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Dendro-dendritic synapses in the suprachiasmatic nucleus of the rat hypothalamus

F.-H. GÜLDNER and J. R. WOLFF

Max-Planck-Institut für biophysikalische Chemie, Abt. Neurobiologie – Neuroanatomie, D-34 Gottingen, G.F.R.

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Summary

Dendro-dendritic synapses have been found in the suprachiasmatic nucleus of the rat. These synapses are similar to 'symmetrical' or Gray-type II synapses. The existence of reciprocal dendro-dendritic synapses was also established. The presynaptic dendrites receive additional synaptic contacts from at least two types of axons.

Introduction

Dendro-dendritic synapses have up to now been found in a number of regions of the vertebrate central nervous system (for references see Lieberman, 1973). In a study of the suprachiasmatic nucleus (SCN) (Güldner and Wolff, 1973) we occasionally observed aggregations of clear vesicles in neuronal processes which otherwise resemble dendrites. There dendrites were found to be presynaptic elements at dendro-dendritic synapses. Since such synapses have not been previously shown to exist in hypothalamic nuclei, preliminary results on their fine structure and arrangement in the SCN are presented.

Material and methods

Male and female Sprague-Dawley rats were perfused with a mixture of 3% glutaraldehyde and 3% paraformaldehyde in 0.05 M sodiumcacodylate buffer. One part of the dissected tissue blocks containing the suprachiasmatic area was washed in 0.1 M buffer and postfixed in 1% OsO_4 solution (conventional treatment). The other part was rinsed for 1 h in the same buffer containing 12% sucrose ($\approx$ 600 mosm) or 24% sucrose ($\approx$ 1000 mosm) and postfixed in 1% OsO_4 solution with likewise enhanced osmolarity (sucrose treatment, see Bodian, 1970; Valdivia, 1970; Lieberman, 1973). The tissue was dehydrated in ethanol and embedded in Epon. Frontal and sagittal thin sections were cut through the SCN and post-stained with an aqueous solution of uranyl acetate (1%) and lead citrate.

Results and discussion

The number of dendro-dendritic synapses which could be unequivocally recognized in a single frontal or sagittal section through the whole SCN is very small; no more than three could be counted, if at all (frontal area about $10^5 \mu m^2$, sagittal area about $1–2 \times 10^5 \mu m^2$). Vesicle aggregations within dendritic profiles, without any membrane specializations, are more often observed (up to 17) in the same area. These could represent sections through presynaptic dendrites outside the active zone.

The *presynaptic dendritic element* (Figs. 1–5) is characterized by a relatively small and isolated aggregation of clear vesicles close to the presynaptic membrane in the mode of *en passant* synapses. The vesicles are spherical (average diameter 500 Å) after conventional treatment (see Methods), but occasionally flattened and discoid after hyperosmotic sucrose treatment (Figs. 2–4). The presynaptic membrane is studded with a few isolated dense projections (Fig. 5). Numerous orderly arranged microtubules, profiles of smooth endoplasmic reticulum, poly(ribo)somes, mitochondria, dense-core-vesicles (diameter 800 to 1400 Å) and multivesiculated bodies occur close to the synaptic vesicle clusters. The first three structures mentioned above are characteristic of dendrites.

The *synaptic cleft* is 130–160 Å wide and is filled with a material of medium electron density, which seems to prevent its collapse, even when the surrounding intercellular clefts are narrow or occluded.

The *postsynaptic dendritic element* shows similar cytological features to the presynaptic one. There is no prominent cytoplasmic density on the postsynaptic membrane, although this can appear more osmiophilic than the usual cell membrane. Significant postsynaptic densities characteristic of asymmetrical (Gray-type I) synapses, have never been observed. Thus the dendro-dendritic synapses of the SCN are similar to the so-called symmetrical or Gray-type II synapses. An inhibitory function of these synapses in the SCN cannot be established by morphological data alone, as the identification of Gray-type I synapses as excitatory and Gray-type II as inhibitory has not yet been proved to be a general phenomenon.

Occasionally, clear vesicles are observed in postsynaptic dendrites, but up to now a reciprocal dendro-dendritic synapse in which both dendrites are presynaptic to each other has been seen only once (Figs. 2–4). Here again no indications for an asymmetrical synaptic complex were found. The same fact was emphasized by Famiglietti (1970) for the reciprocal dendro-dendritic synapses in the lateral geniculate nucleus, by contrast with findings in the external layer of the olfactory bulb (Rall *et al.*, 1966). A synaptic reciprocity between dendrites could well be a general phenomenon in the SCN. But the probability that thin sections will pass through adjacent active zones is low, and in addition the active zones may

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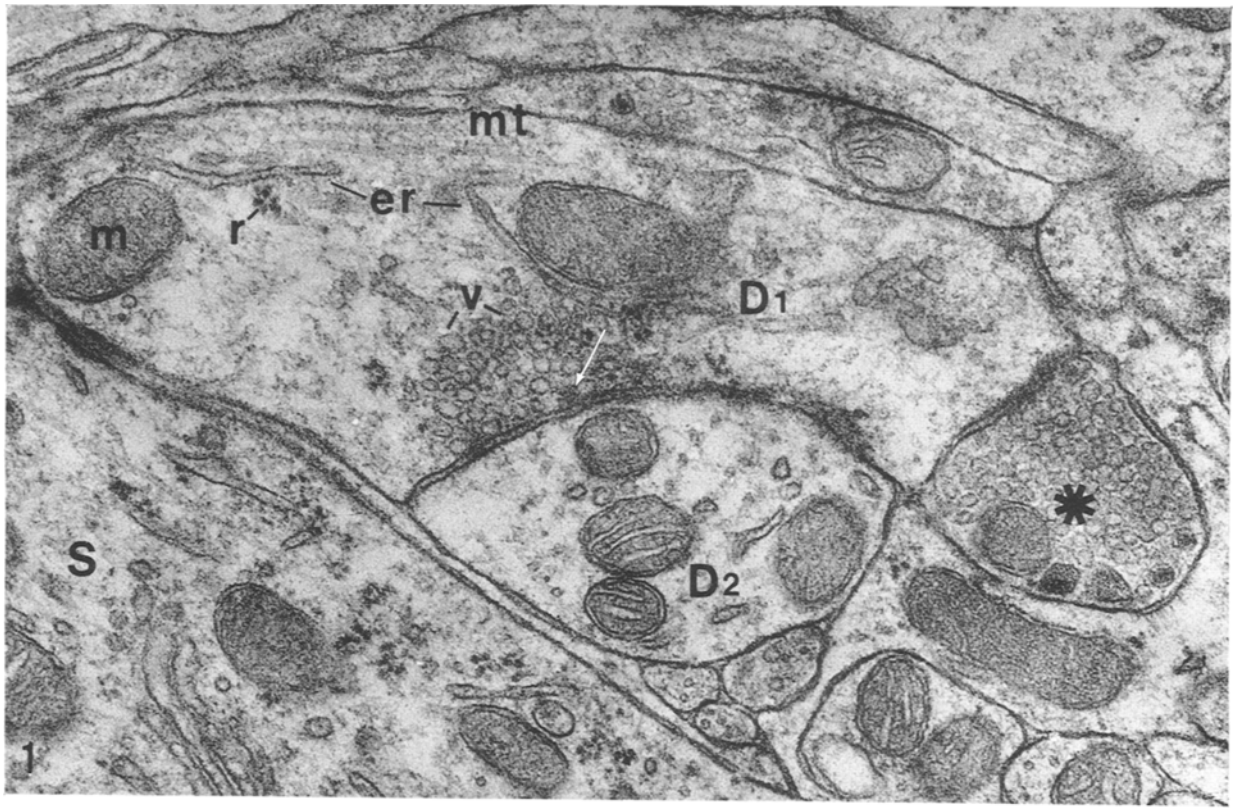
Fig. 1. A dendro-dendritic synapse adjacent to a neuronal soma (*S*) in the SCN (conventional treatment, see Methods). Note ribosomal clusters (*r*), smooth endoplasmic reticulum (*er*) and microtubules (*mt*). The presynaptic dendrite (D1) is postsynaptic to an axonal presynaptic element (asterisk) D_2 = postsynaptic dendrite, *m* = mitochondrion, *v* = clear vesicles, arrow = dense projection. × 54 000.

Figs. 2–4. Serial sections through a dendro-dendritic reciprocal synapse (thick arrows) (sucrose treatment). Some of the clear vesicles are discoid (*d*). × Thin arrow = dense projection. × 46000.


mt
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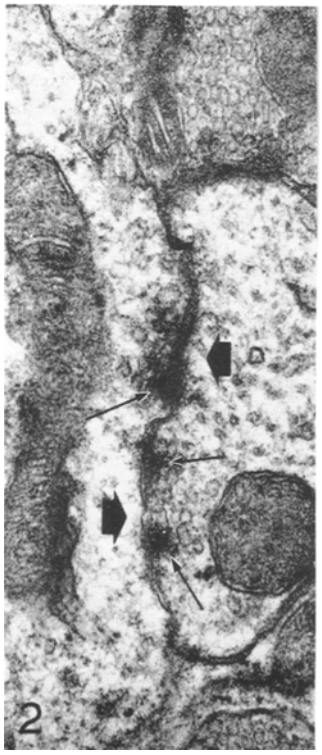
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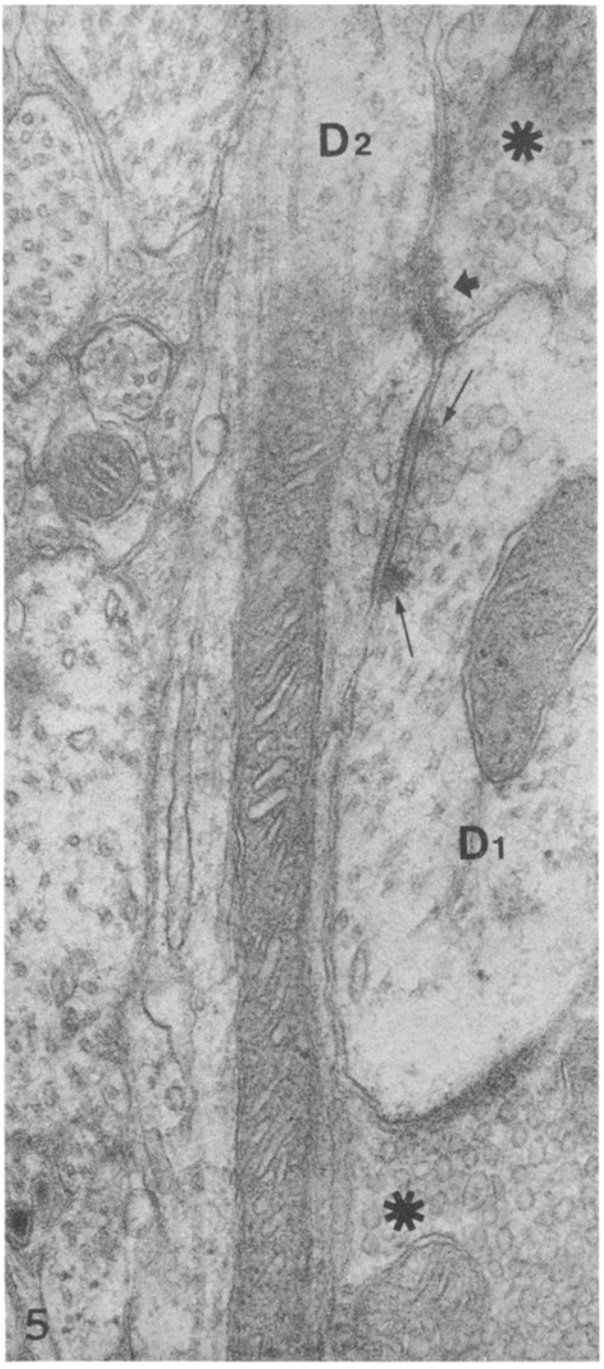
not often lie as close together as found in this one case. This may explain the rarity of this finding. Nevertheless, a mainly unidirectional arrangement of dendro-dendritic sequences (see Reese and Shepherd, 1972) cannot yet be excluded.

The dendrites forming dendro-dendritic synapses are postsynaptic at several types of axo-dendritic synapses. One type is represented by symmetrical synapses (Figs. 1, 5), with few dense-core-vesicles and numerous pleomorphic, i.e. circular, oval and flattened, vesicle profiles in the presynaptic element after sucrose treatment. Another type (Fig. 6) makes symmetrical synapses with spinule-like excrescences of the dendrites. Here the presynaptic element shows only few scattered vesicles of various diameters, which are sometimes flattened after conventional treatment, but are often flattened after sucrose treatment. Dense-core-vesicles also occur. A possible third type forms asymmetrical synapses with dense-core-vesicles and numerous clear vesicles, which remain spherical after sucrose treatment.

The dendro-dendritic synapses are often found within the complex synaptic arrangements of the SCN. (Güldner and Wolff, in preparation), but they are also observed as single synapses in the neuropil.

Dendro-dendritic synapses are formed by amacrine neurons as well as by neurons with short and long axons (see Lieberman, 1973; Reese and Shepherd, 1972). Therefore, the occurrence of such synapses does not correlate with a distinct type of neuron. According to morphological studies of Krieg (1932) and Suburo and Pellegrino de Iraldi (1968) only one class of neuron, probably axon bearing, seems to exist in the SCN. Clattenburg *et al.* (1972) discuss 'two functionally different types of neurons or different functional states of the same kind of cell'. The efferent pathways from the SCN and the target sites are unknown. Up to now only two afferent systems have been described innervating the SCN: one represents fibres from the optic nerve which form asymmetric synapses (Hendrickson *et al.* 1972, Moore and Lenn 1972), the other is a serotoninergic input (Fuxe, 1965; Baumgarten and Lachenmayer, 1972). According to several authors (see Clattenburg *et al.*, 1972; Moore and Eichler, 1972), the suprachiasmatic neurons control the cyclic release of pituitary luteinizing hormone and are involved in the production of rhythmic events which control the hypothalamo-pituitary regulation of adrenal function. However, too little is known about the neuronal composition, synaptology and function of the SCN to suggest at present any functional significance of the dendrodendritic synapses within this nucleus.

Fig. 5. Dendro-dendritic synapse with isolated dense projections (thin arrow) on the presynaptic membrane (conventional treatment). Both dendrites (*D*1, *D*2) involved in this dendro-dendritic synapse are also postsynaptic elements at axodendritic symmetrical synapses (asterisks). Note the desmosome-like structure (intermediate junction ?) near one of these synapses (thick arrow). × 58000.
Fig. 6. Symmetrical synapse between a presynaptic element (large asterisk) with flattened (cylindrical ?) vesicles of various diameter and spinule-like excrescences of a dendrite (*x*). The dendrite contains an aggregation of clear vesicles (*v*) near an indistinct process (conventional treatment). Another presynaptic element (small asterisk) forms a symmetrical synapse (of the type shown in Figs. 1 and 5) with the dendrite. *dcv* = dense core vesicle. × 39000.



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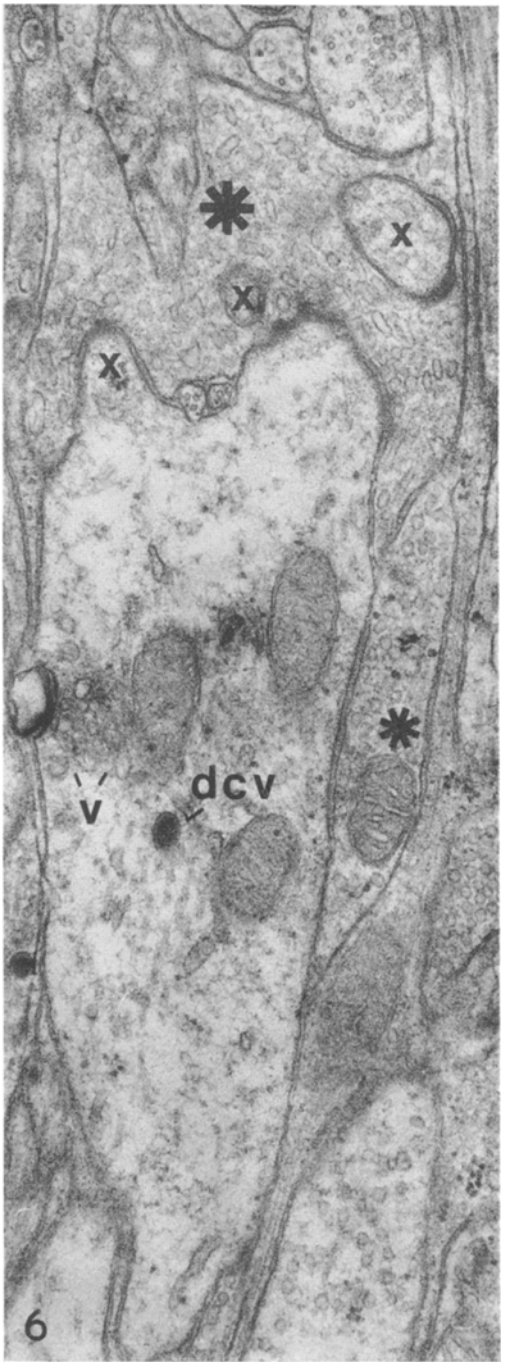

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