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Complex synaptic arrangements in the rat suprachiasmatic nucleus: a possible basis for the "Zeitgeber" and non-synaptic synchronization of neuronal activity

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Abstract. A special type of complex synaptic arrangement occurs in the ventro-lateral portion of the rat suprachiasmatic nucleus. These arrangements are polycentric, with about equal numbers of pre- and postsynaptic elements. Because of an incomplete astroglial covering, these synaptic complexes are connected with each other and form a continuous reticulum or sponge-like system throughout the ventro-lateral region of the nucleus. In two partially reconstructed complex synaptic arrangements, boutons from retinal afferents could be seen to make up the majority of presynaptic elements. They form asymmetric and symmetric synaptic appositions with dendritic elements. Non-optic axo-dendritic synapses of unknown origin with asymmetric and symmetric appositions and dendro-dendritic synapses with symmetric appositions are also seen in complex synaptic arrangements. Within complex synaptic arrangements, dendrites often run in bundles, with some dendrites spiralling around others. Membranes of neighbouring dendrites are closely apposed. These interdendritic appositions are possibly ephapses and may, together with intersomatic contacts, mediate non-synaptic synchronization of neuronal activity in the suprachiasmatic nucleus, as described by other authors. The activity of optic and non-optic synapses in complex synaptic arrangements over a 24 h period may also produce an integrated response that influences the circadian rhythm of neuronal activity in this nucleus.

Key words: Suprachiasmatic nucleus – Complex synapse – Synaptic glomerulus – Ephapse – Dendrites – Synaptic apposition – Biorhythmicity – Rat (Sprague Dawley)

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Introduction

Szentágothai (1970) has defined complex synaptic arrangements (CSAs) as circumscribed accumulations of one or several presynaptic and one or several postsynaptic dendritic elements. If such an arrangement is mostly separated from the surrounding neuropile by glial processes and has a regular round appearance, it is a "glomerular synaptic complex". If, however, such separation is less clear and the appearance of the CSA is irregular, it can be called a "synaptic complex" or "cluster". Subsequently, Wolff and Nemeçek (1971) have defined CSAs as local aggregations of synapses between at least three different types of neurons and distinguish three main groups of CSAs: 1) Centro-axonic CSAs have a central presynaptic axonic element innervating several dendrites (divergent relay). 2) Centro-dendritic CSAs have a central dendrite that is innervated by several presynaptic elements of different origin (convergent relay). 3) Polycentric CSAs comprise an aggregation of several pre- and postsynaptic elements without a recognizable centre. This latter group is variable in different regions of the central nervous system and probably needs to be subdivided further.

Since the first description of such special synaptic arrangements in the lateral geniculate nucleus (Szentágothai et al. 1966), CSAs have been found in many areas of the brain and spinal cord. CSAs in the lateral geniculate nucleus, with synapses of retinal afferents (optic synapses) as major components, continue to attract particular interest (Jones and Powell 1969; Famiglietti and Peters 1972; Lieberman 1974; Spaçek and Lieberman 1974; So et al. 1985). CSAs also occur in another optic target area, the suprachiasmatic nucleus (SCN), as described in a preliminary report by Güldner and Wolff (1977) and later examined again by Card and Moore (1991). Special arrangements of dendrites in these CSAs may provide an explanation for recent findings that neuronal synchronization occurs in this nucleus without calcium-dependent synaptic transmission (Bouskila and Dudek 1993). Therefore, a more detailed study of CSAs within the SCN including 3-dimensional reconstructions is warranted.

Materials and methods

Conventional fixation

Sprague-Dawley albino rats (5 males and 5 females, young adults) were anaesthetized with ether and sacrificed by perfusion through the heart with a mixture of 3% glutaraldehyde and 3% paraformaldehyde in 0.05 M cacodylate buffer after a short preperfusion with cacodylate buffer. Anaesthesia was continued until perfusion was well under way. Perfusions took place during the late morning hours (10:00–12:00) in March. The tissue blocks containing the SCN were washed in 0.1 M buffer, postfixed in 1% osmium tetroxyde and embedded in Epon 812. The entire area of the nucleus was examined in a JEOL 100B electron microscope. From the SCN of one male rat, 45 serial thin sections (estimated thicknesses between 60 and 100 nm) were cut. An area in the ventrolateral region of the SCN was chosen and photographed in all serial sections (final magnification 60000×). Three to four overlapping photographs were necessary to cover the whole area. The outlines of all neuronal elements and astroglial processes from two different CSAs (CSA I, see Fig. 6; CSA II, see Fig. 7) were copied from the photo-montages of every 4th to 5th section on transparent paper, distinguishing between axon terminals (boutons), dendritic elements, astroglial processes, asymmetric synapses (Colonnier 1968) similar to Gray's type I (Gray 1959) where the main characteristic is a postsynaptic density with a thickness >20 nm (Akert et al. 1972), symmetric synapses similar to Gray's type II where the postsynaptic density has a thickness <20 nm, and attachment plaques. Five types of synapses were distinguished. Axonal and dendritic profiles were traced through all serial sections and identified in the copies by numbers and capital letters, respectively. The numbers of pre- and postsynaptic elements, and the numbers of asymmetric and symmetric synaptic appositions were determined in CSA I and CSA II (see Table 1). It should be noted that the numbers given for various parameters in these two (partly) reconstructed CSAs may not be representative for the whole network of CSAs in the SCN.

Cragg's procedure for preservation of the extracellular space

Five male and five female Sprague-Dawley albino rats were anaesthetized with ether. Their skulls were quickly opened and the brains removed. From each brain, a slice of less than 1 mm thickness was cut through the optic chiasm containing the SCN. The slice was then immersed in 50 ml of a solution of 0.135 M sucrose and 0.002 M calcium chloride in 0.135 M sodium phosphate buffer at pH 7.3 in a magnetically stirred beaker. After about 100 s, 50 ml of a solution of aldehydes (2% glutaraldehyde and 2% paraformaldehyde) was slowly poured into the stirred beaker over a period of 3 min. After a 48-h aldehyde fixation, the slices were cooled to 4°C, washed, postfixed in 1% osmium tetroxide for 24 h, washed, stained in 2% uranyl acetate for 2 h, dehydrated, and embedded in Araldite (see Cragg 1979 for further details and reasoning for this procedure).

Results

Conventional fixation

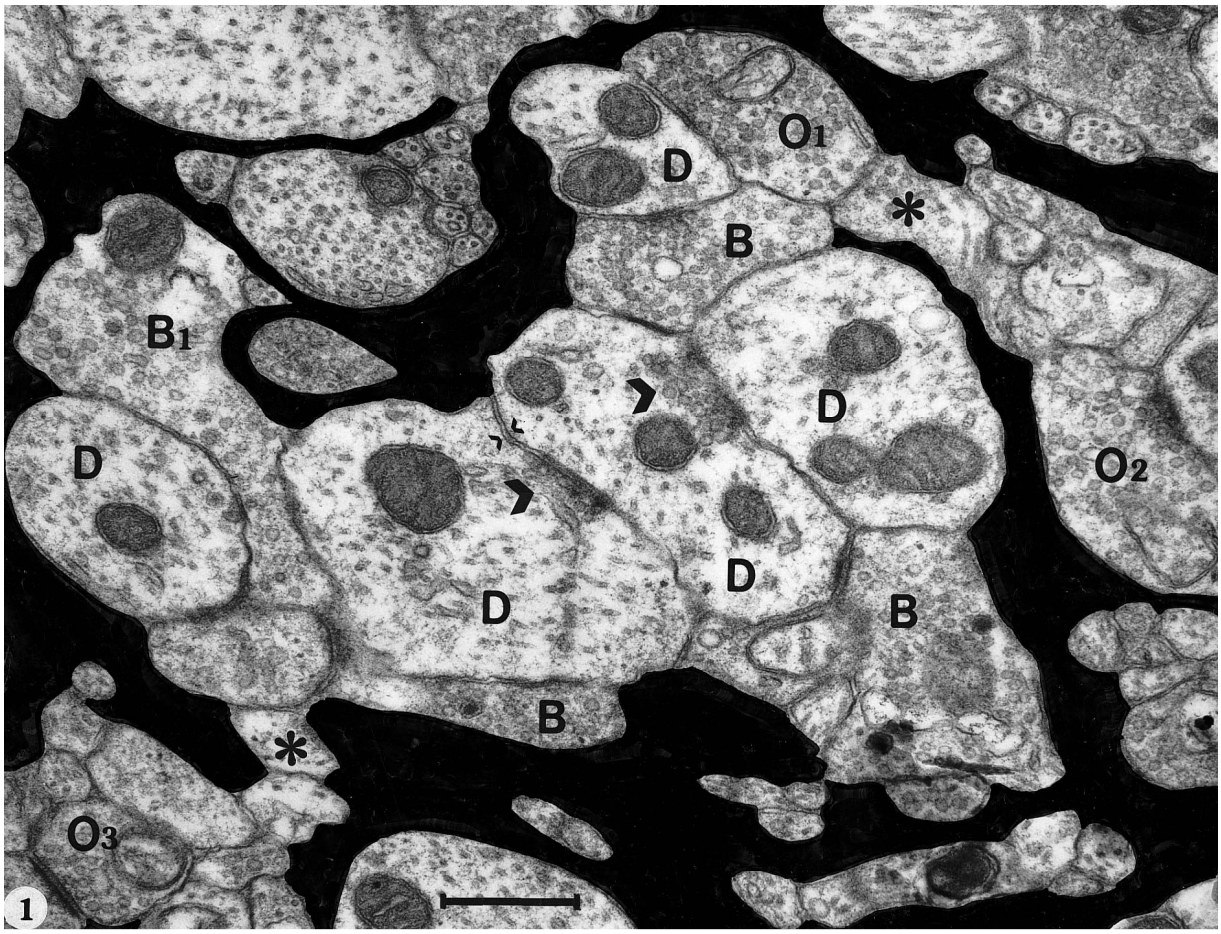
Synapses. The CSAs in the ventro-lateral region of the SCN contain at least five different types of synapses:

1) Optic synapses, i.e. synapses formed by the retinohypothalamic tract (Güldner 1976; Güldner 1978a, b), are the most prominent components. In the conventional and in Cragg's fixation, their presynaptic elements (boutons) are easily recognizable by their striking mitochondria with an electron-lucent matrix and tubular formations of the inner membrane (Figs. 1–3; for boutons 1, 2, 6, 12–14 in CSA I, see Fig. 6; for boutons 1, 2, 4, 8 in CSA II, see Fig. 7). They contain scattered round clear vesicles (mean diameter 45 nm) and various numbers of dense-core vesicles (mean diameter 80 nm). The majority of synaptic appositions are asymmetric (eleven in CSA I and six in CSA II). However, four synaptic contacts were found to be symmetric in CSA I (2-I, 6-K, 12-P, 14-Y; Fig. 6; note that boutons are defined by numbers and dendritic elements are defined by capital letters) and one contact in CSA II (2-C; Fig. 7), respectively. One optic bouton can establish several asymmetric and symmetric synaptic appositions with different dendritic shafts and spines (Güldner 1978 b; Güldner and Wolff 1978). An example is bouton 13 (Fig. 6), which forms five appositions with different dendrites, two of them within CSA I (see also Fig. 3). In CSA I, six optic boutons make fifteen appositions with nine dendritic elements. In CSA II, four optic boutons form seven appositions with six dendrites. Appositions of optic synapses make up 15 out of a total of 22 contacts in CSA I, and 7 out of 13 synaptic contacts in CSA II, respectively (see Table 1).

2) At least one further type of bouton of unknown origin forms up to three asymmetric contacts with dendrites and spines in CSAs (Figs. 4, 5; bouton 5 in CSA I; bouton 7 in CSA II). Boutons 21 and 24 forming "single synapses" outside CSA I are more isolated by astroglial lamellae than are synapses situated within CSA I and other CSAs. The boutons contain clear vesicles of 45-nm diameter, dense-core-vesicles, and dark mitochondria with cristae (see Güldner 1976). In CSA I, one bouton

Fig. 1. A complex synaptic arrangement (CSA) in the ventro-lateral region of the suprachiasmatic nucleus (SCN) with profiles of five boutons (*O* and *B*) and at least five dendrites (*D*). An optic bouton (*O1*) forms a symmetric apposition with the postsynaptic dendrite, which has also a symmetric contact with an unidentified bouton. Note the light mitochondrion in *O1* compared with the dark mitochondria in a non-optic bouton (*B1*) and in the dendrites. Three dendrites form serial dendro-dendritic synapses (*large open arrowhead*). An interdendritic apposition is marked by *small open arrowheads*. The CSA is covered by astroglial processes that are painted *black* in this and the following figures for easier recognition of the special structure of CSAs. Openings of the glial cover (*asterisks*) allow continuity between this CSA and neighbouring CSAs, which are indicated by optic boutons *O2* and *O3*; respectively. The latter form asymmetric contacts. Conventional preparation (compare Figs. 3–5). *Bar*: 0.5 μm

Fig. 2. Cragg's fixation for the preservation of the extracellular space. A CSA with profiles of four optic boutons (only *O1* and *O2* are marked) and five dendrites (*D*). *O1* forms an asymmetric apposition with the postsynaptic dendrite. *O2* contains several dense-core vesicles. The glial cover of the CSA (*black*) opens in two places (*asterisks*) allowing continuity with a neighbouring CSA indicated by *O3*, and access of axons (*lower left*). Note the interdendritic apposition (*small open arrowheads*), which is maintained in spite of the enlarged extracellular space. *Bar*: 0.5 μm



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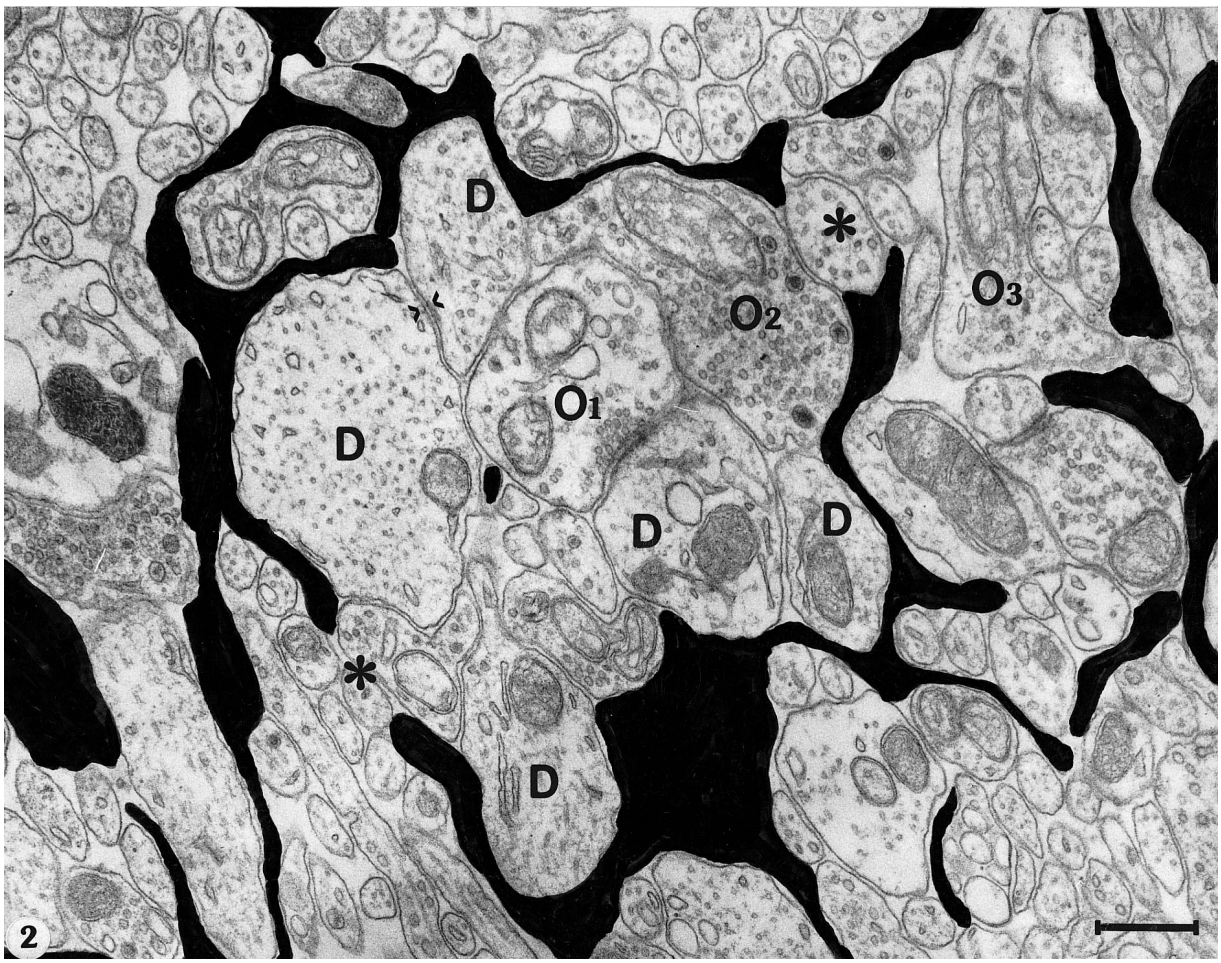

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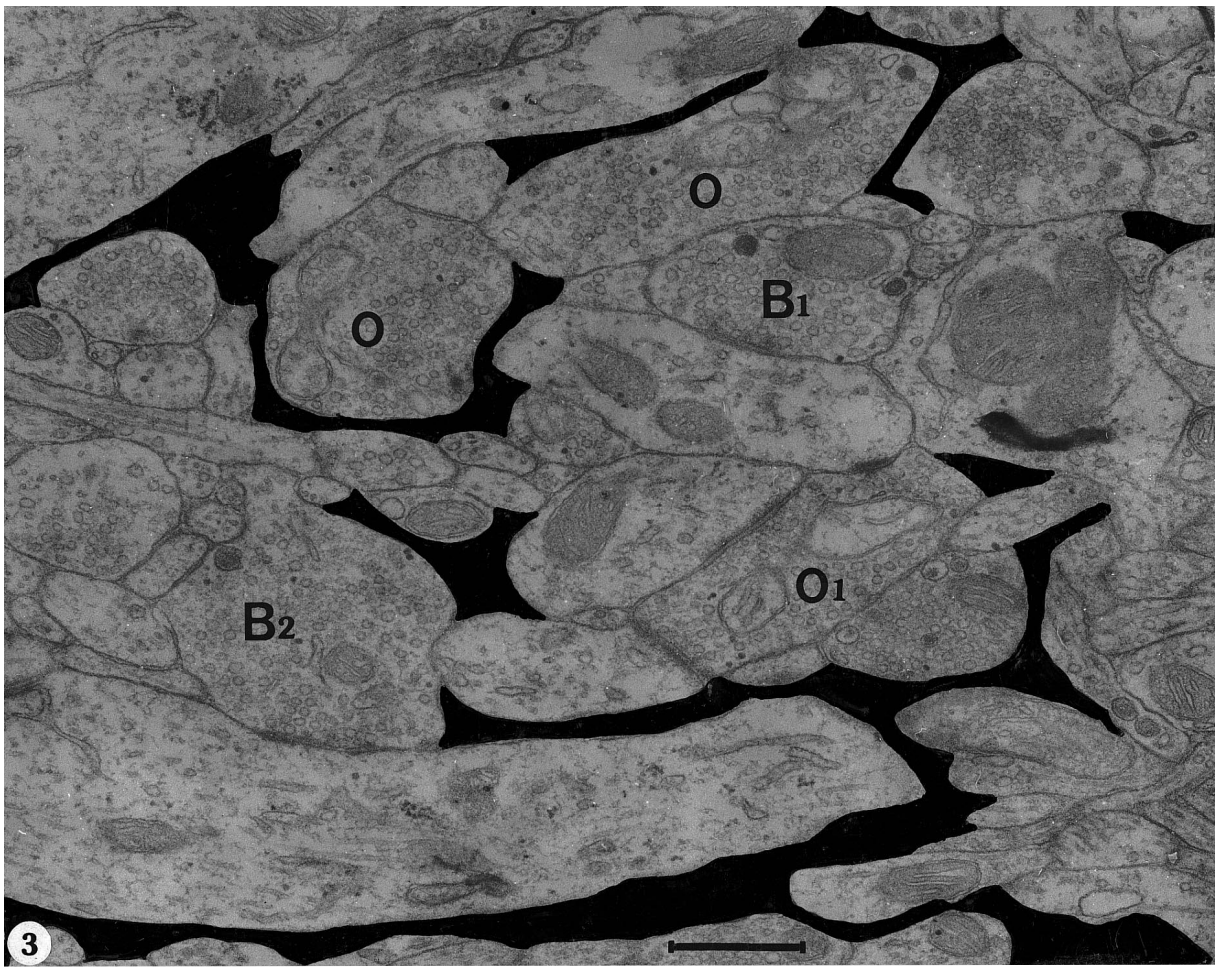
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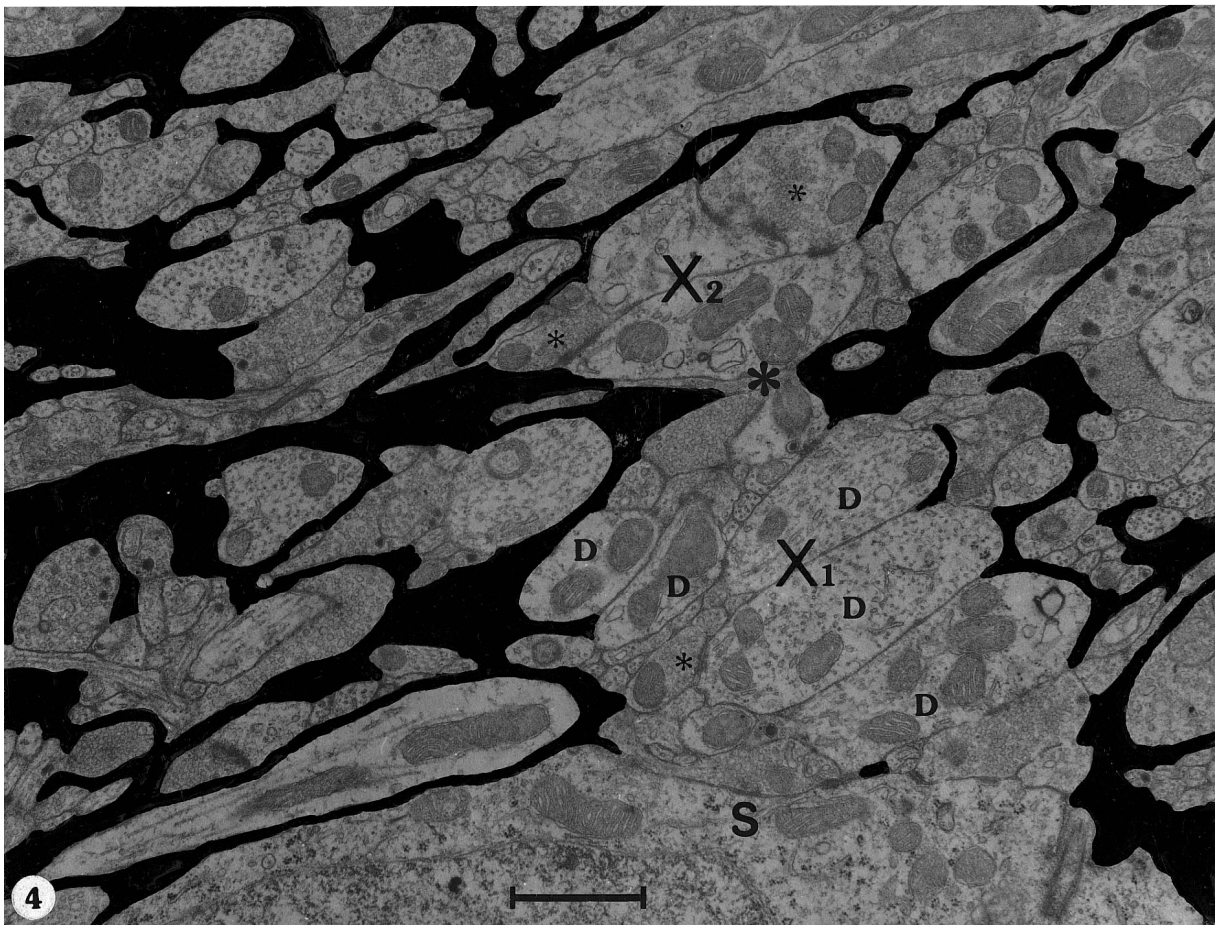
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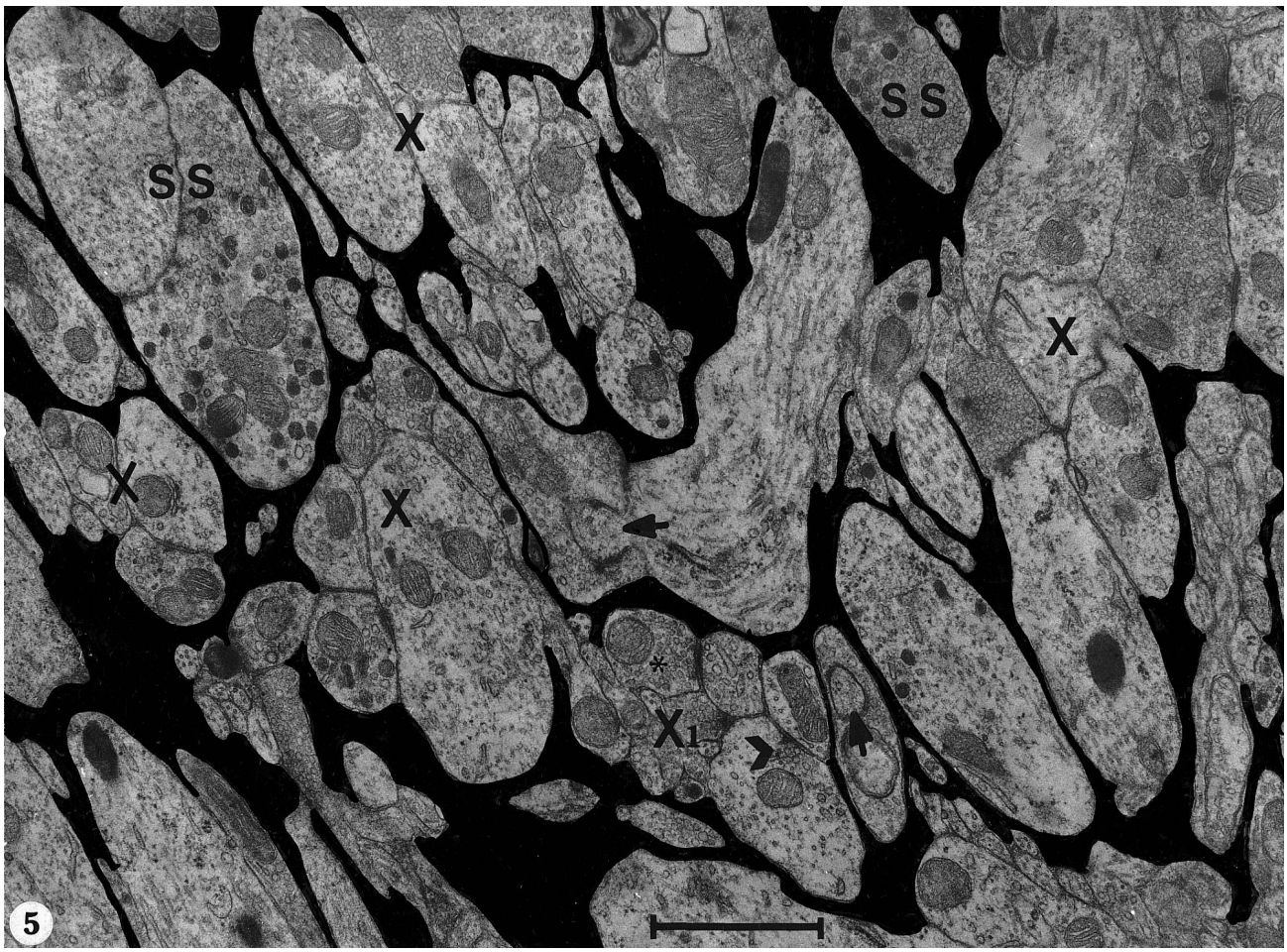


Fig. 5. Several CSAs (*X*) are seen, one of them containing a non-optic asymmetric axo-dendritic synapse (*small asterisk* in bouton) and a symmetric dendro-dendritic synapse (*open arrowhead*). Two "invaginated axo-dendritic synapses" are isolated from the CSAs by astroglial lamellae (single synapse, *arrows* pointing into invaginating dendritic spinule). Another type of single synapse has numerous dense-core vesicles in the bouton (*SS*). Astroglial profiles painted *black*. *Bar*: 1 μm

makes two appositions with two dendrites; in CSA II, one bouton forms one contact. Only 2 out of 22 synaptic appositions in CSA I, and one out of 13 appositions in CSA II belong to this type, respectively.

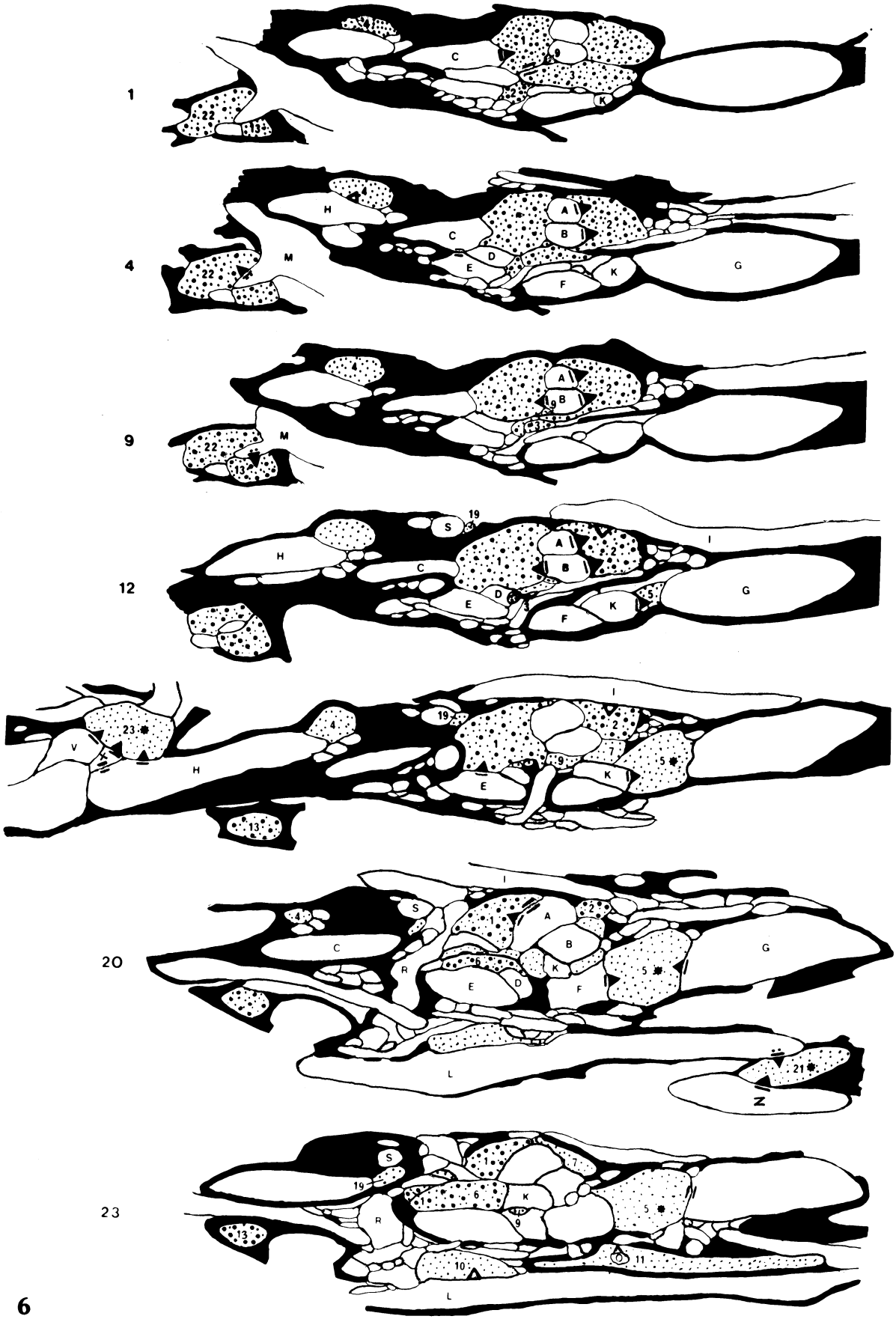
3) At least one type of bouton of unknown origin forms usually one (rarely two) symmetric apposition with dendrites in CSAs (Fig. 3; boutons 7, 9, 10, 18, 19 in CSA I; boutons 5, 6, 9 in CSA II). Other boutons of similar morphological appearance (4, 10, 16 in CSA I) are separated from the CSAs by glial lamellae and represent single synapses. These boutons contain larger clear vesicles (mean diameter 50 nm) than those found in the types of synapses described above, together with dense-core vesicles and dark mitochondria with cristae. Treatment with sucrose-containing buffer after aldehyde fixation causes

Table 1. Numbers (*n*) of synaptic elements in the 3-dimensionally reconstructed parts of two complex synaptic arrangements (CSA I and CSA II) in the rat SCN

	CSA I	CSA II
n Presynaptic elements (boutons)	12	7
n Dendritic elements	12	8
n Synaptic contacts	22	13
n Asymmetric synaptic contacts	12	7
n Symmetric synaptic contacts	10	6

Fig. 3. CSA with several optic (*O*) and non-optic (*B*) boutons. *O1* forms three asymmetric appositions with different dendrites. *B1* and *B2* form symmetric synapses. Note the incomplete covering of the CSA with many openings to neighbouring CSAs. *Bar*: 0.5 μm

Fig. 4. The CSA indicated by *X1* includes a non-optic symmetric axo-somatic synapse (directly above *S*) and a non-optic asymmetric axo-dendritic synapse (*small asterisk in* bouton). Note the bundle of five dendrites (*D*) directly apposed to each other and the opening (*asterisk*) to a neighbouring CSA (*X2*) with two non-optic asymmetric axo-dendritic synapses (*small asterisks in* bouton). Astroglial profiles painted *black*. *S* Neuronal soma. *Bar*: 1 μm



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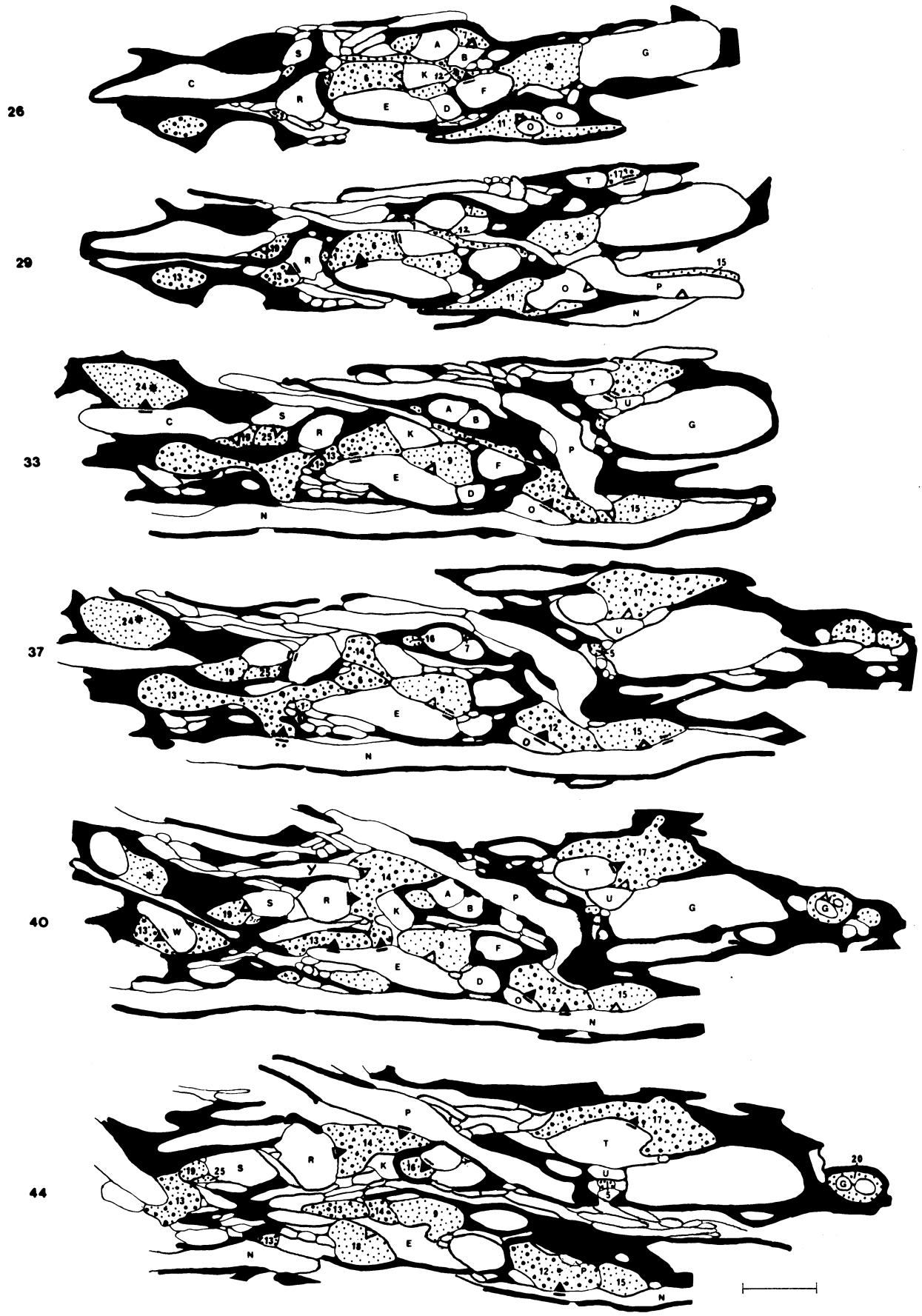

Fig. 6 (Continued)

flattening of variable numbers of clear vesicles in most of these synapses (Güldner 1976). Symmetric axo-somatic synapses can also occur in CSAs (Fig. 4). In CSA I, five such boutons form five appositions with four dendrites. In CSA II, three boutons form four appositions with four dendrites.

4) Dendro-dendritic synapses occur in CSAs (Fig. 1, 5); two of them (O-P and P-N) were found in a CSA next to and continuous with CSA I. These synapses form symmetric appositions, which can be serial (Fig. 1) and reciprocal (Güldner and Wolff 1974; Güldner 1976). Dendro-somatic and somato-dendritic synapses have also been observed. A small number of round clear vesicles (mean diameter 50 nm) accumulates at the synaptic apposition. Sucrose treatment causes flattening in some of these vesicles.

5) A fifth type, called "invaginated axo-dendritic synapses" (Güldner 1976), is sometimes found in CSAs, but is more often isolated from them by glial lamellae (Fig. 5; boutons 11 and 20 in CSA I). The boutons are typically invaginated by one or several dendritic spinules. Most of the boutons contain pleomorphic clear vesicles and dense core vesicles. Some vesicles are flattened even in conventional preparations, whereas sucrose treatment flattens nearly all of them. The mitochondria are usually dark with large cristae or tubules, but sometimes lighter, resembling those in optic synapses. Diagnostic errors can nearly always be avoided because of other distinguishing features (presence of spinule, pleomorphic vesicles). Their synaptic appositions are symmetric in most cases.

The numbers of boutons and dendritic elements forming synaptic appositions in the reconstructed parts of CSA I and II are similar. Slightly more asymmetric appositions than symmetric contacts were found within the CSAs (see Table 1). Boutons may not necessarily establish synaptic appositions with dendrites where both elements come into direct contact with each other (e.g. 1 and 6 with D, 9 and 14 with K in CSA I). Boutons may be true terminals in CSAs or may belong to axons passing through ("en passage").

Attachment plaques. These structures were observed between boutons and dendrites, mostly close to synaptic appositions (1-A, 5-G, 6-E, 6-K, 9-E in CSA I; 15-N and 17-U in neighbouring CSAs). They rarely occurred between two boutons (1–3 in CSA I), but were more often seen between dendrites (C-E, S-R in CSA I and H-X in a neighbouring CSA; F-D in CSA II).

Dendritic bundles and interdendritic appositions. Dendrites run characteristically in small or large bundles through CSAs (Fig. 1, 4; CSA II). Their membranes are apposed for various lengths (1 μm to at least 4 μm) without separation by glial processes. Several dendrites have been observed to spiral around one or more neighbouring dendrites (e.g. C and G around D in CSA II). Thus, dendrite G comes into direct contact with six other dendrites in the reconstructed part of CSA II. Dendrites also cross each other at various angles (e.g. P-O and P-N in CSA I). A given dendrite contacts an average number of 2.6 further dendrites in CSA I, and 3.4 dendrites in CSA II. The average number of boutons forming synaptic appositions with cross-sectioned dendritic stems (the reconstructed length of each stem is about 4 μm) was found to be 2 in CSA I and 1 in CSA I, i.e. 0.5–1 synapse per 2 μm of dendritic length. Dendrites may run away from one bundle to join another, or bundles may be subdivided by glial processes. Dendrites can be seen entering or leaving CSA I (e.g. C and P, probably also A and B) connecting it with neighbouring CSAs.

Individual dendrites receive glial cover as soon as they lose contact with boutons (e.g. F, G, C in CSA I; A in CSA II) or other dendrites. Some dendrites change their diameters dramatically during their course through the reconstructed parts of the CSAs and may become so small that they can easily be mistaken for axons in random sections (e.g. B, C, F, G in CSA II). Other dendrites maintain their size (e.g. A, B, G, F in CSA I; A, D, I in CSA II).

The cleft between closely apposed dendrites is about 2–10 nm wide in conventional preparations (Fig. 1; see also Cragg's fixation, Fig. 2). In some places, it may be filled with an electron-dense material of medium opacity and is then slightly wider than in places where no such material is present. Often, the apposed membranes are strikingly parallel (Fig. 1). Cisternae of smooth-surfaced endoplasmic reticulum are frequently seen under the apposed membranes. Although the outer leaflets of apposed dendritic membranes can sometimes come very close to each other and are then frequently connected by thin strands of electron-dense material, no clear morphological signs of electrotonic coupling between neuronal elements have been found in the material studied so far.

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Fig. 6. Half-schematic drawings from selected serial sections through CSA I. Only the outlines of boutons and some axons (*dotted, numbered*), dendritic elements (*white, capital letters*) and astroglial profiles (*black*) are depicted. In order to avoid cluttering, *numbers* boutons and *letters* dendrites appear only in every *second* depicted section, except at the levels of synaptic contacts, where *numbers* and *letters* are given throughout. Unidentified profiles are left *blank*. Asymmetric synaptic appositions are symbolized by *black triangles* on the presynaptic membrane and a *bar* underneath in the postsynaptic dendrite (△); *two dots* under the *bar* indicate subjunctional bodies. Symmetric synaptic appositions are represented by *open triangles* in the presynaptic element (Ω). Attachment plaques are indicated by a *double bar* (=). Optic boutons are filled with *large* and *small dots*, whereas non-optic boutons contain *small dots*, only. An *asterisk* indicates non-optic boutons forming asymmetric appositions (type 2). CSA I is defined in sections *1* and *4* by boutons *1–3* and dendrites *A – F* and *K*. In section *44*, CSA I is defined by boutons *9,13,14,18,19* and dendrites *D,E,P,R,S,Y* (compare section *40*). Several neighbouring CSAs are also shown to demonstrate their continuity with CSA I via dendritic bridges (e.g. dendrite *P* running from CSA I to a CSA containing boutons *12,15*) or more directly (bouton *5* and dendrite *G* connecting CSA I with a CSA containing bouton *17*). Note the dendro-dendritic synapses *O-P* and *P-N* in section *29*, and single synapses isolated by astroglial processes (see boutons *4,11,20,24*). Boutons *11* and *20* form "invaginated axo-dendritic synapses". Bouton *16* cannot be classified because of the absence of a mitochondrial profile. *Bar*: 1 μm

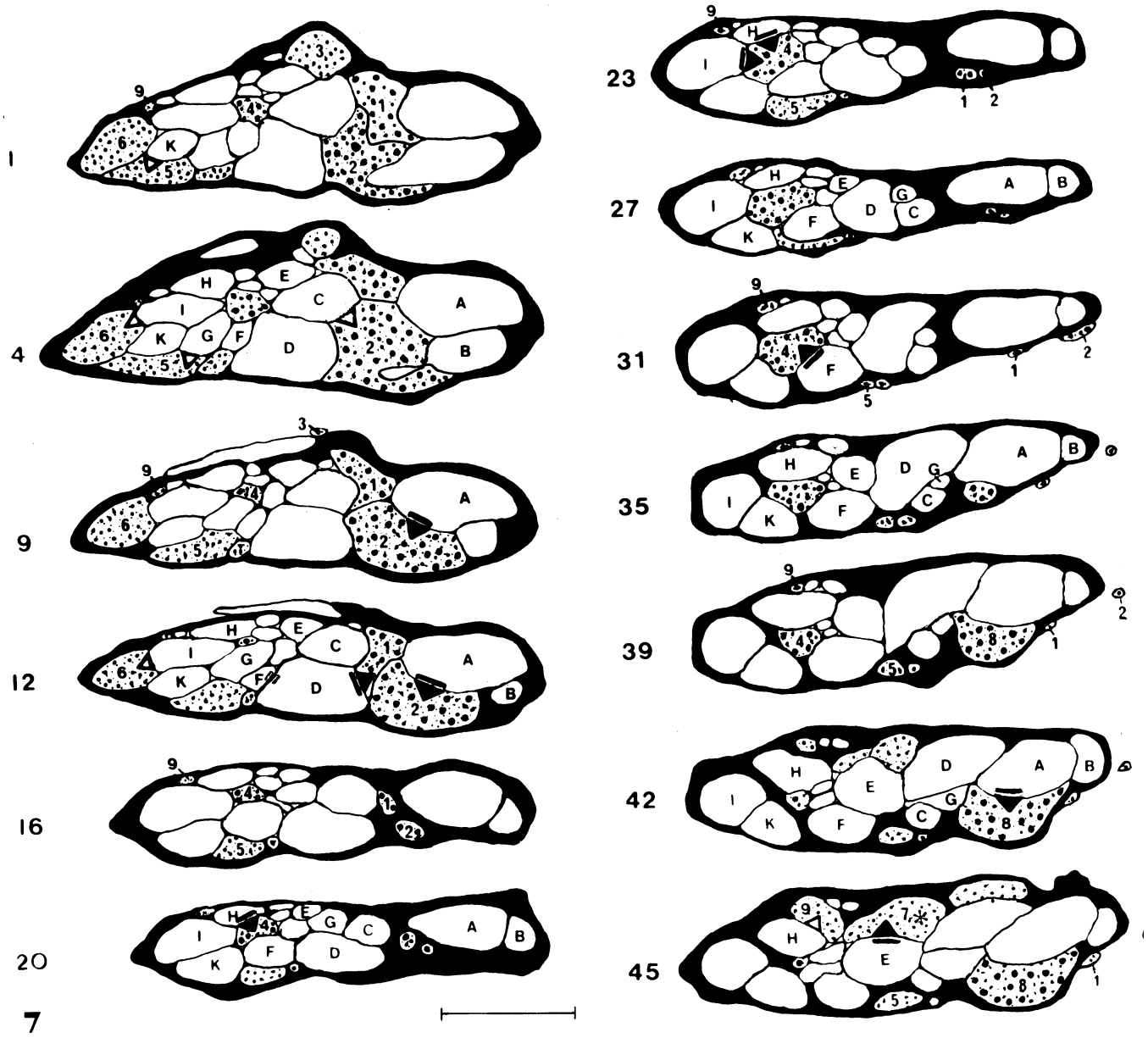

Fig. 7. Half-schematic drawings from serial sections through CSA II. Symbols are the same as those used for CSA I in Fig. 6. In this direction of sectioning, connections between CSA II and neighbouring CSAs cannot be seen. Note dendritic bundles *A,B* and *E,C,D,F,G,K,I,H*; both bundles unite in section *35*. Dendrites *C* and *G* spiral around *D*. Note also the changes in size of several dendrites (e.g. *C* and *F*). *Bar*: 1 μm

Conversely, typical gap junctions are often seen between astroglial processes. Attachment plaques between dendrites have often been observed (see above).

Astroglia. Astroglial processes separate CSAs in the SCN in an incomplete fashion. Although, in some sections, glial processes seem to cover CSAs completely (e.g. CSA II), it can be seen that CSAs, when cut in other directions, continue either directly or via dendritic and axonal "bridges" into neighbouring CSAs (CSA I, Fig. 1, 3, 4; see also Cragg's fixation, Fig. 2). Single synapses are isolated from the surrounding neuropil by astroglial processes, which can sometimes be multilamellar. Such multilamellar covering occurs more often in albino rats compared with hooded strains (F.H. Güldner, unpublished results).

Cragg's fixation

The structural characteristics of the neuronal and glial elements are similar to those observed after conventional fixation. However, the larger volume of the extracellular space in Cragg's preparation (Fig. 2) allows better recognition of firm membrane adhesions in contrast to loose appositions. The coherence of CSAs as special

glia-covered subunits of a whole network can be clearly seen. The specificity of interdendritic and synaptic adhesions is also obvious. However, other encounters between dendrites, between astroglial processes and dendrites, astroglia and boutons, and between boutons and dendrites, often look more irregular, with the opposing membranes not being parallel. Nevertheless, membranes of neighbouring neuronal and glial elements can still be seen to be very close to each other for short or long distances.

Discussion

As they contain about equal numbers of pre- and postsynaptic elements, the CSAs in the suprachiasmatic nucleus belong to the polycentric type (Wolff and Nemeçek 1971). Because of their continuity with neighbouring CSAs, they appear to form a reticulum or sponge-like structure throughout the ventro-lateral portion of the SCN. The gaps of this "sponge" are filled by blood vessels, neuronal and glial cell bodies, large glial processes, axon bundles and single synapses, the latter being covered individually by astroglial lamellae. These characteristics make CSAs in the SCN different from those in other areas of the central nervous system where CSAs have been described as islands within a neuropile formed by single synapses (Szentágothai et al. 1966; Szentágothai 1970; Spaçek and Lieberman 1974). In the SCN, however, single synapses resemble islands between CSAs. Unfortunately, true single synapses can only be recognized with the aid of serial sections, as in random sections an apparently single synapse might belong to the periphery of a CSA. It is therefore difficult to estimate the overall proportion of single synapses. Sometimes, optic synapses seem to be well isolated by astroglia in random sections. However, for the above reasons, it is unclear whether true single optic synapses exist in the SCN, and if so, whether this insulation is a constant or a transient phenomenon. Insulation of certain types of synapses, and even "hyperinsulation" by multilamellar glial wrapping (Güldner and Wolff 1973), appears to be functionally as important as keeping them together in groups, and may result from the special nature of substances released and/or from their overall activity.

Optic boutons predominate in CSAs and are the major source of divergent excitatory influence. However, there is morphological and electrophysiological evidence for direct inhibitory action of about 25%–30% of symmetric optic synapses (Güldner 1978 b; Güldner and Ingham 1979; Sawaki 1979). The ratio of asymmetric (excitatory) and symmetric (inhibitory?) synaptic appositions in the SCN, including optic synapses, is sexually dimorphic, with female rats having a greater relative number of symmetric appositions than male rats (Güldner 1982). After long-term light stimulation, the relative number of symmetric optic synapses is greater than that observed in SCNs from animals kept in constant darkness (Güldner and Ingham 1979).

Except for intrinsic dendro-dendritic synapses, it is still unknown which of the numerous types of non-optic afferents to this nucleus and other intrinsic axons (for literature, see Meijer and Rietveld 1989) participate in CSAs, which types form single synapses, and which synapses receive multilamellar glial cover. At least some of the "invaginated axo-dendritic synapses", which are mostly single symmetric synapses, may be vasopressinergic (Castel et al. 1990). A percentage of boutons with pleomorphic vesicles, some dense-core vesicles and dark mitochondria may belong to the geniculo-hypothalamic tract as they resemble those that display neuropeptide-Y immunoreactivity (Card and Moore 1991). The latter authors have also observed that synapses of the geniculo-hypothalamic tract can be symmetric and asymmetric, with the majority being asymmetric. However, it is possible that the non-optic asymmetric and symmetric synapses in CSAs with dark mitochondria in the boutons are formed by several afferent and intrinsic systems. Thus, the serotoninergic input from the raphe nuclei to the SCN may also participate in the formation of CSAs, as its synapses are found on the same dendrites that are contacted by neuropeptide-Y-containing boutons (for further description and literature, see Van den Pol 1991). This may hold for the synapses of intrinsic and inter-SCN connections, and for the numerous γ-aminobutyric acid-containing boutons that display dense-core vesicles and dark mitochondria, and that mostly form symmetric contacts, although 3% of the cases show asymmetric appositions (Van den Pol 1991). Further characterization of the origin of non-optic synapses in CSAs and of single synapses is therefore needed using tracers or immunocytochemical methods.

Glial processes cover single synapses completely, except for the access of axons and dendrites. They also contact all pre- and postsynaptic elements within CSAs, albeit to a lesser degree. Dendrites are immediately covered by glia if they do not form synapses or non-synaptic contacts with other dendrites.

Dendritic bundling via extensive appositions between dendritic membranes in CSAs is of special interest, as it suggests functional interaction between dendrites of different neurons. It has previously been proposed by Fleischhauer (1974) that dendritic bundles in the cortex facilitate the synchronization of neuronal activity (for a more recent discussion and literature, see Wyss et al. 1995). Recently, Bouskila and Dudek (1993) have found synchronization of neuronal activity independent from synaptic transmission in the rat SCN. The authors discuss three possible conditions that may account for such a mechanism: 1) electrotonic coupling of neurons via gap junctions, 2) electrical field effects via ephapses (Bennet and Auerbach 1969; Faber and Korn 1989), and 3) changes in extracellular ion concentrations. In agreement with Van den Pol (1980), we have found no convincing evidence for typical gap junctions between neurons in our material. Although another type of electrotonic coupling cannot be completely ruled out at present, options 2) and/or 3) appear to be the more likely cause for non-synaptic synchronization in the SCN.

The interdendritic appositions in CSAs may indeed be ephaptic. Van den Pol (1980) has previously discussed the possible existence of ephaptic contacts be-

tween chains of neuronal somata in the dorso-medial portion of the SCN. Many of these neurons are vasopressinergic (Vandesande et al. 1975); extensive intersomatic and interdendritic appositions have recently been observed between such vasopressin-containing neurons (Castel et al. 1990). Intersomatic appositions may therefore account for the synchronization of neuronal activity in the dorso-medial part of the nucleus; however, somata tend to be more separated in the ventro-lateral portion. Nevertheless, the numerous, possibly ephaptic contacts between dendrites in CSAs, in addition to the intersomatic contacts, could connect all neurons concerned to a synchronously firing network.

Most of the five major types of neurons in the suprachiasmatic nucleus described by Van den Pol (1980) have only two sparsely branched dendrites, with one type ("spiny neuron") showing numerous spines and more irregular excrescences. In the same classical study, Van den Pol (1980) measured a mean total dendritic length of 432 μm per neuron, which he considered to be an underestimate. In the 4-μm depth (approx.) of our reconstructions, one dendrite establishes contacts with three other dendrites on average. A dendrite running through the reticulum of CSAs may thus form up to 75 contacts with other dendrites per 100 μm of its length, although a certain percentage of this length would be occupied by single synapses. A given neuron may then have the opportunity to contact about 300 other neurons. The total number of neurons in one SCN has been estimated to be between 8000 and 13 000 by various authors (for literature, see Güldner 1983).The premise of these morphometrical deliberations is that our reconstructed CSAs are representative of all CSAs in the SCN; this, of course, may not be the case. Nevertheless, the above calculations indicate the orders of magnitude involved and may be of interest for future electrophysiological studies.

Interdendritic and intersomatic appositions together with synchronicity of neuronal activity appear to be a stable feature of the SCN, although whether the dendritic contact area changes under different functional situations or in aging animals remains to be examined. Oxytocin and/or vasopressin release in the supraoptic nucleus during parturition, lactation and dehydration has been shown to cause retraction of astroglial processes from the surface of neuronal somata and dendrites (Perlmutter et al. 1984; Montagnese et al. 1988). This results in increased numbers of direct intersomatic and interdendritic appositions (dendritic bundles). The phenomenon is reversed upon cessation of the above stimuli. The question arises as to whether there is an increase or decrease of non-synaptic synchronicity in the supraoptic nucleus under the above conditions. An electrophysiological and morphological examination of the Brattleboro rat supraoptic nuclei and SCN would also be worthwhile in this respect.

The 3-dimensional reconstructions of CSA I and II were done in a male rat in order to avoid a possible influence of the oestrous cycle on the morphology of CSAs in females. Comparisons of a great number of CSAs from random sections did not show any differences in their basic structure between males and females. Nevertheless, it needs to be morphometrically determined whether there are any changes in astroglial covering of neuronal elements during the various phases of the oestrous cycle.

Interdendritic appositions in CSAs are clearly maintained in Cragg's preparation (Cragg 1979), which claims to preserve the true volume of the extracellular space (up to 20% of the total brain volume, see Van Harreveld 1972). This method shows that the interdendritic appositions are specific and are not just caused by dendrites being closely pressed together. It also demonstrates that interdendritic contacts have a highly regular appearance in some places but are more irregular in others. This could mean that only a part of the contact area between two dendrites is specific, and possibly ephaptic.

CSAs are generally thought to be subcellular integrating units that produce relatively stable responses after processing information from various sources (Wolff and Nemeçek 1971). In the CSAs of the SCN, the rhythmic activity of retinal afferents over the 24 h period, modified by the activity of non-retinal inputs, influences the circadian rhythm of neuronal activity ("pacemaker", see Turek 1985) in this nucleus. This influence is the "Zeitgeber" (=time-giver, Aschoff 1954), which can change the rhythm of the pacemaker according to the relative length and timing of light and darkness, and the activity of the non-retinal inputs. The "Zeitgeber" proper may therefore ultimately lie in the CSAs of the SCN.

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