (see also Colonnier, 1968, and Akert *et al.*, 1972), the type I (GTI or asymmetrical) synapses are characterized by a significant density (> 90 Å wide) on the postsynaptic membrane and a synaptic cleft (160–200 Å wide), whereas in the type II (GTII or symmetrical) synapses the postsynaptic density is very small (< 90 Å) or even absent (see also Adinolfi, 1971), and the synaptic cleft is not wider than 160 Å.

1. Gray-Type I-Synapses (Figs. 1, 2, 6, 7c, 8, 10, 14, 15)

The *presynaptic elements* (PreEls) are axonal enlargements. Serial sections revealed that the PreEls can be spherical, fusiform or discoid with little tendency to conform to the surface of surrounding cellular processes, whilst other PreEls are more irregularly shaped, or even branched, with a strong, glia-like tendency to be moulded around the surrounding neuropile structures. Cross sections through PreEls usually have a diameter of 0.8–1.3 μm, but also sizes of up to 2–3 μm were sometimes found. PreEls can be of "boutons terminaux" or of "en passant" type.

The PreEls contain spherical or slightly oval *clear vesicles* with a diameter ranging between 320–600 Å (mean diameter about 450 Å) (Fig. 6). The distribution of the mean diameters of the vesicle populations in 47 PreEls of three animals is quite uniform, so that PreEls with significantly different vesicle sizes could not be distinguished among the GTI-synapses (Fig. 6a). In some PreEls, the size of the clear vesicles was found to be strikingly variable. After sucrose-treatment, the clear vesicles tend to remain spherical, although some may be oval or flattened (Fig. 2e). Being more or less closely aggregated, the clear vesicles often fill large portions, if not the whole volume, of the PreEls (Figs. 1c, 2a, b). However, there are also PreEls with few scattered vesicles or small vesicle aggregations near the active zone (Fig. 2a). Rarely, the synaptic vesicles were found in an extremely close aggregation forming a hexagonal array (see Fig. 4e).

In some preparations, neighbouring vesicles were often seen to be connected by a bridge, consisting of a trilaminar membrane (about 70 Å thick, Fig. 1f). The two outer dense layers of this membrane are continuous with the outer

layer of the vesicular membrane, the inner electron-lucent layer of the membranous bridge is continuous with the medial layer of the vesicle membrane. Two or more clear vesicles appear to be connected in this way. Besides clear vesicles a few coated vesicles also occur in the PreEls.

In up to 45% of the sections through PreEls one to several *dense core vesicles* (dcv) with a diameter of about 700–1,000 Å occurred (see Table 1, Figs. 1a–c, 2a, c). Occasionally, the size of the core is small and/or has low density. Clear vesicles (or vacuoles) of corresponding size can also occasionally be found. The shape of the dcv is usually spherical, but sometimes it can be oval or even sausage-shaped. In a few PreEls small dcv with a diameter of only about 500 Å also occur, often situated near the active zone. Serial sections through 15 synapses revealed that there are indeed PreEls which do not contain any dcv (four cases); eight PreEls had 1–3 dcv, whereas the remainder were filled with 8–16 dcv. In all PreEls the dcv were found to be associated with clear vesicles. Occasionally, axonal varicosities were found to be filled nearly exclusively with numerous dcv, but these varicosities never showed active zones.

In a single PreEl 1–4 *mitochondria* usually occur, which can be branched. However, sometimes they can be entirely absent, as ascertained in reconstructed synapses.

There are essentially two different types of mitochondria in the PreEls of GTI synapses: the first one (type L, Fig. 1b, e, 2c, 14/2) is comparatively large (thickness 0.25–0.5 μm). The matrix is more or less electron lucent. The relatively few profiles of the inner membrane are mostly tubular. The tubules are irregularly distributed within the matrix. They can be branched and connected with each other forming, at least partly, a reticular system. These mitochondria can also be found in non-synaptic parts of axons. Axonal processes or synapses containing type L mitochondria were mainly found in the ventral areas of the SCN near the fibers of the optic chiasma. Structure and localization of type L mitochondria do not change after various fixation procedures (such as after perfusion fixation with 4% paraformaldehyde in 0,1 % sodium cacodylate buffer +2% dextrane) or treatment with solutions of various osmolarity before osmification. The second type (type D, Figs. 1a, 2d, 6b, 14/3) is smaller (thickness about 0.15–0.25 μm) containing longitudinally oriented cristae and a matrix of medium electron density. PreEls with type D mitochondria are distributed in all portions of the SCN.

Occasionally, a possible third type of mitochondria can be observed in PreEls. These mitochondria are relatively large (0.3–0.4 μm thickness) and contain an electron-lucent matrix. However, the inner membrane forms cristae which are oriented in parallel.

In addition to the organelles described above, a few *glycogen granules, vacuoles* and irregular profiles of the *smooth surfaced endoplasmic reticulum* and/or a *multivesiculated body* also occur in the PreEls.

The "active zone" of the PreEl is indicated by triangular or polygonal *dense projections* (height 650–850 Å) lying on the presynaptic membrane.

The *synaptic cleft* is about 180–240 Å wide and contains an electron dense material.

The *postsynaptic elements* (PostEls) of GTI synapses are mainly dendritic shafts of all size classes and spines (Fig. 2c), but only very occasionally somata (Fig. 2d). In rare cases, the PostEl invaginates the PreEl with a spine-like protrusion of variable size. A distinct cytoplasmic density of variable thickness (up to 300 Å) adheres to the postsynaptic membrane, although sometimes a clear distinction from the GT II synapses (see below) becomes difficult when the postsynaptic density is less distinct (< 100 Å).

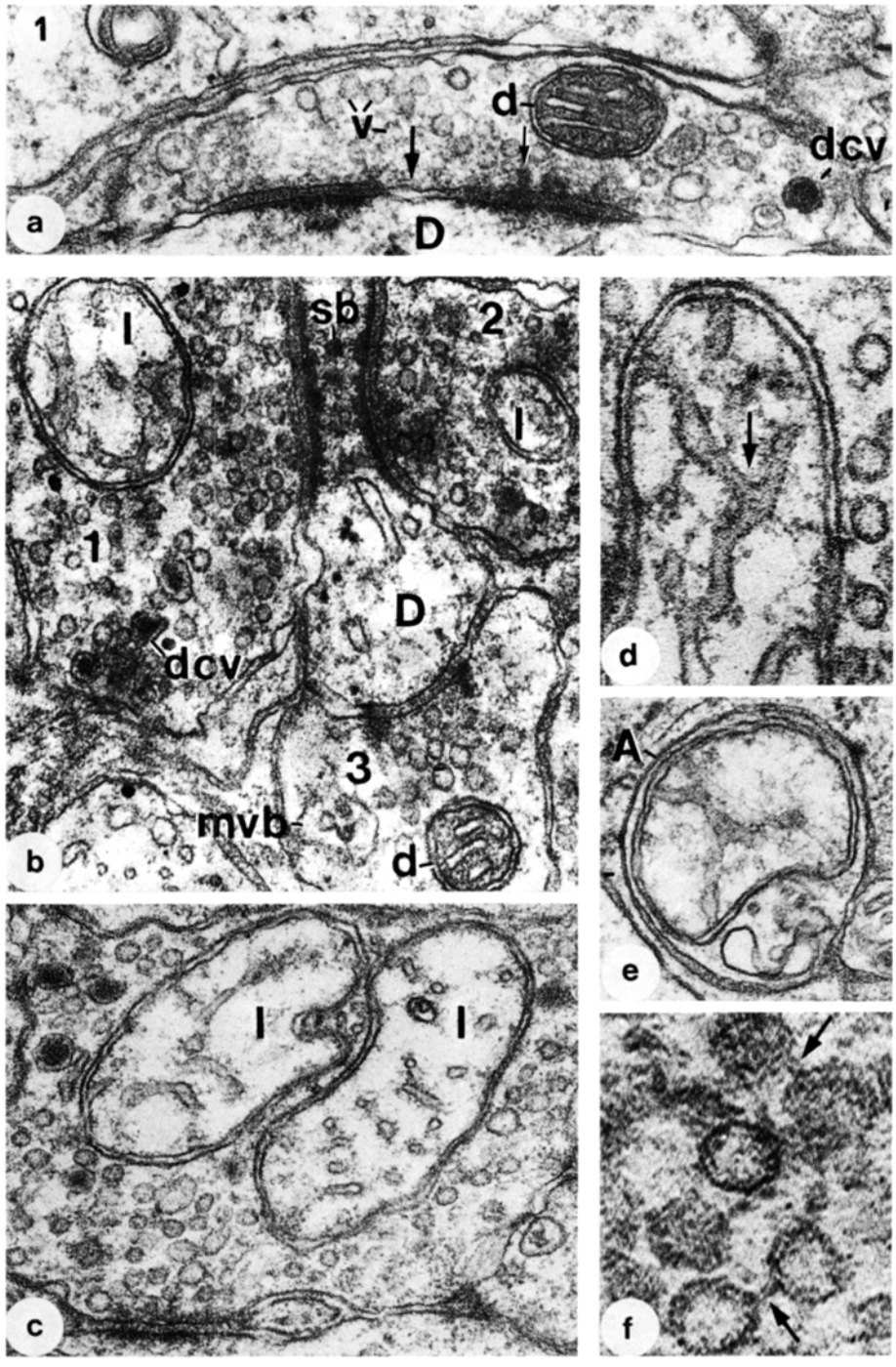
In the various sections through the SCN of male and female animals, about 10–50% of the GTI synapses show one to several *subjunctional bodies* (sb) (Figs. 1b, 2a, b, d, e). These are approximately spherical, electron dense bodies with a diameter of about 300–400 Å, arranged in a two-dimensional plane below the postsynaptic density. The distance between their centre and the postsynaptic membrane is constant, about 600 Å. As confirmed in serial sections, their number in the various synapses is variable. In two cases where one PreEl made contact with two dendritic shafts, it was observed and confirmed with serial reconstructions that only one of the two PostEls possessed sb (Fig. 15). At this point it is noteworthy to mention that the rare axo-somatic GTI synapses can also have sb in the SCN.

Occasionally, so-called *double plug-crest synapses* (Akert, 1972) can be observed, where one spine-like dendritic element bears two presynaptic elements facing each other, both "using" the same set of sb (Fig. 1b). As the distance of the sb from the postsynaptic membrane is quite constant (see above), the thickness of the dendritic element in this region is regularly about 1,600 Å. Here the distance of the sb from the postsynaptic membrane is about 700 Å, which is somewhat larger than in the other "simple" GTI synapses. The "use" of one set of sb by two opposite PreEls is not the rule. In spines the facing PreEls normally have their own set of sb.

In about 10% of the sections through GTI synapses the active zone can be interrupted by one or even two gaps without any synaptic specializations (Figs. 1a, 2b). The length of the gaps is very variable, so that they could

Fig. 1a–f. Gray-type I or asymmetrical synapses (=GTI synapse): (a) GTI synapse with type D-mitochondrion (*d*). Note the interruption of the active site (*thick arrow*). *Thin arrow*, dense projection. ×63,000. (b) Two GTI synapses ("double-plug crest synapse") (*1* and *2*) with type L-mitochondria (*l*). The presynaptic elements abut on a crest-like protrusion of a dendrite both "using" the same set of subjunctional bodies (*sb*). Compare the GTII-synapse (*3*) showing a type-D mitochondrion (*d*) and clear vesicles being larger than those in the GTI synapses. ×63,000. (c) GTI synapse with two type L-mitochondria (*l*). In the left mitochondrion, the tubules of the inner membrane are cut more longitudinally, in the right one more transversely. ×63,000. (d) Higher magnification of a type L-mitochondrion. Note the branching of the longitudinally cut tubules (*arrow*), as also seen in Fig. 1e. ×120,000. (e) Type L-mitochondrion in an axon (*A*). ×63,000. (f) Clear vesicles in a GTI synapse. Note the membranous bridges between two pairs of vesicles (*arrow*). ×240,000

List of Abbreviations in the Figures: A axon, *cv* coated vesicle, *d* type D mitochondria, *D* dendrite, *dcv* dense core vesicle, *e* endoplasmic reticulum, *G* astroglial process or lamella, *g* Golgi apparatus, *l* type L mitochondrion, *m* microtubules, *mi* mitochondrion, *mvb* multivesiculated body, *N* nucleus, *P* presynaptic element, *r* ribosomes, *S* soma, *sb* subjunctional bodies, *v* clear vesicle, *va* vacuole.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indicate "perforated" active zones (Peters and Kaiserman-Abramof, 1969), irregularly formed ones (e.g., horseshoe-shaped) or truly separated complexes (see also Andres, 1975). The diameter of the whole active zone usually ranges in random cross sections from 0.25–0.85 μm (average 0.5 μm), in rare cases up to about 1.5 μm.

One PreEl can form synaptic complexes with one to several postsynaptic dendritic elements (up to four have been found, Fig. 15).

Rarely, small dendrites can be encompassed by the PreEl, and in one extreme case the embracing processes touched each other beyond the dendrite.

GTI synapses can be situated directly beside GTII synapses (see below) without their being separated by a glial sheath (Fig. 15).

Three, four, five and six days after removal of one eye, degenerating GTI synapses were found in the SCN, mainly in the contralateral nucleus. The mitochondria observed in the degenerating GTI synapses are about 0.2–0.4 μm in diameter and contain a relatively electron lucent matrix. The inner membrane is represented mostly by circular (*i.e.*, tubular) profiles. However, 12, 20 and 50 days after bilateral eye removal a significant fraction of GTI synapses remains preserved on the dendritic shafts, spines and somata. Although PreEls with type L mitochondria do not entirely disappear, their number appears to be considerably reduced. Whereas the myelinated fibers of the optic tract showed all signs of degeneration, other myelinated fibers running through the SCN at different levels and in different directions appeared quite normal. At least a fraction of these fibers also seems to innervate the nucleus, as some were found to lose their myelin sheath. However, the unmyelinated portions of these fibers could not be traced to bouton-formations, so that their origin and destination still remains uncertain.

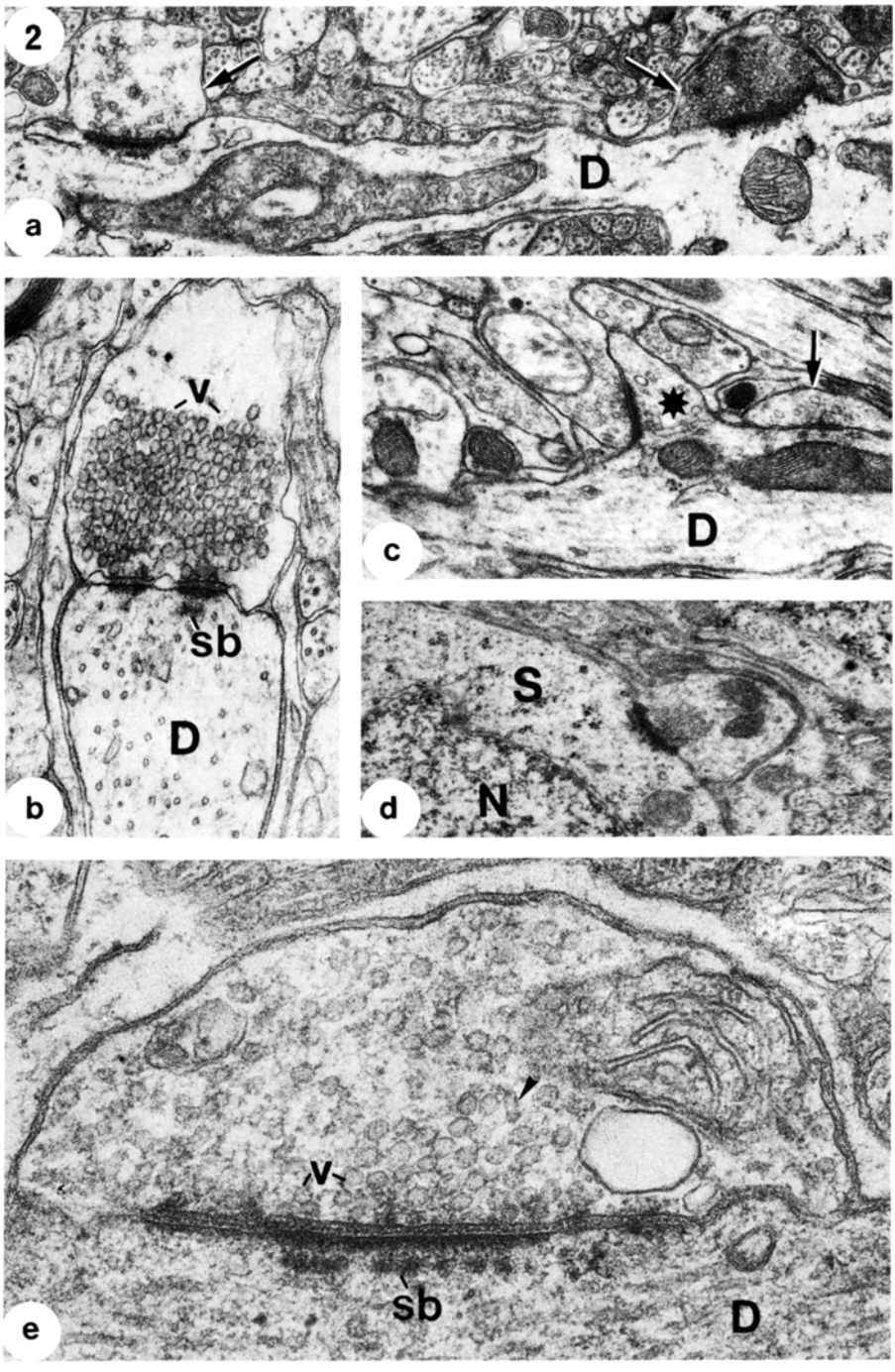
2. *Gray-Type II-Synapses* (Figs. 3, 4, 6, 14, 15)

a) Synapses between Axonal Presynaptic Elements and Dendritic Shafts or Somata = AD Synapses

Presynaptic Elements: The PreEls are spherical or spindle-shaped axonal enlargements with a diameter of about 0.7–1.4 μm.

They contain *clear vesicles* with a diameter ranging in size between 420–670 Å (500 Å on an average) (see Fig. 6). As in the GTI synapses, the distribution of the mean diameters of vesicle populations in 48 PreEls is uniform so that different types of PreEls could not be distinguished on the basis of the vesicle

Fig. 2a–e. GTI synapses: (a) Two GTI synapses (*arrow*), the right one being completely filled with clear vesicles, the left one containing only few scattered vesicles. Both presynaptic elements show dense core vesicles. Note the ring-shaped, branched mitochondrion in the postsynaptic dendrite (*D*). ×10,000. (b) GTI synapse with a compact aggregation of clear vesicles (*v*) which does not fill the whole volume of the presynaptic element. Note the interruption of the active site with subjunctional bodies just beneath the postsynaptic density. ×38,000. (c) GTI synapse between a presynaptic element with L-mitochondrion and a dendritic spine (*asterisk*). A GTII synapse is seen at the right margin (*arrow*). ×25,000. (d) Axo-somatic GTI synapse with type D-mitochondria. ×16,000. (e) GTI synapse after sucrose treatment. The clear vesicles tend to remain spherical. Only one flattened vesicle can be seen (*arrow*). ×58,000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size. In most of the PreEls the clear vesicles are generally larger than those in the GTI-synapses (Fig. 6). The clear vesicles tend to be more oval than those in GTI-synapses of conventional preparations, whereas after sucrose treatment a considerable portion of them become flattened in most of the PreEls (Fig. 4a–c). The ratio between flattened and more circular vesicle profiles varies among individual PreEls.[1] Clear vesicles often fill the whole volume with the other organelles in more or less close aggregations. Sometimes there are also PreEls with only few vesicles scattered in the cytoplasm and/or concentrated near the active zone. In a few PreEls the clear vesicles were found to be very closely aggregated forming a hexagonal pattern (Fig. 4e).

In these PreEls neighbouring vesicles could also be connected by a small membranous bridge (see GTI synapses, compare Fig. 1f).

In up to 53% of the sections through GTII synapses one to several *dcv* occur with a diameter ranging from about 800 to 1,300 Å (see Table 1, Fig. 3). They possess a circular or elongated shape. As in the case of the GTI synapses the size and/or the density of the core may vary (Fig. 3c). The 12 synapses studied by serial sections contained between 6 and 45 dcv. In some cases, PreEls may contain even more of them.

One to four *mitochondria* usually occur in the PreEls mostly belonging to type D mitochondria, but sometimes to type C- and rarely to type L mitochondria (see above: GTI synapses).

Additionally, vacuoles or irregularly formed profiles of the smooth surfaced *endoplasmic reticulum, glycogen particles* and *coated vesicles* can be observed. In one case a coated vesicle was seen to fuse with a sac of the endoplasmic reticulum.

The presynaptic membrane is studded with triangular or polygonal *dense projections* with a length of about 650–750 Å.

The *synaptic cleft* is about 100–150 Å wide and contains a material of medium electron density.

Postsynaptic Element: The PreEls of GTII synapses occur on dendritic shafts of all sizes and neuronal somata, but they have not so far been found on spines. The postsynaptic membrane appears more electron dense than the adjacent cell membrane. The *postsynaptic density* is usually very thin (<90 Å) and often absent. Sometimes, a clear distinction from GTI synapses cannot be made.

Also in these synapses the active zone can be interrupted by a gap of variable length which does not show any specializations. As in the GTI synapses this occurs in about 10% of the sections through a synapse. The diameter of the whole active zone ranges from about 0.24–0.6 µm.

Small dendrites can be encompassed by the PreEls and, in extreme cases, the embracing processes touch each other beyond the dendrite forming a "meso" (Fig. 4d). In serial sections it was found that one PreEl can contact up to three different dendrites (Fig. 15). A fraction of these GTII synapses are "en passant" synapses (Fig. 3d).

[1] In a few preparations the average degree of flattening was obviously less pronounced, although no variations concerning the technique could be detected. This suggests that there may be unknown critical points in the procedure or that premortal conditions of the tissue may influence the specific artefact of flattening of the synaptic vesicles.

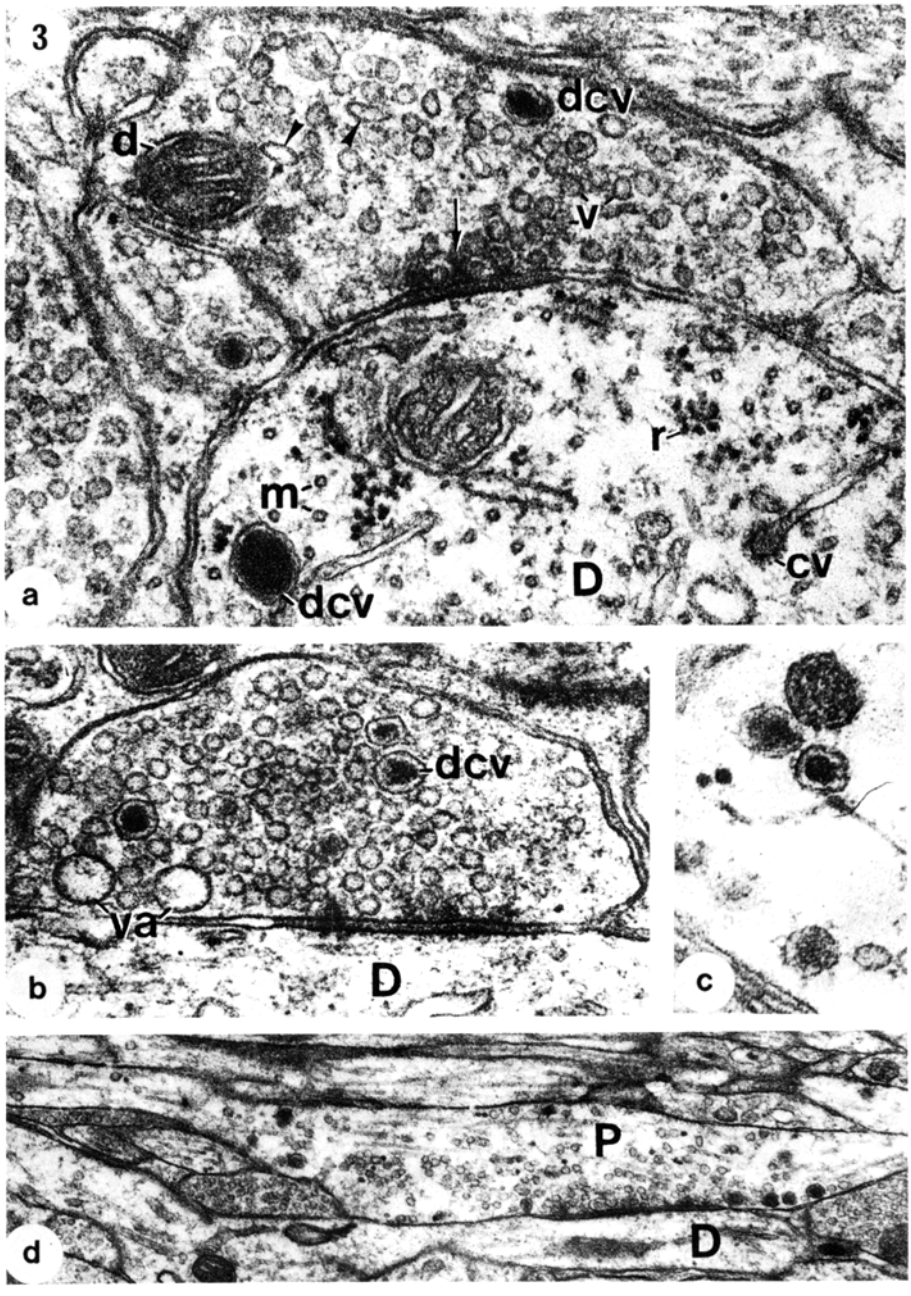


Fig. 3a–d. Gray-type II or symmetrical synapses between axonal enlargements and dendrites or somata = AD synapses. (a) AD synapse with type D mitochondrion (*d*). Oval or flattened clear vesicles (*arrow head*) occur in the PreEl even after conventional preparation. Note dense core vesicle (*dcv*) and a coated vesicle (*cv*) fused with a sac of the endoplasmic reticulum. *Thin arrow*: dense projection. ×63,000. (b) AD synapse with several dense core vesicles (*dcv*) and two empty vacuoles (*va*). ×63,000. (c) Four dense core vesicles in an AD synapse. The electron density and the state of filling of the dense core can be variable. ×82,000. (d) An "*en passant*" AD synapse. ×27,000

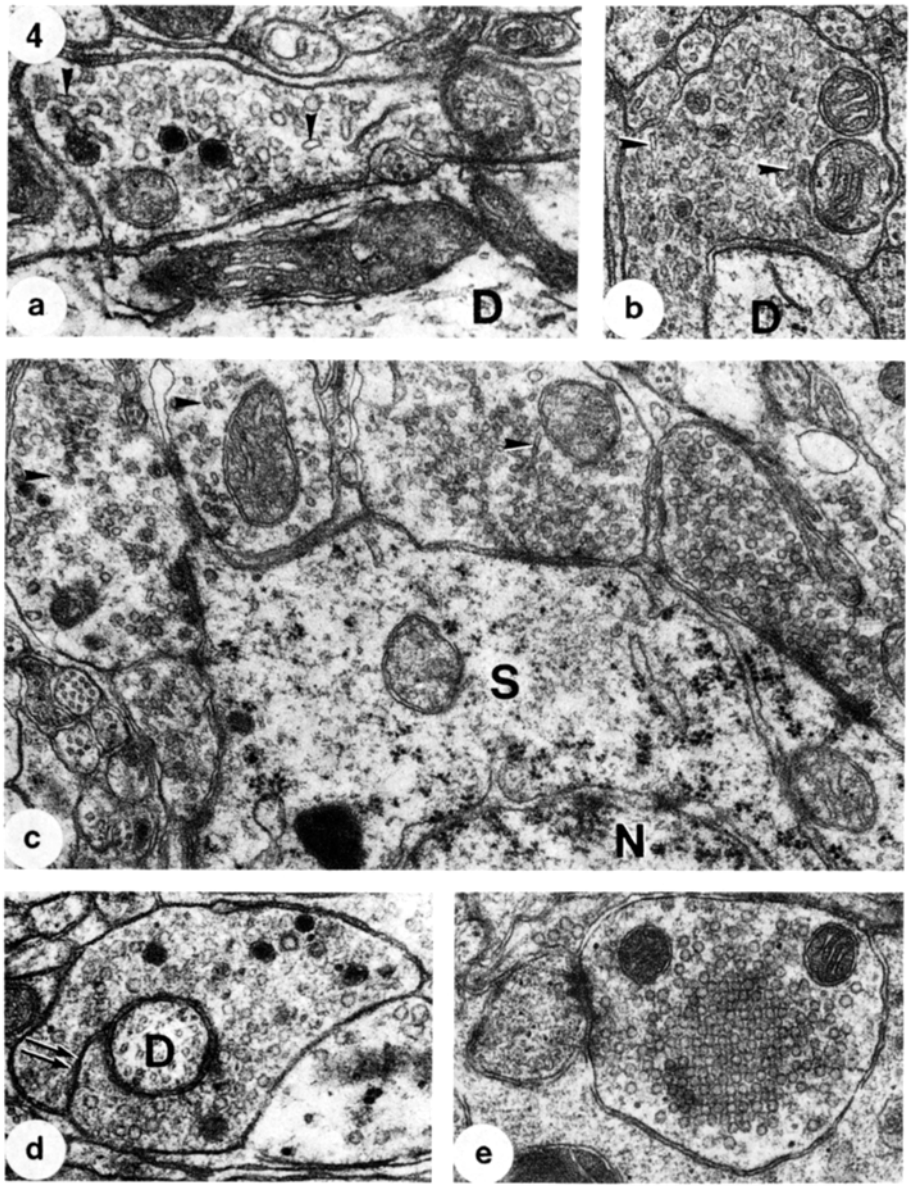


Fig. 4. (a–c) AD synapses after sucrose treatment. A considerable number, but not all, of the clear vesicles have become flattened (*arrow head*). In (c) a group of axo-somatic synapses is shown: Three presynaptic elements show flattened (*arrow head*) and spherical vesicles, but the fourth PreEl only spherical or slightly ovoid vesicles. (a) ×42,000; (b) ×42,000— (c) ×37,000. (d) The presynaptic element of an AD synapse surrounds the small dendrite (*d*) forming a "meso" (*double arrow*). (e) Sometimes, the clear vesicles are so tightly packed, that a hexagonal pattern results. ×31,000

b) Synapses between Axonal Presynaptic Elements and Invaginating Dendritic Protrusions = IAD synapses (Figs. 5, 6, 14, 15)

Presynaptic Elements: The PreEls are about 0.7–2.0 μm in cross section and often have an elongated shape. In most cases, they contain relatively few *clear vesicles* scattered in the cytoplasm and which appear only slightly aggregated near the active zone. In most cases the size of the vesicles is strikingly variable, ranging from 380 up to about 1,100 Å (Fig. 5). As vesicles are frequently more numerous and equal in size, IAD synapses may be confused with GTII-synapses. Sometimes, in conventional preparations, but regularly after treatment with sucrose buffer, a large fraction of the vesicles becomes flattened, especially the larger ones (Fig. 5d).

One to several *dcv* (diameter of about 1,000–1,700 Å) also occur in up to 33% of the sections through PreEls (Fig. 5b, Table 1). Their shape can be spherical or oval. Three-dimensional reconstructions of six synapses showed that one to more than 20 dcv can be present in one PreEl.

The *mitochondria* (up to four could be counted in one PreEl) are about 0.2–0.5 μm wide. The inner membrane mostly forms irregularly distributed circular and tubular profiles which can belong to the cristae, and are leaf-like or tubular in structure. The matrix usually contains a flocculent material of variable electron density, but can sometimes appear electron lucent resembling the type L mitochondria in GTI synapses.

This is the only type of synapse, in which mitochondria possessing different structural aspects could be observed more frequently in one PreEl (Fig. 5b).

Additionally, often *coated vesicles* and *vacuoles,* and less frequently the more irregular profiles of the *smooth surfaced endoplasmic reticulum* are found in the PreEls. In one case the latter were found to be in connection with the outer membrane of a mitochondrion (Fig. 5e). Sometimes, *microtubules* can be seen in the PreEl. Most of the spinule synapses seem to be of the "en passant"-type.

The presynaptic membrane is studded with few *dense projections* (length about 650–850 Å).

Table 1. Number of dcv (n dcv) found in one section through PreEls of GTI and GTII-, AD and IAD-synapses (%)

GTI-synapses (130)		GTII AD-synapses (90)		GTII IAD-synapses (70)	
n dcv in	% of PreEls	n dcv in	% of PreEls	n dcv in	% of PreEls
0	55	0	47	0	67
1	32	1	38	1	20
2	5	2	8	2	0
3	0	3	6	3	5
4	5	4	0	4	2
≧5	3	≧5	2	≧5	5

The *synaptic cleft* is not wider than 160 Å and contains a material of medium electron density.

The *postsynaptic elements* are represented by spine-like protrusions of larger and smaller dendrites and only infrequently by somata, which invaginate the PreEl. They are mostly plug-shaped or leaf-like with a short thin neck and a roundish or flattened head (Fig. 5) (diameter about 0.2–0.6 μm; length of the whole spinule 0.4 μm to about 1.0 μm). Sometimes, the head of the protrusion is branched (Fig. 5c). One PreEl can be invaginated by up to three protrusions arising from different dendrites. Only parts of their membrane area belong to active zones, being interrupted by undifferentiated gaps, so that the whole extent of the active zones cannot be estimated. The postsynaptic membrane itself shows an enhanced electron density, but the postsynaptic density is very delicate and mostly absent.

The dendritic protrusions often contain spherical or elongated clear vesicles, coated vesicles and sometimes free ribosomes. In rare cases, a vacuole, a multivesiculated body or a dcv is present. IAD synapses can lie directly adjacent to GTI and other GTII synapses without being separated by astroglial processes.

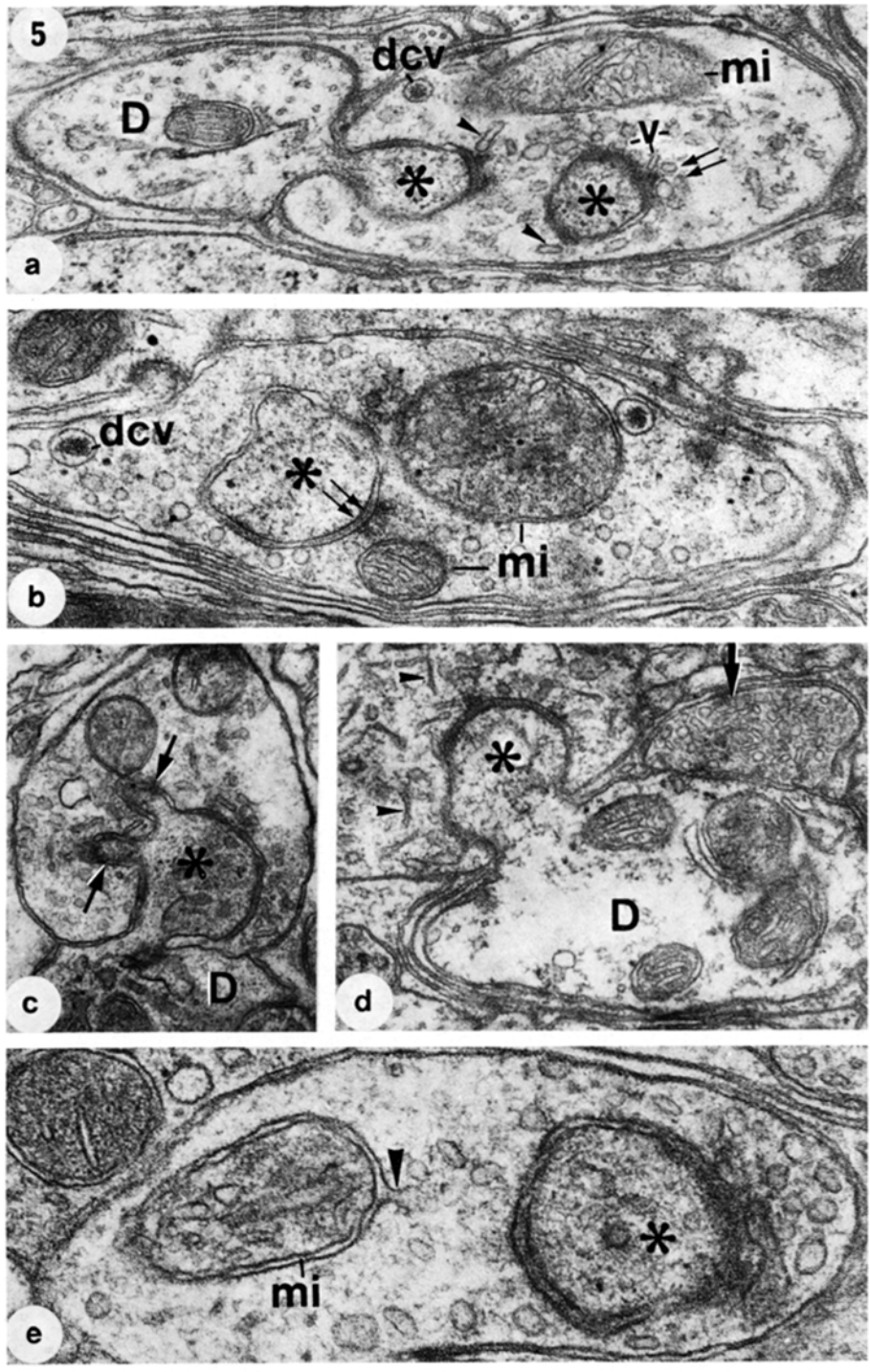
Sometimes, this striking synapse-type cannot be clearly distinguished from the GTII-AD synapses described above, as the clear vesicles can be more uniform in size, and the latter synapses and GTI synapses can also, although rarely, be invaginated by a small dendritic protrusion.

c) Dendro-dendritic, Dendro-somatic and Somato-dendritic Synapses = DD Synapses (Figs. 7, 8, 14, 15)[2]

The *presynaptic elements* are predominantly formed by dendritic shafts of a distinct size (diameter about 0.5–1.5 μm). They show a small and isolated aggregation of *clear vesicles* upon the presynaptic membrane. In seven reconstructed PreEls an actual count of about 80–150 clear vesicles could be made. One somatodendritic synapse contained nearly 20 vesicles (Fig. 8). In conventional preparations, the vesicles are usually circular or slightly oval (diameter 440–

[2] Preliminary results on DD synapses in the SCN have previously been published (Güldner and Wolff, 1974). The ultrastructure and additional findings concerning the arrangement and connexions are described.

Fig. 5a–e. Invaginated axo-dendritic and axo-somatic Gray-type II synapses = IAD synapses. (a) The presynaptic element is invaginated by two clublike protrusions (*asterisk*) from different dendrites. The size of the clear vesicles is very variable; some of them are already flattened after conventional preparation (*arrow head*). The clear vesicles are scattered in the cytoplasm and are only slightly aggregated near the small active zones (*double arrow*). × 34,000. (b) IAD synapse showing a dendritic protrusion (*asterisk*) with one active zone (*double arrow*). Note dense core vesicle (*dcv*) and the variable appearance of the two mitochondria (*mi*). × 45,000. (c) The dendritic protrusions (*asterisk*) in the presynaptic element of the IAD synapse can be branched (*arrow*). × 25,000. (d) After sucrose treatment, especially, the larger clear vesicles become extremely flattened (*arrow heads*). On the right an AD synapse with flattened vesicles (*thick arrow*). × 36,000. (e) The outer membrane of the mitochondrion within the presynaptic element is connected (arrow) with an element of the smooth surfaced endoplasmic reticulum (clear vesicle?). × 75,000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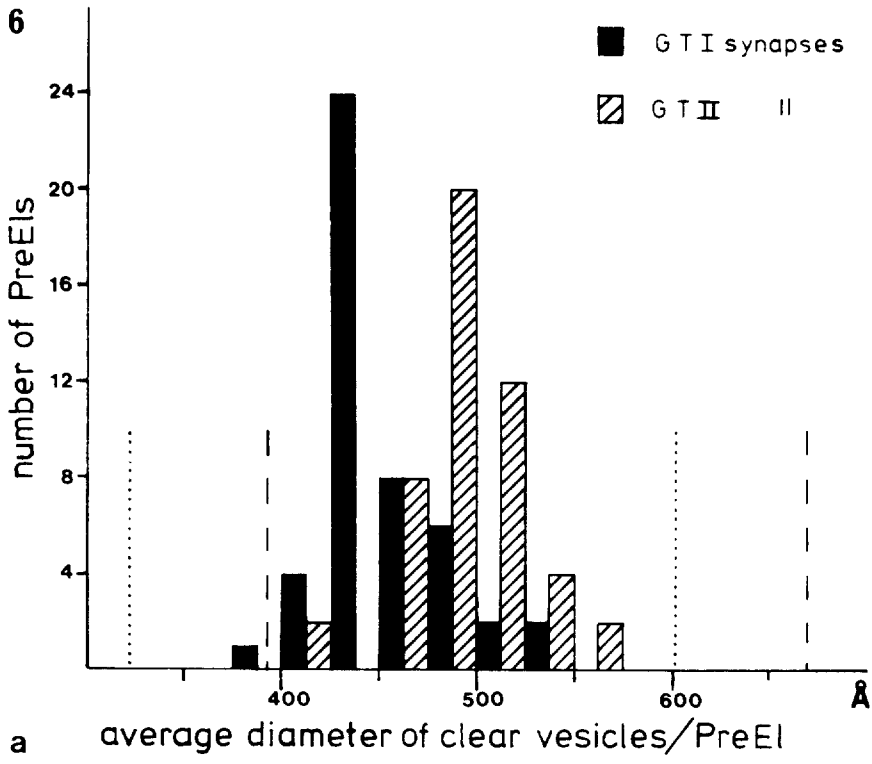


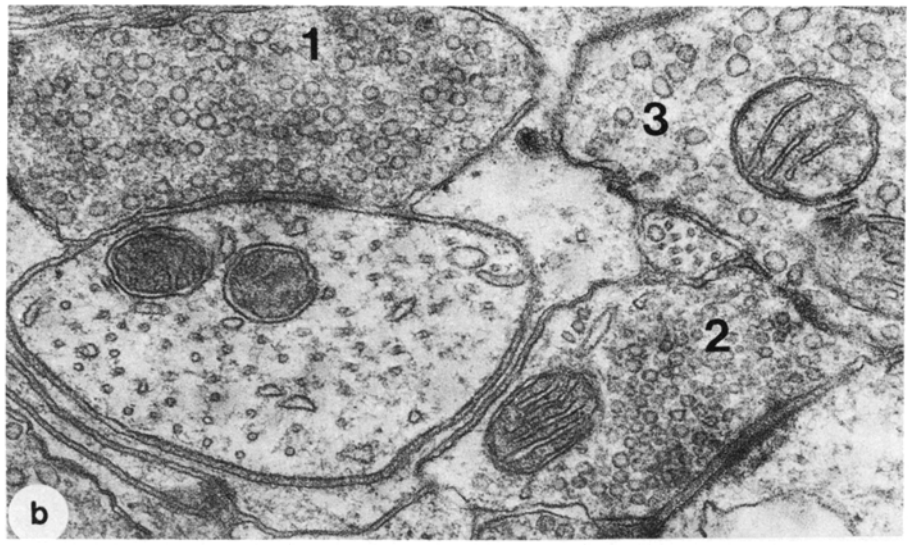


Fig. 6. (a) Histogram showing the distribution of the average sizes of clear vesicles measured in the presynaptic elements of 47 GTI- and 48 GTII synapses. In total, the diameters of 1150 clear vesicles in GTI synapses, ranging between 320–600 Å (*dotted lines*), average diameter 450 Å, and 910 clear vesicles in GTII-AD synapses, ranging in size between 390–670 Å (*dashed lines*), average diameter 500 Å, were measured. The difference of the two distributions is highly significant ($\alpha < 0.001$). (b) The clear vesicles in the GTII-AD synapse (*1*) are obviously larger on an average than those in the GTI synapse (*2*). Compare them with the variable size of clear vesicles in the IAD synapse (*3*). × 50,000

650 Å; average 500 Å). After treatment with sucrose buffer, they occasionally appear flattened due to their discoid shape (Güldner and Wolff, 1974: Figs. 2–4). *Dcv* (diameter 750–850 Å) were also found to occur close to or within the aggregation of clear vesicles, sometimes situated directly upon the presynaptic membrane. Size and density of the cores can be variable, as in PreEls of the other types of synapses.

Approximately five to ten *dense projections* occur on the presynaptic membrane. Their height ranges between 600–800 Å as in axonal PreEls.

The diameter of the active zone ranges from 0.25 to about 0.6 μm.

Near the synaptic complex, *microtubules,* profiles of the *smooth endoplasmic reticulum, poly(ribo)somes, mitochondria,* dcv (diameter 800–1,400 Å) and *multivesiculated bodies* often with surrounding vesicles occur, the first three structures mentioned above being typical for dendrites.

The *synaptic cleft* resembles that of the other GTI synapses (width: 130–160 A; electron dense band).

The *postsynaptic elements* are dendritic shafts crossing the presynaptic dendrite at various angles (Fig. 7a). They show similar ultrastructural features. The postsynaptic membrane appears more osmiophilic than the adjacent parts of the cell membrane. No prominent cytoplasmic density could be observed on the postsynaptic membrane. The whole synaptic complex has a diameter of about 0.4–0.5 μm.

Reciprocal DD-synapses have also been found to occur, *i.e.,* both dendrites are presynaptic to one another (Fig. 7b). Both the synaptic complexes are always of the symmetrical type (GTII synapse). The active zones can be situated closely side by side, but also more separated (a distance of about 2 μm could be estimated in a three-dimensional reconstruction, see Fig. 15), so that the probability of cutting both zones in one section is very small.

In a three-dimensional reconstruction of an area close to the myelinated fibers of the optic chiasma, a neuron could be found which innervates one dendrite, and which is innervated by two other dendrites. These two dendrites innervate each other, forming a reciprocal synaptic arrangement (Fig. 15: D1 and D2). As only a part of the neuron could be reconstructed, it is unclear whether the former dendrite also innervates the neuron and/or whether the neuron innervates the two other dendrites. The dendrites forming DD synapses are postsynaptic in both GTI and GTII synapses (AD and IAD synapses, Figs. 7a, c, 15). The reconstructed part of the neuronal soma was found to be postsynaptic only for GTII synapses. Although boutons of GTI synapses come into direct contact with the reconstructed part of the soma, they seem to innervate only (or preferentially) dendrites (Fig. 15). In this context it should be mentioned that the cytoplasm of the reconstructed neuron contains a "nucleolus-like body" (Le Beux, 1970).

Axo-axonal synapses could not be detected within the SCN, although their possible existence cannot yet be denied in marginal regions of this nucleus (see Methods).

III. General Cytological Features of the SCN-Synapses

Attachment Plaques: These structures, also called "intermediate junctions" (Brightman and Reese, 1969) which have the appearance of primitive desmosomes, are often formed between:

1. PreEl and PostEl near the active zone and, thus, occasionally the source of diagnostic error;
2. between two adjacent dendritic and/or somatic elements (Fig. 7) or
3. rarely between two adjacent PreEls (Fig. 12).

Postsynaptic Elements

Neuronal Somata: Their cytology has already been described by Suburo and Pellegrino de Iraldi (1969, rat) and Clattenburg *et al.* (1972, rabbit). At present, only a few observations will be noted which were observed in the material of this study. The perikarya occasionally contain the so-called *nucleolus-resembling bodies* (see Le Beux, 1971), concentric membranous bodies and structures similar to the "*dark cisternal fields*" of Anzil *et al.* (1971, see below under Dendrites).

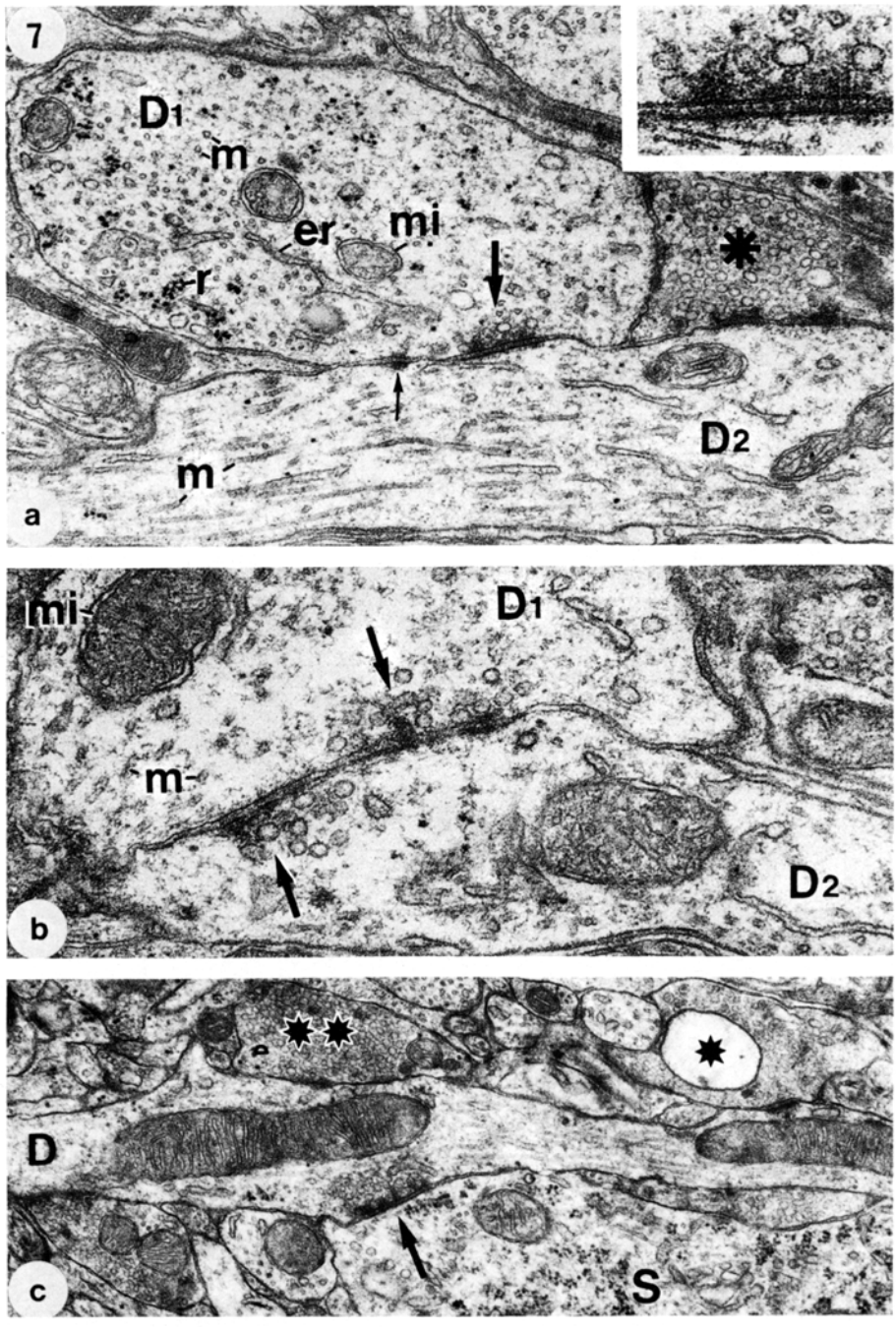
Dendrites: In addition to the organelles and structures typical of dendrites, such as microtubules, smooth surfaced endoplasmic reticulum and especially ribosomes, mitochondria (similar to type D in GTI synapses), dcv, clear and coated vesicles, multivesiculated bodies and sometimes concentric membranous bodies also occur in the postsynaptic dendritic elements.

An interesting fact is that the *multivesiculated bodies* (Fig. 9, insets) have been found to be more numerous (up to 40 times) in the dendrites of female rats than in the male. Coated vesicles could be observed to fuse with multivesiculated bodies (Fig. 9, inset).

The *spines* (Fig. 2c) are pointed or club-shaped (length about 0.5–1 μm). They usually contain only spherical or elongated clear vesicles and coated vesicles. An obvious spine apparatus has not been observed.

In some of the postsynaptic dendritic elements the *number of mitochondrial profiles* is unusually large (Fig. 10c–e). In three dimensional reconstructions it could be shown that in these elements some mitochondria are U-shaped or branched, forming Y-, H-, or coral-like configurations (Fig. 10a, b). In a

Fig. 7e–c. Dendro-dendritic (a and b) and dendro-somatic GTII synapses (c). (a) Two crossing dendrites (D_1 and D_2) form a synapse (*thick arrow*), D_1 being the presynaptic element. Compare the size of the vesicle aggregation with that of the AD synapse, formed between the axonal presynaptic element (*asterisk*) and D_2 (very probably also with D_1); *thin arrow:* attachment plaque between D_1 and D_2 near the active zone. × 31,000. Note microtubules (*m*), polyribosomes (*r*) and endoplasmic reticulum (*er*). *Inset:* Higher magnification of the active zone of the dendro-dendritic synapse shown in (a). × 85,000. (b) Reciprocal dendro-dendritic synapse (*thick arrow*), D_1 and D_2 being both pre- and postsynaptic elements. (*arrows*, active sites). × 50,000. (c) Dendro-somatic synapse (*thick arrow*). The presynaptic dendrite (*d*) is postsynaptic in a GTI synapse (*single asterisk*) and a GTII synapse (*two asterisks*). × 25,000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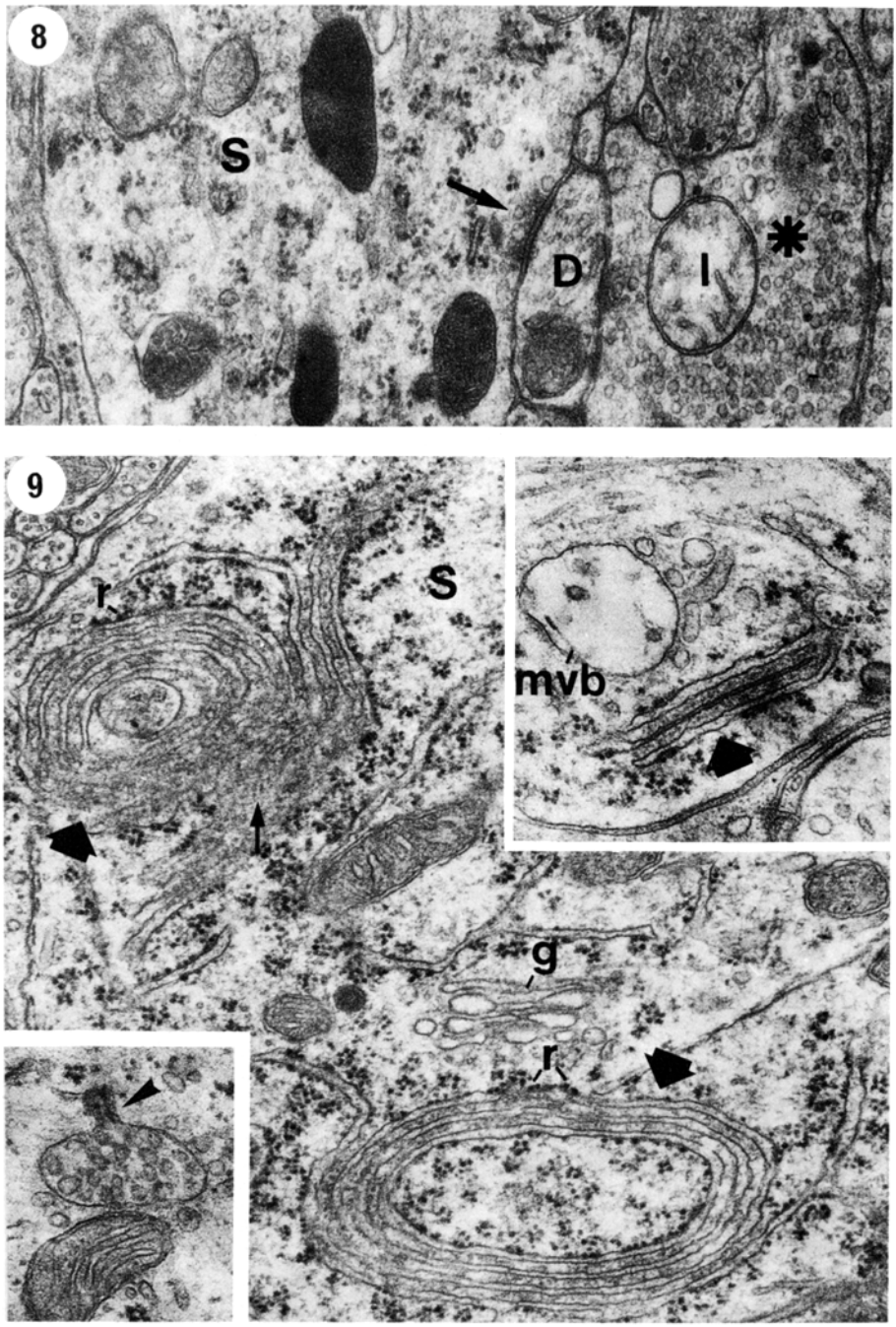

Fig. 8. Somato-dendritic synapse (*thick arrow*). The soma (*s*) is also postsynaptic in the dendro-somatic synapse shown in Fig. 7c (see also Fig. 15). The postsynaptic dendrite is also postsynaptic in a GTI synapse (*asterisk*) as revealed by serial sections. Note type-L-mitochondrion (*I*) in the presynaptic axonal element. × 42,000

few extreme cases dendritic profiles are packed with mitochondria and glycogen granules. Occasionally, *subsurface cisternae* occur.

In some dendrites and neuronal somata specialized profiles of the *endoplasmic reticulum* were found (Fig. 9): three to seven cisternae are closely packed, delimiting a cytoplasmic space with a constant width of about 300 Å (260–360 Å), and contain a flocculent material of medium electron density, being significantly denser than the surrounding cytoplasm. In tangential sections the intercisternal cytoplasm appears to contain dense particles arranged in a regular pattern. The cisternae are usually smooth surfaced, although sometimes the outer membranes are studded with ribosomes, or they are connected with rough surfaced endoplasmic reticulum. The content of the cisternae is electron lucent, but sometimes the membranes lie tightly together forming a band of high electron density (Fig. 9, inset). The cisternae can appear to be separated, piled together or connected with each other at their sides, forming single stacks or spherical (or tube-like?) complexes with concentric layers. Free single or polyribosomes are accumulated near these complexes and in several cases a multivesiculated body was also found near this area.

Some dendrites are "beaded" with varicosities, which often appear nearly "empty" and possess narrow connections with concentrated microtubules. However, most of the other dendrites are nearly straight. Some dendrites demonstrate enlargements alternating with constrictions, the enlargements being filled with the organelles described above. In two cases, dendrites have been found to be interfolded in a complex way (Fig. 13).

IV. Relationships between Synapses and Astroglia

The extent of glial covering of synapses in the SCN is variable. Within complex synaptic arrangements (see below) synapses can show no glial covering. Single synapses have only a partial or complete covering by one glial lamella. Beyond that, a certain number of synapses are partially or totally enveloped by a multilamellar astroglial arrangement (Fig. 11a, b). Up to five glial layers could be found, surrounding one synapse. This arrangement results from interdigitations of different lamellar extensions of astrocytic processes, from overlapping "embracement" by two or more lamellae arising from one process, or, sporadically, from a spiral cover of one extended lamella.

Fig. 9. Special arrangements of the endoplasmic reticulum, called "dark cisternal fields" (*thick arrow*) in a neuronal soma. The cytoplasmic sheath between the cisternae of endoplasmic reticulum shows a relatively higher electron density than the remaining cytoplasm. In tangential section (upper dark cisternal field, *thin arrow*) indications for a regular pattern of electron dense particles in the intercisternal space can be seen. Only the outermost membranes of the cisternae are studded with ribosomes (*r*). *g* Golgi apparatus. ×38,000. *Inset* in the upper right: dark cisternal field (*thick arrow*) in a dendrite near a multivesiculated body (*mvb*), which is surrounded by vesicles. Note the close approximation of the two membranes of the medial cisterna. ×37,000. *Inset* in the lower left: A coated vesicle fuses with the membrane of a multivesiculated body in a dendrite (*arrow*). ×42,000

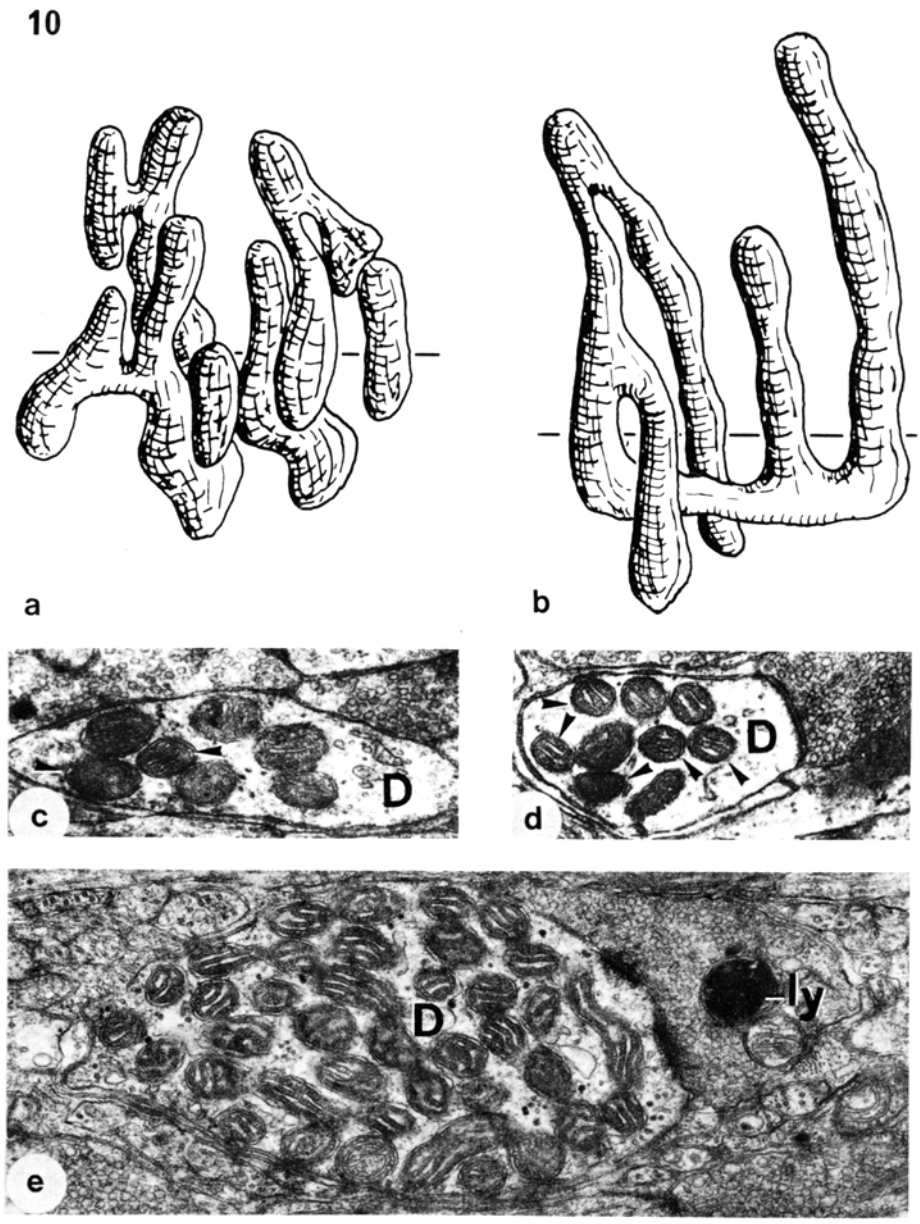

Fig. 10. (a) and (b) Three-dimensional reconstruction of mitochondria occurring in two postsynaptic dendritic elements (c) and (d) which show a strikingly enhanced number of mitochondrial profiles. (c) Section level indicated by a line in (a), ×27,000. (d) Section level indicated by a line in (b). In (a), all mitochondria cut in (c) are reconstructed, in (b) only one. Profiles belonging to this branched mitochondrion in the relevant section are indicated by *arrow heads*. × 27,000. (e) Postsynaptic dendritic element with an exceptionally high number of mitochondrial profiles. Some glycogen particles are also present. The presynaptic element contains a lysosome (*ly*). ×29,000

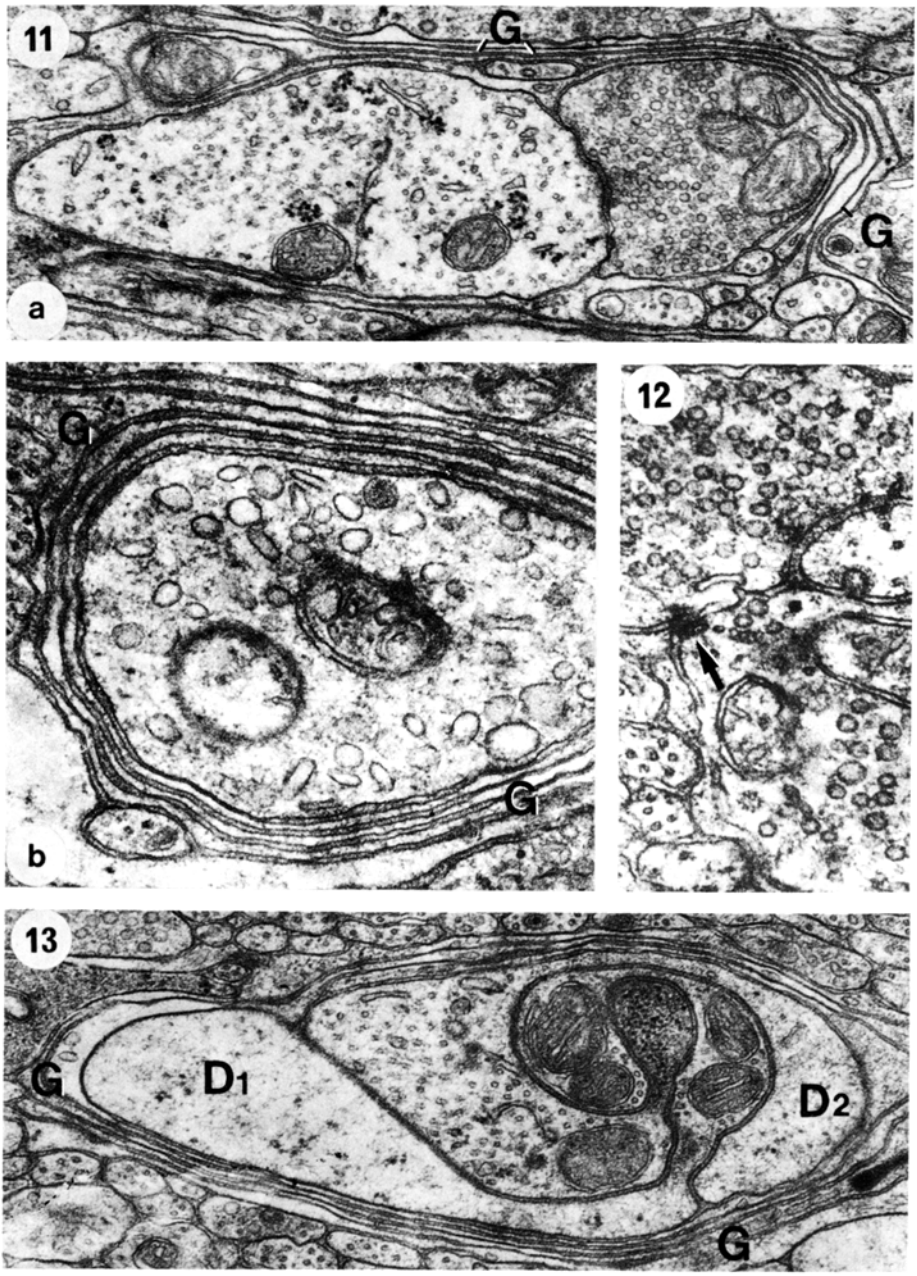

Fig. 11. (a) GTII-AD synapse covered by up to four astroglial lamellae (*g*). ×30,000. (b) GTII-IAD synapse covered by up to five astroglial lamellar processes. ×42,000

Fig. 12. Attachment plaque (*arrow*) formed between two presynaptic elements. ×60,000

Fig. 13. Complex interfolding of two dendrites (D_1, D_2), as observed twice in this study. Both dendrites are enveloped by multilamellar astroglial formations (*G*). ×32,000

A multilamellar envelope was only rarely seen around GTI synapses, but more often around axo-dendritic GTII synapses. The IAD synapses, especially, were found to be surrounded by more than one glial layer (about 10% on an average).

In the normal SCN, the synapses covered by multilamellar astroglial formations did not show any signs of degeneration. Additionally, the segments of neuronal somata and dendrites which are free of synapses are also often enveloped by similar multilamellar astroglial arrangements (Fig. 13).

V. Complex Synaptic Arrangements

The various types of synapses can appear as single, isolated synapses which form the majority of synapses in the dorsal part of the SCN. However, most of these types of synapses can be aggregated without any glial interference in complex synaptic arrangements, which as a whole are covered by astroglial lamellae. These complex synaptic arrangements mainly occur in the ventral regions of the SCN. Only the IAD synapses tend to be separated from these complexes by glial barriers.

The complex synaptic arrangements of the SCN are described in detail in a forthcoming paper (Güldner and Wolff, in preparation).

VI. Some Quantitative Aspects

In the various frontal sections through the SCN the following *relative frequencies of the different types of synapses* are found:

Table 2

GtI synapses	
"simple"	25–30%
"double plug" crest synapses	0–2%
GTII synapses	
AD synapses (axo-dendritic)	20–40%
AD synapses (axo-somatic)	2–10%
IAD synapses	30–50%
DD synapses	0–2%

The size of the boutons and the extent of the active zone is not significantly different in GTI- and GTII-synapses, except for the IAD synapses, where all sections through dendritic protrusions were counted. As the total area of the protrusions is larger than the active zones of the other synapses, the chance to be cut is likewise higher, so that their relative proportion is probably overestimated.

The reconstruction of several longitudinally cut dendritic elements demonstrated that about one synapse occurs per 1.5–2 μm of dendritic length. Accord-

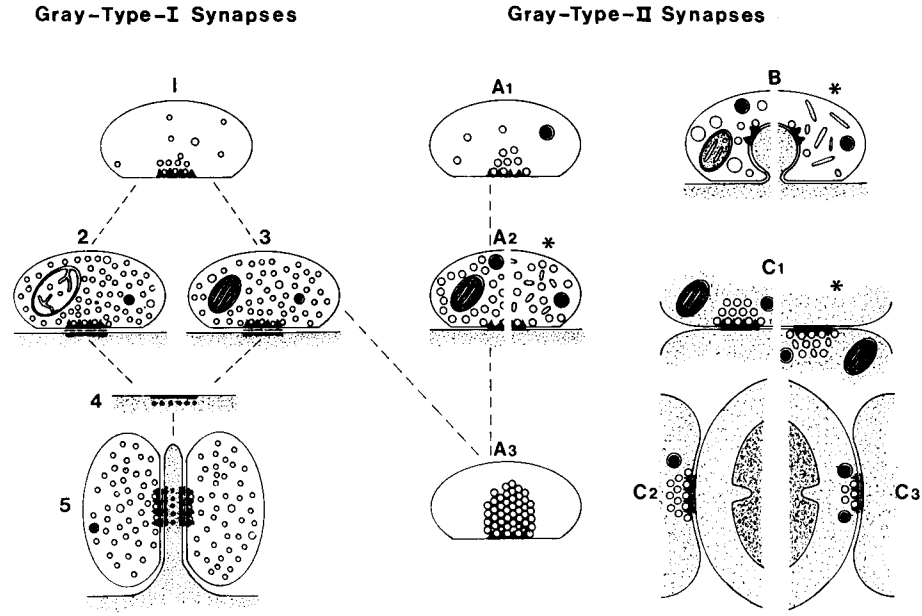

Fig. 14. Schematic drawing of the types of synapses found in the SCN of the rat: *1–5*: Gray-type I (GTI) synapses. *2*: GTI synapse with type L mitochondrion, *3*: GTI synapse with type D mitochondrion. In both of these types of synapses the number of clear vesicles can be more or less decreased (*1*), although an extreme diminution of the clear vesicles is very rare in synapses with type L mitochondria. Dense core vesicles occur in the presynaptic elements in most cases, but they can be absent throughout the whole bouton, which is indicated in *1* and *5* (right). Both types (*2* and *3*) can also show dense projections just beneath the postsynaptic density (*4*); in rare cases both types can form a "crest" synapse (*5*) "using" the same set of subjunctional bodies. *A1–3*: Axo-dendritic and axo-somatic Gray-type II synapses: (=AD synapses). They have mostly somewhat larger clear vesicles which are partly flattened after sucrose treatment (*asterisk*) (*A2*). The number of clear vesicles can be small (*A1*). Sometimes the aggregation of clear vesicles forms a hexagonal pattern (*A3*) as also seen on rare occasions in GTI synapses (indicated by a connecting dashed line). *B*: Invaginated axo-dendritic and axo-somatic GTII synapses (IAD synapses), showing clear vesicles of variable size, most of which are flattened after sucrose treatment (*asterisk*). *C1*: Dendro-dendritic (=DD) synapse. Some of the clear vesicles become oval or flattened after sucrose treatment (*asterisk*). *C2*: Dendro-somatic synapse. *C3*: Somato-dendritic synapse

ing to Golgi-Cox impregnations, SCN neurons can have two to five dendrites, which are about 150–350 μm long showing only few branches. These findings suggest that there are between 300 to 1,200 synapses on at least most of the neurons. Although exact stereological measurements have not yet been made, a calculation from the data mentioned above of the number of synapses per tissue volume suggest that the *average number of synapses per cell* is nearer to 300 than to 1,200. In random sections through the SCN only every fifth soma on an average showed an axo-somatic synapse (only sections through active zones were counted). Thus, between five and 25 axo-somatic synapses can be expected to occur on suprachiasmatic neurons. This does not yet exclude

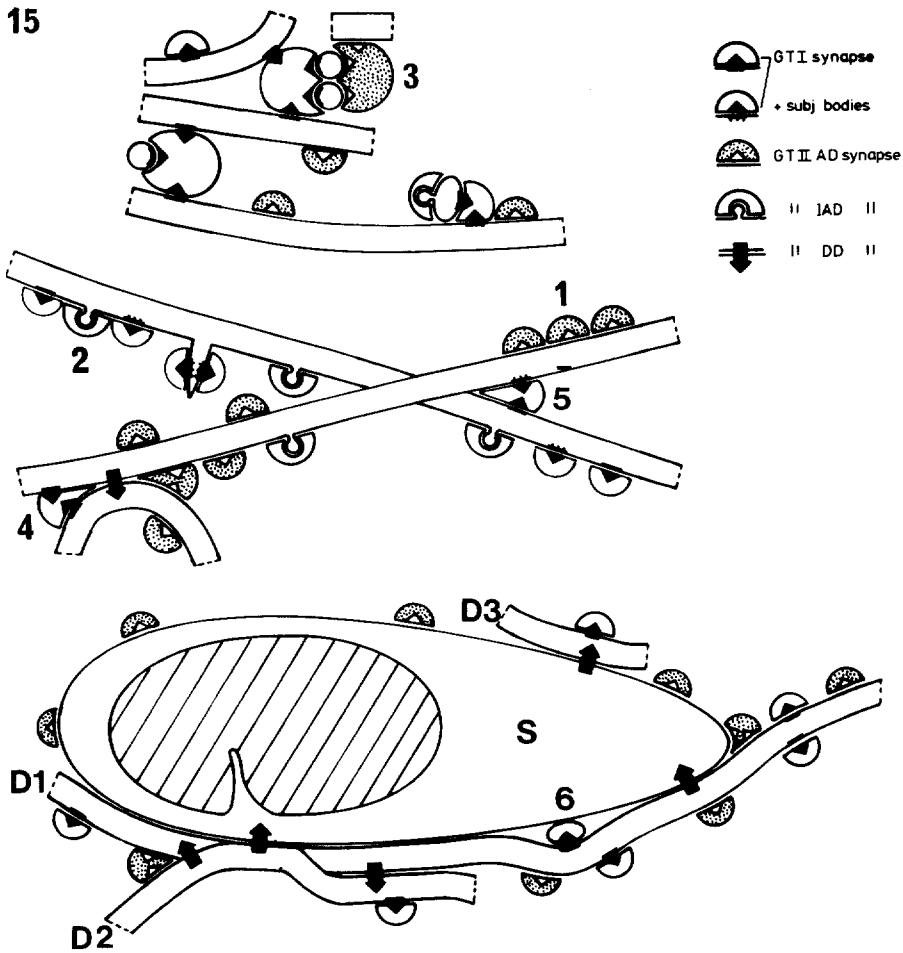

Fig. 15. Half-schematic drawing of principles of synaptic connections, as found in two areas of the SCN reconstructed by 50 serial thin sections. The dendrites appear to be innervated randomly by the different types of synapses, although aggregations of one type can occur (*1*). Different types of synapses can lie directly side by side (*2*), often without being separated by glial processes. One presynaptic element can innervate up to four different dendrites (*3*). Two dendrites can innervate each other where they cross (*thick arrows*) and both can be innervated at the same time by a GTI and/or a GTII-AD synapse at this point (*4*). Note that in one GTI synapse where the presynaptic element innervates two dendrites, only one of the postsynaptic elements shows subjunctional bodies (*5*). The neuronal soma (*S*) is innervated by two dendrites D_1, D_2 (*thick arrows*) which also innervate each other with reciprocal synapses (*thick arrows*). The soma itself innervates a third dendrite (D_3). Otherwise, the soma appears to be innervated only by GTII-AD synapses. The presynaptic element of a GTI synapse touching the somal membrane without glial interference only innervates D_1 (*6*). As the whole soma could not be reconstructed, it remains unclear, whether the soma also innervates the two first dendrites, or whether it is innervated by the third dendrite, or a GTI synapse

the possibility that single somata have more synapses, as on a few perikarya local aggregations of four to six PreEls could be observed.

Discussion

The synapses of the suprachiasmatic nucleus (SCN) can be divided into two main groups, the GTI (or "asymmetrical") and the GTII (or "symmetrical") synapses (see Gray, 1959; Colonnier, 1969; Akert *et al.*, 1972). However, a relatively small portion of the synapses could not be classified with certainty, corresponding to the findings of Colonnier (1969) in the cerebral (visual) cortex.

1. GTI Synapses

As known from the degeneration studies of Hendrickson *et al.* (1972), Moore and Lenn (1972) and Hartwig (1974) optic fibers innervate the SCN through GTI synapses. After bilateral eye enucleation, a considerable number of GTI synapses remain preserved which contain only type D mitochondria. This gives evidence that the GTI synapses of the SCN are formed by more than one type of fiber. The optic synapses cannot yet be recognized with certainty in normal tissue. Although they probably form those PreEls which often conform to the surrounding neuronal processes, containing large numbers of clear vesicles and mainly type-L mitochondria, a definite decision could only be made by tracer experiments. Whether in the remainder of the GTI synapses the variations in number and size of clear vesicles and presence or absence of dcv correspond to different types of synapses and to different physiological states of one type remains to be evaluated. After application of 6-OH-DA, Baumgarten (personal communication) observed degenerating PreEls forming asymmetric synapses in lateral regions of the SCN. This observation might suggest that noradrenergic terminals form GTI synapses, which corresponds to findings of Calas (1973) who showed an uptake of tritiated noradrenaline in asymmetrical synapses within the median eminence (see also Aghajanian and Bloom, 1967; Lenn, 1967). Due to the fact that GTI synapses are also preserved in the medial areas of the SCN after degeneration of the optic afferents, at least three different synaptic systems of this type can be assumed to be present.

In this respect it is worthwile to note that PreEls of GTI synapses contain a very varying number of dcv, as revealed by three dimensional reconstructions. Therefore, it is conceivable that dcv may disappear completely under certain states of synaptic activity. Consequently, a classification of a given synapse may not be possible on the basis of one section through the PreEl or even on the basis of a three-dimensional reconstruction, either in the SCN or in other regions of the central nervous system, if additional criteria do not exist. To make this problem more difficult, disturbances of the blood brain barrier lead to the appearance of structures quite similar to dcv in glial and neuronal processes, *i.e.*, also in PreEls (Wolff *et al.*, 1975). This phenomenon could occur, to a minor extent, under "normal" conditions, so that PreEls containing dcv may not always be "specific".

Further, the presence of subjunctional bodies (sb) does not appear to be indicative of a special type of synapse, as they are seen in GTI synapses showing considerable ultrastructural variations. Moreover, in synapses formed between one PreEl and several dendrites the sb can be present in one PostEl, but absent in others. The same phenomenon was recently described by Andres (1975).

Thus the formation of sb seems to be dependent on the state of the PostEl concerned. Whereas the occurrence of "simple" GTI synapses with sb has been established in many regions of the central nervous system (see Akert *et al.*, 1972), the so-called double plug crest-synapses where two opposite PreEls use the same set of sb have been observed only in the subfornical organ, the habenular nucleus, the preoptic area, the nucleus tractus solitarii and in the substantia gelatinosa Rolandi (see Akert, 1972; Prince and Jones-Whitters, 1974; Andres, 1975; Chiba and Doba, 1975). Their significance is unknown.

2. *GTII Synapses*

The *AD-synapses* formed between axonal PreEls and dendritic shafts or somata pose similar problems as the GTI synapses. As they show in principle similar variations in the appearance of the PreEls, they are also suspected of being composed of more than one type of fiber. Nevertheless, at least a large fraction of these synapses represents the serotoninergic input into the SCN, demonstrated by Fuxe (1965), Baumgarten and Lachenmayer (1972) and Saavedra *et al.* (1974). Baumgarten and Lachenmayer (1972) found with the aid of degeneration studies that the degenerating PreEls of the serotoninergic fibers abut on neuronal somata and dendritic shafts forming symmetric synapses (personal communication). Thus, at least a large fraction of the GTII-AD synapses should belong to the serotoninergic system.

Strikingly, the size of the clear vesicles and dcv is in most GTII synapses on an average larger than that in GTI synapses. This is quite contrary to findings in other regions of the CNS, e.g., in the cerebral cortex (Colonnier, 1968). However, as there is a considerable range of overlap not only in the size of single clear vesicles, but also in their average size in individual PreEls, boutons of GTI- and TGII-Synapses cannot always be distinguished from each other by comparing the vesicle size alone.

After treatment with sucrose buffer the clear vesicles of AD synapses are far more often oval or flattened than those in GTI synapses. As in freeze-etched preparations the vesicles remain spherical (Akert *et al.*, 1972), the flattening of clear vesicles seems to be a specific artefact caused in distinct types of synapses during the fixation procedure. The flattening of clear vesicles is thought to result from osmotic forces (see Korneliussen, 1972), but Gray (1969, 1975) proposed an alternative hypothesis, namely that the flattening could be imposed on the vesicle from material (stereo-framework) in the surrounding cytoplasm, which becomes reorganized as a result of reaction with aldehyde. The ratio between flattened (discoid) vesicles and circular (spherical) vesicles can vary among the individual synapses of one animal as well as in the SCN of different animals. It would be worthwhile studying more exactly whether the extent

of vesicular flattening is dependent on the degree of filling by the corresponding transmitter substance as it was also recently discussed by Tigges *et al.* (1975). Heuser and Reese (1973) demonstrated the "pinocytotic" vesicular re-uptake of membrane material in PreEls during synaptic activity. Whittaker and his coworkers (Zimmermann and Whittaker, 1974a, b; Whittaker *et al.*, 1975) could show that in synaptosomes of the electric organ of *Torpedo* the filling or refilling of synaptic vesicles with transmitter molecules is completed only after a considerable time after release. Although this time should be reduced *in vivo* and at higher temperature, the ratio: flattened/spherical vesicles could possibly give some indication of the actual synaptic state of activity at the time of fixation.

The meaning of the membranous bridges between neighbouring clear vesicles within GTI and GTII synapses is unclear. Van Harreveld and Trubatch (1975) describe clear vesicles connected by "stalks" with the presynaptic membrane in a small fraction of stimulated synapses. In some figures of their publication (e.g., Figs. 3, 18), two vesicles are seen to be connected by bridges, although the authors do not emphasize this fact. The "stalks" are formed by a fusion of the electron-dense cytoplasmic lamella of the vesicular membrane and the cytoplasmic lamella of the synaptic membrane, bordering a median electron-lucent lamella, which is continuous with the median lamella of the unit membranes. This unit membrane-like appearance of the "stalks" resembles that of the bridges between two or more clear vesicles. In this respect, van Harreveld and Trubatch discuss the possibility of the "cytonet" as suggested by Gray (1973), guiding or even moving the vesicles towards the presynaptic membrane, but they tend more to the opinion that the "stalks" are formed during the process of recycling of vesicles. The stalks would be the last connection of the newly formed pinocytotic vesicle with the presynaptic membrane, shortly before it snaps and the vesicle moves away from the membrane. Whether the bridges between synaptic vesicles appear during the process of formation of new synaptic vesicles from larger vesicles, vacuoles or irregular profiles of the smooth surfaced endoplasmic reticulum (see Heuser and Reese, 1973) or whether they are the morphological manifestation of another mechanism, is unclear.

Due to their relatively constant structural features the *IAD-synapses* seem to belong to one distinct type of fiber. Their origin is unknown. In most of these synapses, the variable size of the clear vesicles is striking. Nevertheless, in some cases the size of the vesicles can be more homogeneous and their number can vary, so that a differentiation from other axo-dendritic GTII synapses may become difficult. Similar vesicle populations in some other types of synapses have been described, e.g., in the amygdala by Wakefield and Hall (1974) and in the preoptic area by Prince and Jones-Witters (1974). The latter authors discuss the possibility that the large clear vesicles, having the dimensions of vacuoles, are special functional states of dcv with a "masked" or emptied content. Support for this interpretation could be the findings of Morris and Cannata (1972, 1973), who showed that the electron density of the core of dcv in the neurohypophysis depends on definite conditions, e.g., the pH of the fixation medium. As, however, after sucrose treatment these vesicles are extremely flattened with nearly touching opposite membranes, the presence of a "masked" content seems unlikely.

The factors inducing the invagination of PreEls by protrusions or excrescences of the postsynaptic dendrites (see also Němeček, 1972; Andres, 1975) are still unknown. Nevertheless, there are indications that this phenomenon is caused by a certain activity level of the afferent system: Van Harreveld and Trubatch (1975) observed increasing numbers of dendritic and glial invaginations within PreEls after long lasting experimental activation (see also Párducz *et al.*, 1974; Fig. 5). Thus, the IAD synapses could represent a system with a special, perhaps high, activity pattern quite distinct from the rest of afferents in the SCN. PreEls of GTI- and axo-dendritic GTII-synapses can also, in rare cases, show small dendritic invaginations, which could indicate that some axons of a distinct afferent system have an activity level different from the remainder.

The *DD synapses* in the SCN are exclusively symmetrical, *i.e.*, GTII synapses. They are only rarely found in random sections through this nucleus. Nevertheless, one neuron can form at least two presynaptic sites on one dendrite, as could be shown by three-dimensional reconstructions. Supposing that each of the approximately 10,000 neurons (Raisman, personal communication) in one nucleus forms such synapses, a minimal amount of 20,000 DD synapses should be present. Taking into account that each active zone (diameter about 0.4–0.6 μm) can be spread over about four to eight thin sections and the length of the ovoid SCN is about 500 μm, then approximately 10–20 DD synapses should appear in each frontal thin section. Instead, only 0 to three were found in any one section (frontal section, area $10^5\ \mu m^2$, sagittal section, area $1 \cdot 2 \times 10^5\ \mu m^2$). Consequently, DD synapses must be formed by only a smaller number of the SCN neurons. As these neurons probably have more than two presynaptic sites (one SCN-neuron can have two to five main dendrites with some branchings), their number should be less than 2,000.

This provides further evidence that there may be several functionally different types of neurons in the SCN (see also Clattenburg *et al.*, 1972; Vandesande *et al.*, 1974). Unfortunately, such neurons cannot be distinguished with certainty from ultrastructural features alone (see Suburo and Pellegrino de Iraldi, 1969).

Other points of discussion concerning DD synapses have been treated in a preliminary paper on this topic (Güldner and Wolff, 1974).

3. Considerations on Some Cytological Features of PreEls and PostEls within the SCN

Indications for a *sexual dimorphism* could not yet be detected in this study, except for the fact that in females the PostEls show in most cases far more *multivesiculated bodies* than in males. As coated vesicles contribute to the formation of multivesiculated bodies (Fig. 9, inset) (Birks *et al.*, 1972), it might be concluded that SCN neurons in females form considerably more coated vesicles. Whether this phenomenon is correlated with a higher (cyclic) neuronal activity in females remains to be evaluated.

Occasionally, special arrangements of rough and smooth surfaced endoplasmic reticulum could be observed in neuronal somata and dendrites which resemble the so-called "unique lamellar configurations" described by Adinolfi (1969)

in the entopeduncular nucleus (cat), the "dark cisternal fields" studied by Anzil *et al.* (1974) in striatal neurons (rat) and some forms of the "ribbons" and "ribbon rolls" recently demonstrated by King *et al.* (1974) in the arcuate nucleus, especially in female rats during diestrus. Similar structures were found by Bogusch and Lindner (personal communication) in the so-called "Ringbindenmuskelfasern" of tongue, pharynx, thymus, eye, larynx and heart as well as in skeletal muscles after denervation. The role of these structures is unclear. Anzil *et al.* regard them as transient formations of the endoplasmic reticulum in a special functional state. King *et al.* discuss the possibilities that these structures could be involved in "the synthesis and packing of neurosecretory material in conjunction with the Golgi apparatus", or that they "could involve detoxication of surplus hormones or their metabolites".

Several types of mitochondria could be found in the various neuronal elements. Among these, the large type L mitochondria with their "empty" matrix are expecially conspicuous, being mainly localized in PreEls of GTI synapses in ventral regions of the SCN near the fibers of the optic chiasma. Their inner membrane gives rise to tubules or finger-like structures, which can branch forming a reticulum by interconnections. Since it is improbable that the typical features of the inner membrane represent a fixation artefact, it may indicate a special state of the mitochondria, possibly due to a distinct activity pattern of the axons concerned. Similar mitochondria seem to exist also in the optic terminals in other primary visual centers: Szentágothai (1973) described in the optic axon terminals in the lateral geniculate nucleus "large sparsely cristated mitochondria that may easily swell even in well fixed material" (cf., Lieberman and Webster, 1974; Fig. 36B). Tigges and Tigges (personal communication) recently observed "light mitochondria" in the optic boutons of primate superior colliculus.

4. Multilamellar Glial Sheaths

In the SCN as well as in a number of other hypothalamic nuclei (Güldner and Wolff, 1973) neuronal elements can be surrounded by more than one astroglial process. Additionally, perikarya and dendrites, 5 to 20% of the spinule synapses, and, more rarely, also asymmetric axo-dendritic GTI-synapses have been observed to be covered to a variable extent. Similar findings were also made in extrahypothalamic areas e.g., olfactory bulb (Price and Powell, 1970); lateral geniculate nucleus (Peters *et al.*, 1970); n. trochlearis (Bak and Choi, 1974); n. oculomotorius (Waxman and Pappas, 1971); cerebellar cortex (Palay and Chan-Palay, 1974); oliva inferior, n. gracilis and rarely cerebral cortex (Wolff, personal communication).

The meaning of this phenomenon is still uncertain. Studies are now in progress to reveal whether the degree of astroglial affinity to neuronal elements is dependent on the activity level of the neurons concerned as proposed by Güldner and Wolff (1973) and recently discussed by Bak and Choi (1974), who found glial envelopes composed of several astrocytic processes around *en passant* synapses in the trochlear nucleus of the cat. According to Kuffler

and Nicholls (1966) and Baylor and Nicholls (1966) who assume that glial processes near synaptic zones serve as a barrier against the diffusion of the potassium ions, Bak and Choi conclude "that the well-developed glial barrier around the synapses *en passant* is a morphological improvement of an enhanced synaptic transmission".

5. Distribution of the Synapses within the SCN

PreEls and PostEls of the various types of synapses are not evenly distributed within the SCN. The optic and serotoninergic synapses are concentrated in more ventral regions, as revealed by autoradiographic and fluorescence microscope studies (Fuxe, 1965; Hendrickson *et al.*, 1972; Moore and Lenn, 1972). In the present study the DD synapses could only be observed within about the ventral half of the nucleus. The IAD synapses occur in all regions of the SCN, but sometimes they also appear to be accumulated to some extent in ventral regions, as do the complex synaptic arrangements. As for the postsynaptic side, Vandesande *et al.* (1974) demonstrated that neurophysin-containing neuronal somata, which react on adrenalectomy, are localized mainly in the medial and dorsal parts of the SCN. Baumgarten (personal communication, see above) found noradrenergic boutons in lateral areas of the SCN.

6. Some Aspects of Function and Meaning of the Types of Synapses

Function and meaning of most of the synapses described in the present paper are still unclear. The GTI synapses can be only tentatively regarded as excitatory and the GTII synapses as inhibitory. However, it is still uncertain whether this concept (Uchizono, 1965; Akert, 1972) is generally valid, although no evidence against it has yet been put forth. In this respect it is worth mentioning that the asymmetrical optic synapses in the lateral geniculate body are established as excitatory (see Creutzfeldt, 1970); the asymmetrical and symmetrical DD reciprocal synapses in the external layer of the olfactory bulb have been shown to be excitatory and inhibitory, respectively, by electrophysiological methods (see Reese and Shepherd, 1972); and the symmetrical serotoninergic synapses are inhibitory (Bloom *et al.*, 1972). Thus, provided that the above-mentioned concept is generally applicable, in the SCN only about one-third of the synapses would be excitatory, but two-thirds of them would be inhibitory. In this respect it is noteworthy that Makara *et al.* (1975) did not find accumulated radioactivity in the SCN region of rats after ^{3}H-GABA infusion into the third ventricle. Thus, GABA-ergic terminals do not seem to play any role in the SCN.

7. Synaptic Systems

To date *five synaptic systems* in total have been distinguished in the SCN. There are most probably more systems existent which have yet to be discerned.

One of these, the DD synapses, is *"intrinsic"*, although possible connections with neighbouring nuclei via long dendrites cannot yet be excluded. Two others, the visual input and the serotoninergic system, are knwon to be *"extrinsic"*, *i.e.*, afferents from neurons in the retina and the raphe nuclei, respectively. Recently, the ventral lateral geniculate nucleus has been recognized to be a third region sending afferent fibers to the SCN (Swanson and Cowan, 1974; Ribak and Peters, 1975). The nature of their PreEls is still unknown. Additionally, Field (personal communication) has found a projection from the hippocampal area to the SCN via the medial corticohypothalamic tract (cf. also Záborszky *et al.*, 1973). Thus, the origins of the remaining systems, one representing asymmetrical (GTI) synapses, the other one forming the symmetrical IAD synapses, remain to be elucidated.

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