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Retinal afferents on Golgi-identified vertical neurons in the superior colliculus of the rabbit. A Golgi-EM, degenerative and autoradiographic study

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Summary. The characteristics and distribution pattern of retinal afferent terminals making synaptic contacts on narrow field vertical neurons in the stratum griseum superficiale of the rabbit superior colliculus were studied using the Golgi-gold substitution technique in combination with either autoradiographic or degenerative methods. At the level of light microscopy, identified gold-toned vertical neurons showed similar features to those previously described by others. Although their axons were frequently seen rising from the basal dendritic tuft, they could also emerge from an apical dendrite, but rarely from the cell body. The electron microscopic study revealed that these neurons received radiolabelled or degenerated profiles with typical features of retinal terminals, while more proximal parts seemed to receive fewer terminals but of larger size. Axo-somatic synaptic junctions were very rare and, interestingly, retinal terminals were not seen forming synapses on the basal dendritic arborization. The present results indicate that the narrow field vertical cells are targets for retinotectal projection.

Key words: Retinal terminals, Superior colliculus, Golgi-EM autoradiography, Golgi-EM degeneration, Rabbit

Introduction

The superior colliculus (SC) of the mammalian brain is a relay centre for the visual system in which numerous afferents coming from different brain areas converge into its seven well-defined layers. The three superficial layers, named from the pial surface to the depth as stratum zonale (SZ), stratum griseum superficiale (SGS) and stratum opticum (SO) are known to receive mostly visual afferents from the retina and the visual cortex in many species (Sterling, 1971, 1973; Lund, 1972; Mathers, 1977a; Vrensen and De Groot, 1977; Pollack and Hickey, 1979; Behan, 1981, 1982, 1984; Holländer and Schönitzer, 1983; Mize, 1983; Schönitzer and Holländer, 1984; Sefton and Dreher, 1985; Hofbauer and Holländer, 1986; Crabtree, 1989; Gayer et al., 1989; Harvey and Worthington, 1990).

Not only are afferent systems well defined in the SC; indeed, the use of neuroanatomical techniques has allowed a description of many different neuronal cell types classified according to their morphology, location and axonal distribution (Ramón y Cajal, 1911; Valverde, 1973; Langer and Lund, 1974; Tokunaga and Otani, 1976; Labriola and Laemle, 1977; Mathers, 1977b). Among these, the typical, and numerous narrow field vertical cells located in the SGS are of special relevance since they are considered as extracollicular projecting neurons (Valverde, 1973; Mathers, 1977b) with axons sending collateral branches to the deep collicular layers (Mooney et al., 1988).

In the rabbit, as in other mammals, a massive contingent of retinal afferents penetrate into the superior colliculus and take an ascending trajectory to reach their targets, mainly in the uppermost part of the SGS (Mathers, 1977a; Vrensen and De Groot, 1977; Holländer and Schönitzer, 1983; Schönitzer and Holländer, 1984; Hofbauer and Holländer, 1986).

Due to the special relation in the SGS between retinal axons and narrow field vertical cells, it has been speculated that retinal terminals might synapse on the dendritic tree of narrow field vertical cells (Langer and Lund, 1974; Mathers, 1977a). However, morphological evidence supporting this assumption has not been established yet.

In this work, we have attempted to study, at the level of electron microscopy, the presence of synaptic contacts between retinal terminals and narrow field vertical cells in the SGS of the rabbit superior colliculus. For this purpose, we have used the Golgi-gold substitution technique (Fairén et al., 1977) on silver-stained neurons in combination with either degenerative or

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autoradiographic methods.

Materials and methods

Thirty-day-old male albino rabbits (New Zealand 300-390 g, n=37) were used for this study. After sedation with ketamine hydrochloride (40 mgs/kg, i.m., Ketalar, Parke Davis) they were deeply anaesthetized with 20% aqueous urethane (0.75 g/kg body weight, i.p.). Thirteen animals received an intravitreous injection into the right eye of 400 µCi of ^{3}H-L leucine (Sigma Chemical Co.) dissolved in saline (0.15 ml) while twenty-two were subjected to a right retinal removal. The survival time for animals injected with ^{3}H-L leucine was 2 days and up to 9 days for those with unilateral retinal removal. Besides, two control animals were used for conventional electron microscopy.

All animals were perfused through the heart, under deep 20% urethane anaesthesia, firstly with a brief rinse of phosphate-buffered saline at 37 °C and subsequently with 1l of a mixture containing 1% formaldehyde (freshly generated from paraformaldehyde; Robertson et al., 1963) and 1.25% glutaraldehyde, in 0.1M sodium cacodylate buffer (pH 7.4).

Contralateral superior colliculi were removed and processed for the Golgi staining method (Valverde, 1973). After silver impregnation, tissue blocks were gradually dehydrated in glycerol, encased in paraffin and coronally sectioned at 125 µm on a microtome and collected in pure glycerol.

To study single-stained neurons at the electron microscopic level, the Golgi-gold substitution technique was adopted (Fairén et al., 1977; Somogoyi et al., 1979). Briefly, sections were rehydrated and transferred to a solution of 0.04% gold chloride from between 40 and 50 minutes followed by 1% sodium thiosulphate for 90 minutes. Gold-toned vertical neurons were postfixed in 2% osmium tetroxide prepared in sodium cacodylate buffer (0.1M, pH 7.4), dehydrated in graded alcohol, embedded in Durcupan and, after polymerization, photographed and drawn under a camera lucida.

Ultrathin sections (gold interference colour) were cut from gold-toned vertical cells and picked up onto Formvar-coated single slot copper grids. Those processed for autoradiography, were covered with a single layer of carbon and coated with Ilford L-4 nuclear emulsion diluted 1/1 in distilled water. After three months of exposure in the dark at 4 °C, autoradiographs were developed using Microdol X (Kodak) and fixed in 25% sodium thiosulphate.

All ultrathin sections were contrasted with 2% aqueous uranyl acetate and lead citrate and examined in a Jeol 100sx electron microscope.

Results

Light microscopic observations

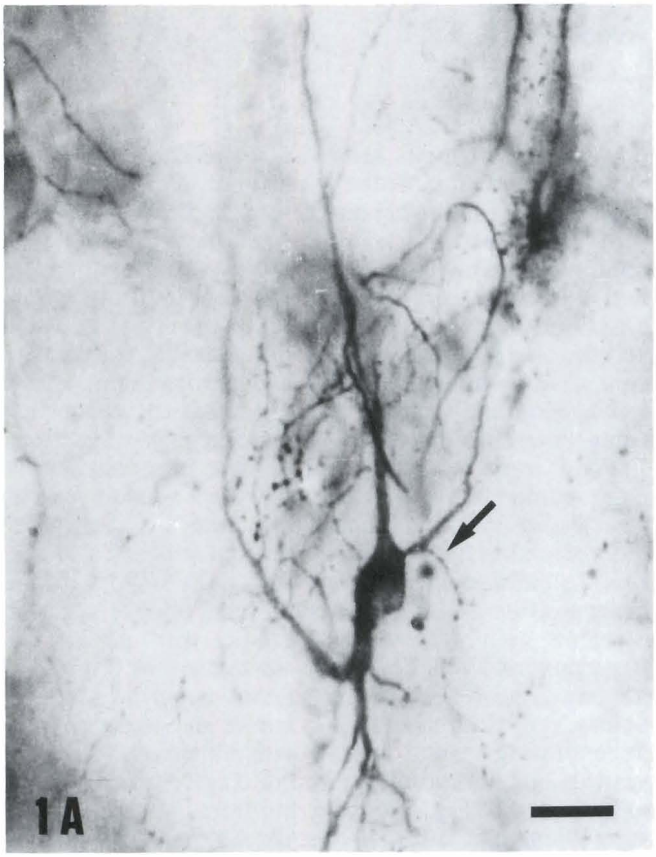
Gold-toned neurons of different morphology were seen throughout the SC. In the SGS, the most common labelled cells were vertical-type neurons located at different depths of the layer (Figs. 1A, 2A). They exhibited fusiform cell bodies with a major axis oriented perpendicularly to the pial surface. Two thick dendritic trunks arising from the apical and basal poles of the perikarya took respectively an ascending and descending trajectory outlining together with their branches a narrow cylindrical field. The calibre of the apical dendrite remained almost constant and, after a certain distance, it gave off a dendritic tuft with few smooth branches of uniform diameter which extended into the SZ. Spines were only present on the most distal dendrites. The basal dendrite was usually thicker and shorter than the apical one which often divided in a basal dendritic tuft of thinner and uniform branches restricted to the SGS, but they were also frequently observed entering into the SO. Occasionally, a basal dendrite turned upwards running to the surface (Fig. 1A). No spines were observed on basal dendrites.

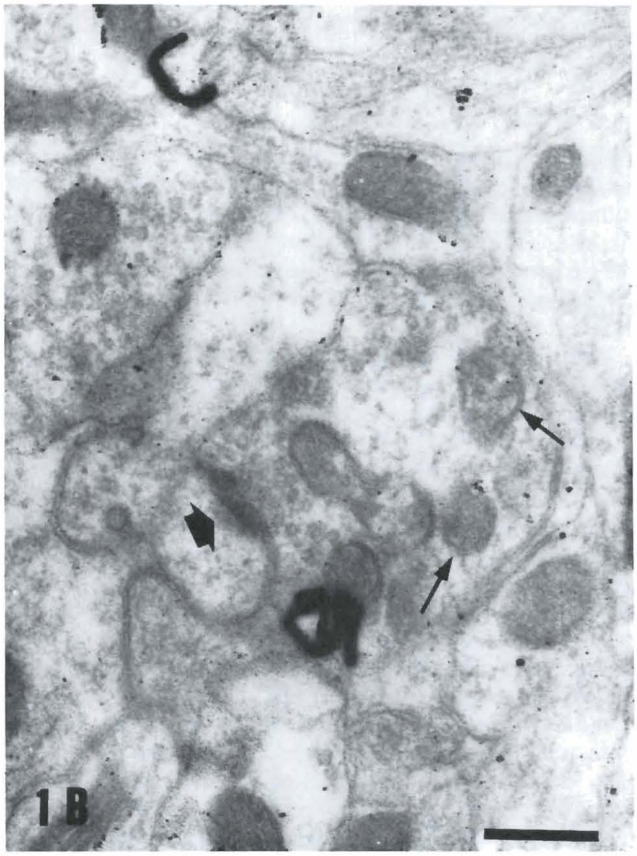
These vertical neurons had thin varicose axons which showed a noticeable variability in origin. They often arose from the basal dendritic arborization, but the axon could also emerge from an apical dendrite and, very rarely, from the cell body (Figs. 1A, 2A). Although these axons could ramify profusely giving off numerous varicose collaterals distributed within the superficial layers, they were more often seen leaving the SGS with only a few collaterals remaining in it.

Electron microscopic observations in control autoradiography and in autoradiography combined with the Golgi-gold substitution technique

Ganglion cell axons of the rabbit retina cross the midline in the optic chiasm to reach the contralateral superior colliculus. Thus, injections of ^{3}H-L leucine into the right eye labelled numerous retinal terminals distributed in the superficial layers of the left SC, but which were more abundantly accumulated in the uppermost part of the SGS. They were rather large

Fig. 1. Autoradiographic retinal terminals in the rat superior colliculus. **A.** Golgi-gold toned vertical neuron in SGS showing the origin of a thin varicose axons (arrow) from an ascending dendrite. Scale bar: 10 µm. **B.** Labelled-retinal terminal in contralateral SGS following an injection of ^{3}H-L leucine into the right eye. It contains clear round vesicles densely accumulated at the presynaptic site of an asymmetrical synaptic contact (arrow) made on a dendritic profile. The terminal exhibits typical mitochondria characterized by a low electron density and a wide space between the inner and outer membranes (thin arrows). Scale bar: 0.5 µm. **C - E.** The identified vertical neuron showed in A, seems to receive labelled retinal terminals at distal (C, arrowheads) and at proximal parts of the ascending dendritic tree (D, arrow) as well as at the cell body (E, empty arrows). Note in E, an autoradiographic grain over the postsynaptic profile (lower empty arrow) and gold precipitate restricted to the cytoplasm. The nucleus is indicated by asterisks. Scale bars in C, D: 1 µm, and in E: 0.5 µm.


1A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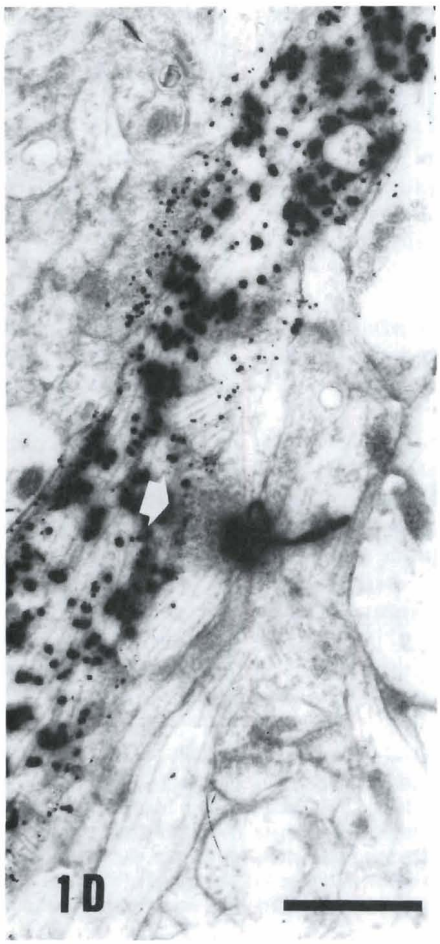
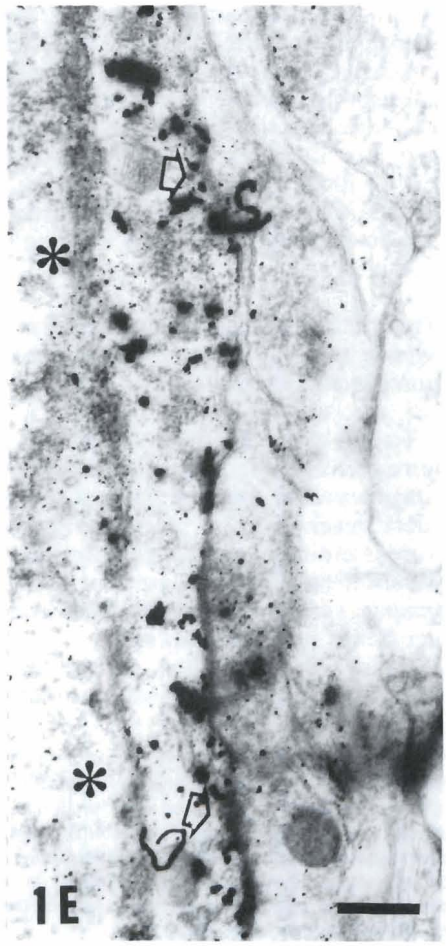
1C


1D


1E

scalloped profiles characterized by numerous round clear vesicles densely packed at presynaptic sites of asymmetrical synaptic junctions often established on dendrites (Fig. 1B). Furthermore, these retinal terminals contained several electron-translucent mitochondria with a wide space between inner and outer membranes (Fig. 1B).

When autoradiography was combined with the Golgi-gold substitution technique ultrathin sections including portions of an identified gold-toned vertical neuron (Fig. 1A) showed a high density of small gold particles accumulated in the cytoplasm of proximal and distal dendritic profiles (Figs. 1C, 1D). However, gold deposit in cell bodies was exclusively restricted to the cytoplasm leaving the nucleus free. The apical dendritic tree and the neuronal somata were the only elements of the Golgi-identified narrow field vertical cells seen that received profiles with autoradiographic grains over their surface. Furthermore, radiolabelled boutons showed distinctive characteristics when compared to typical retinal terminals. Thus, the most distal dendritic branches were contacted by numerous profiles of small size which had many clear round synaptic vesicles and no mitochondria (Fig. 1C). Similar radiolabelled terminals of larger size were observed making asymmetric synapses on more proximal dendrites of thicker diameter (Fig. 1D). Although the apical dendrites of these vertical cells received the majority of those supposed retinal terminals, very rare axo-somatic contacts were also seen between radiolabelled boutons and identified cell bodies (Fig. 1E). Occasionally, radioactive material was detected over the cytoplasm of a perikaryon just at the postsynaptic site of the synapse, suggesting a trans-synaptic transport (Fig. 1E). Interestingly, we could not find labelled retinal elements forming synapses on basal dendrites.

Electron microscopic observations following retinal degeneration and in combination with the Golgi-gold substitution technique

To allow a correct identification of degenerative elements following a retinal removal, a period from 7 to 9 days was estimated the optimal survival time in which a dark degeneration characterized by an increase of the axoplasmic density had already taken place but with preservation, in most cases, of synaptic vesicles and synaptic specializations. At these times, signs of glial engulfment were not detected.

Under these conditions, on the SC contralateral to a complete retinal removal, numerous degenerating profiles with features reminiscent of retinal terminals were distinguished in the superficial layers, but with higher density in the uppermost part of the SGS. Indeed, they exhibited an irregular contour and contained relatively clear mitochondria with a prominent space between inner and outer membranes and numerous electron-translucent spherical vesicles mostly accumulated at presynaptic sites. Sometimes, few dense-core vesicles appeared intermingled with them. These retinal terminals showed several asymmetrical synaptic contacts with neighbouring dendritic elements (Fig. 2B).

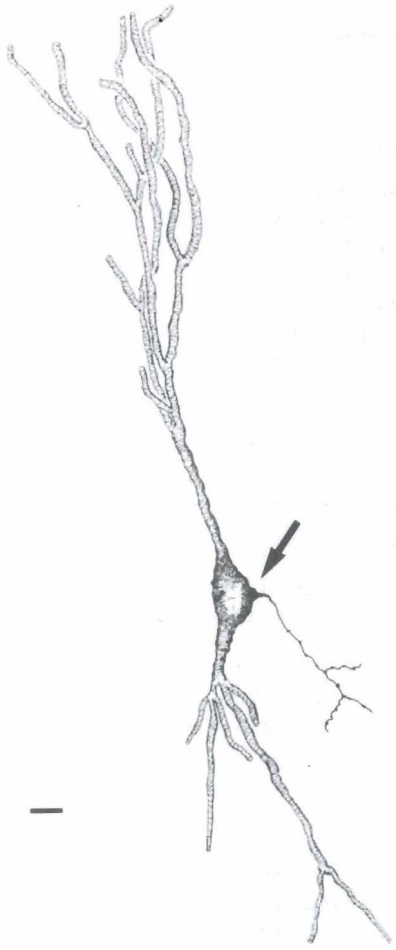
As in cases following ^{3}H-L leucine injections, small degenerating retinal terminals with clear round synaptic vesicles, no mitochondria and asymmetric synapses were more abundantly distributed on distal processes of the apical dendritic tuft of identified gold-toned vertical neurons (Fig. 2C). To a lesser extent, relatively larger elements with similar characteristics were present on more proximal dendrites of the same neurons (Fig. 2D). Amongst the few retinal terminals synapsing on cell bodies, it was remarkable to observe the presence of a degenerating retinal profile with numerous synaptic vesicles and several mitochondria closely related to the axon hillock (Fig. 2E). Furthermore, similar to the autoradiographic material, degenerating retinal terminals were not seen synapsing on the basal dendritic arborization.

Discussion

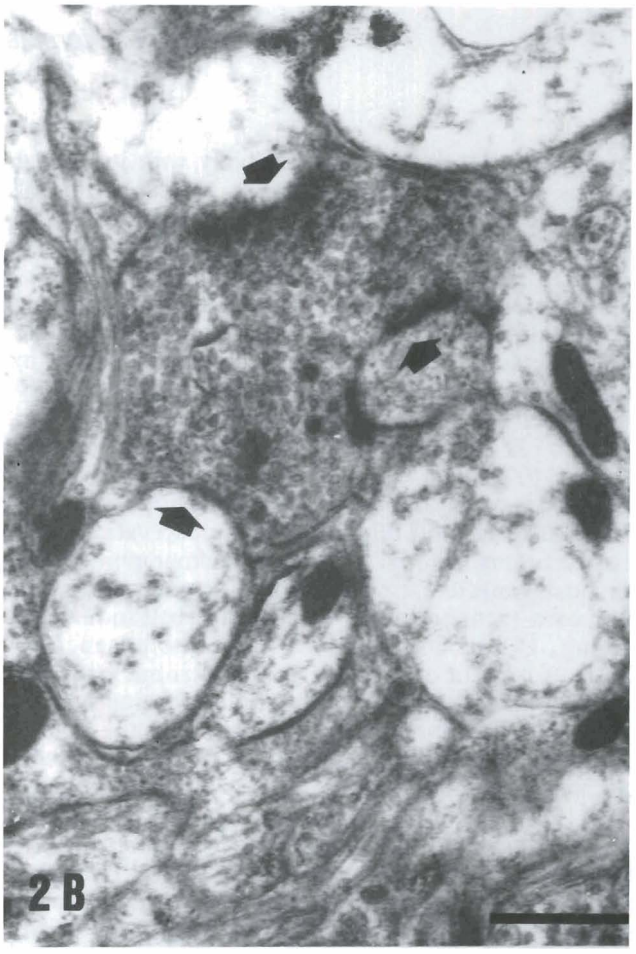
In the present work, we have analyzed the characteristics and distribution pattern of retinal afferent terminals on identified vertical neurons in the SGS of the rabbit superior colliculus. The Golgi-gold substitution technique (Fairén et al., 1977; Somogyi et al., 1979) in combination with either degeneration or autoradiography proved to be a suitable method since it allowed the simultaneous study of the morphology of target neurons identified at the light microscopical level, and the ultrastructural characteristics as well as distribution of degenerated or radiolabelled afferent terminals onto those neurons previously characterized.

Thus, in the SGS we could observe gold-toned vertical fusiform neurons with their typical morphology already described using Golgi methods (Ramón y Cajal, 1911; Langer and Lund, 1974; Tokunaga and Otani, 1976; Labriola and Laemle, 1977; Mathers, 1977a). However, in addition to the well-known origin of the axon from the descending dendritic tree (Caldwell and Mize, 1981; Mooney et al., 1985), some vertical neurons had the axon rising from the ascending dendritic

Fig. 2. Degenerated retinal terminals in the rabbit superior colliculus. **A.** Camera lucida drawing of a narrow field vertical neuron of the SGS with two polarized dendritic trunks placed at different planes of focus. The axon is raised from the cell body (arrow). The drawing allows a view of the entire neuronal morphology. Scale bar: 10 μm. **B.** Degenerating retinal terminal in the SGS with numeorus synaptic vesicles mostly accumulated at several asymmetrical synaptic junctions established on dendrites (arrows). Scale bar: 0.5 μm. **C - E.** The identified gold toned neuron showed in A is contacted by degenerating retinal terminals at distal (C, arrow, asterisk indicates the postsynaptic element) and at proximal (D, arrow) portions of the ascending dendritic arborization. Scale bars: 0.25 μm. E. Observe an ongoing retinal terminal degeneration (arrow) synapsing on the cell body close to the axon hillock (thick arrow). Scale bar: 0.5 μm.



2A



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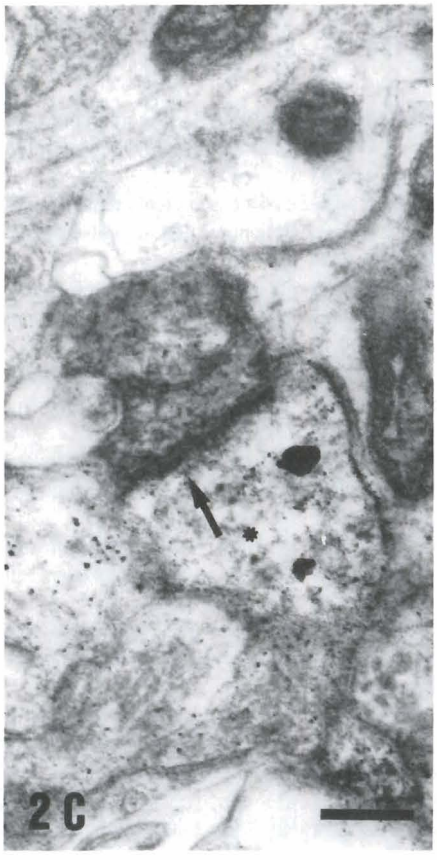
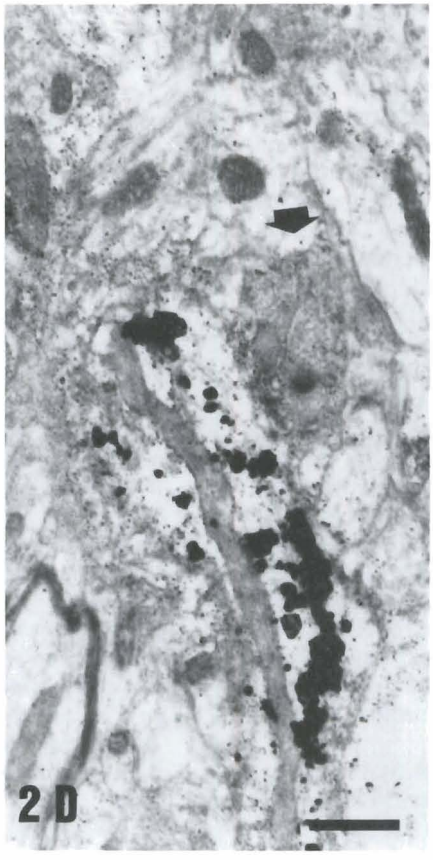
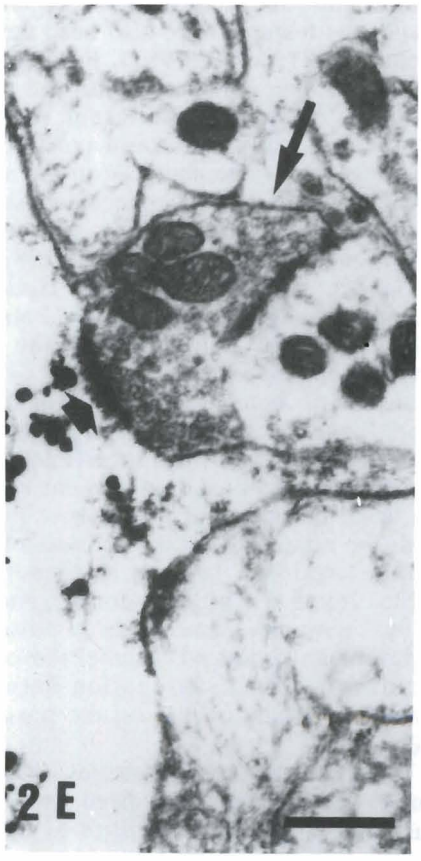


2C



2D



2E

arborization or even from the cell body with its axonal branches remaining within the layer, similar to that of the vertical short axon neurons (Valverde, 1973). These observations were in contrast with previous studies (Mathers, 1977b) showing extracollicular axons exclusively emerging from vertical neurons. We presume that these differences are mostly due to the unpredictable staining obtained with the Golgi techniques and not to the age of our animals, since these neurons have already reached their mature properties by postnatal day 9 (Spear et al., 1972) and their adult morphology by day 15 (Mathers, 1977b).

The use of autoradiographic methods had the adventage of a good ultrastructural preservation. The presence in our material of few autoradiographic grains over postsynaptic elements, would strongly speak in favor of a transneuronal transport (Holländer and Halbing, 1980). On the other hand, the absence of labelling in glia and in nuclear neuronal profiles confirmed the selective character of this technique.

Radiolabelled retinal terminals displayed the typical morphology already described in the rabbit and several other species (Lund, 1969, 1972; Sterling, 1971; Tigges et al., 1973; Valverde, 1973; Vrensen and De Groot, 1977; Graham and Casagrande, 1980; Behan, 1981; Mize, 1983; Schönitzer and Holländer, 1984; Hofbauer and Holländer, 1986). Interestingly, we could observe that retinal terminals located at upper parts of the apical dendritic tree of identified vertical neurons lacked mitochondria and were smaller compared to deeper ones. Analogous features have also been described in the cat (Behan, 1981; Mize, 1983). Furthermore, this depth-dependent size correlated with the density of retinal terminals. Although quantification was not carried out in the present study, we could corroborate that retinal terminals were more abundant in the uppermost zone of the SGS, with gradually reduced density in depper parts of the layer (Mathers, 1977a; Vrensen and De Groot, 1977; Holländer and Schönitzer, 1983; Schönitzer and Holländer, 1984; Hofbauer and Holländer, 1986). Of special relevance was that we could not observe labelled retinal profiles synapsing on the basal dendritic arborization, even though dendrites of some cells, or at least part of them, were located in the retinorecipient zone. Since the visual cortical projection is though to terminate throughout the superficial layers but mainly deeper than the retinal terminals one might speculate that the basal dendrites of the narrow field vertical cells could be contacted by this projection, but not by the retinal terminals. In fact, Hofbauer and Holländer (1986) found a very rare convergence of retinal and cortical terminals onto the same postsynaptic element. Experiments conduced to label the cortical input will decide whether a segregation between retinal and cortical afferents occurs on the postsynaptic vertical cells.

In the degenerative material, the optimal survival time to detect changes was from 7 to 9 days. This period was longer than those described by others (Lund, 1972; Sterling, 1973; Mathers, 1977a; Pollack and Hickey, 1979; Behan, 1982) and was represented by an increase in the axoplasmic electrondensity but with good preservation of the presynaptic elements. At shorter survival time, however, we could only notice a vesicular accumulation in the retinal terminals, which very likely reflected an early morphological stage of degeneration. On the other hand, periods over 9 days were too long to allow the identification of retinal profiles since at this stage only signs of a late degeneration were observed. The performance of retinal removals matched the results of the autoradiographic material with regard to morphology, distribution, size and density of retinal terminals reinforcing, thus, the data obtained with that technique.

In conclusion, the Golgi-gold substitution method, combined with either autoradiographic or degenerative techniques, has allowed us to describe the ultrastructural features and the distribution of retinal axon terminals on identified vertical neurons in the SGS of the rabbit superior colliculus. Further studies are required to elucidate whether other neuronal cell types located in the retinorecipient layers are also targets for the retinal ganglion cells.

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