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The axon initial segment of corticocollicular neurons in the rabbit visual cortex: an electron-microscope study with HRP

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Summary. The synaptic connections of the axon initial segment (IS) of retrogradely labeled corticocollicular neurons in the rabbit visual cortex were studied using the HRP-EM method. Identified IS showed relatively few synaptic boutons unevenly distributed along their surface. All these axo-axonic terminals contained pleomorphic vesicles and formed symmetrical synaptic junctions with the IS. The possible origin and chemical nature of these synaptic boutons are discussed.

Key words: Visual cortex - Corticocollicular neurons - Axon initial segment - HRP - EM study - Rabbit

Introduction

It is well established that the primary visual cortex in mammals sends projections to subcortical structures (for review see Swadlow, 1983) amongst them to the superior colliculus (SC). As in other species (Swadlow, 1983; Jones, 1984), in the rabbit, visuocortical neurons projecting to the SC are located in layer V though some are also in layer IV (Swadlow and Weyand, 1981). All of them have a pyramidal shape (Jones, 1984). Available information from pyramidal neurons includes ultrastructure (for review see Feldman, 1984), and the type and distribution of synaptic contacts which receive the cell body of the cat corticocollicular neurons (Kennedy, 1982). However, little is known about synapses of the axon initial segment (IS) of identified corticocollicular neurons. The purpose of the present work was to study the number, distribution and type of synaptic contacts on the IS of neurons in primary visual cortex which were retrogradely Golgi-like labeled following injections of horseradish peroxidase (HRP) in the rabbit SC. Part of the results reported here have been published in abstract form (Matute et al., 1983).

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Materials and methods

Twelve pigmented rabbits (1.5-2 Kg body weight) anesthetized with ketamine (40 mg/Kg body weight, s.c.) followed by urethane (0.75 g/Kg body weight, i.p.) were used for injections. After removal of the left occipital cortex and the underlying hippocampus 1-2 µl of an 70% w/v HRP (type VI, Sigma) aqueous solution were injected with a 5 µl Hamilton syringe in 3-4 different sites of the right SC under visual control. Two days later the rabbits were deeply reanesthetized and perfused first with 500 ml of saline and then with 1.5 liters of a solution containing 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The brains were dissected out of the skull, and stored overnight in the same fixative at 4° C. Histochemical processing of 100 µm thick Vibratome slices (Vibratome, Oxford Instruments) from a block of tissue containing the visual cortex was carried out according to the cobalt chloride method described by Adams (1977). Sections were analyzed under light microscopy and Golgi-like labeled neurons drawn by camera lucida. Selected sections were osmicated, stained with uranyl acetate, dehydrated and flat embedded in an Epon-Araldilt mixture. Silver-gold serial thin sections were cut, collected in one-single slot gride coated with formvar, stained with lead citrate, analyzed and photographed with a Philips 201 electron microscope. Drawings of the outlines and synapses of the IS were obtained from projected negatives, and then the IS was manually reconstructed (Fairén and Valverde, 1980). The results reported below were obtained from one corticocollicular neuron whose IS could be completely followed. Three other neurons were studied, however, the full length of their IS's could not be observed.

Results

Following injections of HRP in the rabbit SC a large number of retrogradely labeled cells were found in the primary visual cortex. Most of them were located in layer V and some in layer IV, a result which is consistent with previous reports (Swadlow and Weyand, 1981; Pinilla et al., 1984). Some of these neurons were Golgi-like labeled and, therefore, was possible to observe both the dendritic tree and the descending part of their axon. Figure 1 shows a camera lucida drawing of the corticocollicular neuron whose IS was fully analyzed. Since the HRP reaction product is electron-dense, identification of labeled cells was relatively easy as shown in figure 2.

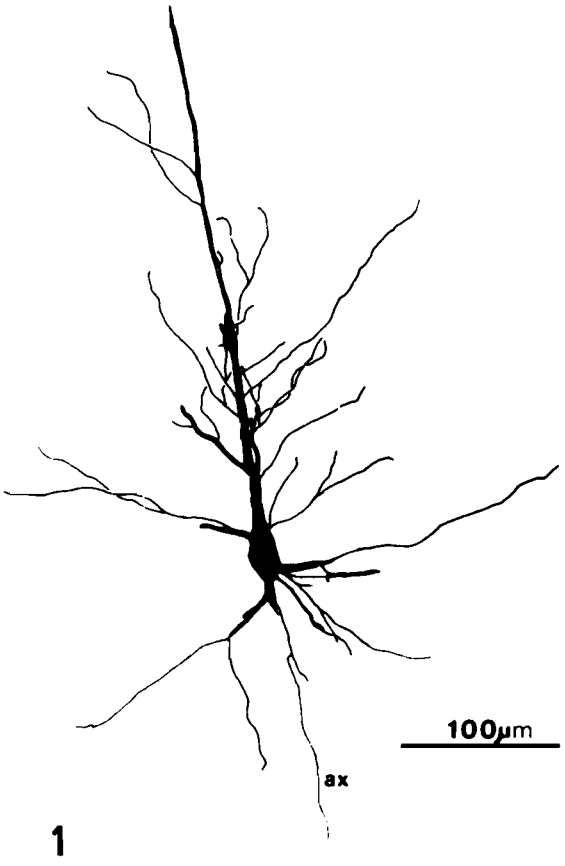

Fig. 1. Camera lucida drawing of a Golgi-like labeled neuron in the rabbit visual cortex following injection of HRP in the superior colliculus. Note the pyramidal shape of this cell together with a descending portion of the axon (ax)

The criteria to define the IS included both the presence of the undercoating of the axonlemma and the microtubule bundles (Westrum, 1970; Palay et al., 1976; Sloper and Powell, 1979). The lenght of the IS of the corticocollicular neuron was approximately 40 µm (Fig. 4) which is similar to other studied IS's from pyramidal cells in the cortex (Westrum, 1970; Sloper and Powell, 1979). Ten synapses unevenly distributed along the IS

Fig. 2. Photomontage from a level of the pyramidal neuron (pyr) drawn in figure 1. Note numerous electron-dense elements (arrows) corresponding to the HRP-reaction product. Bar: 2µm.

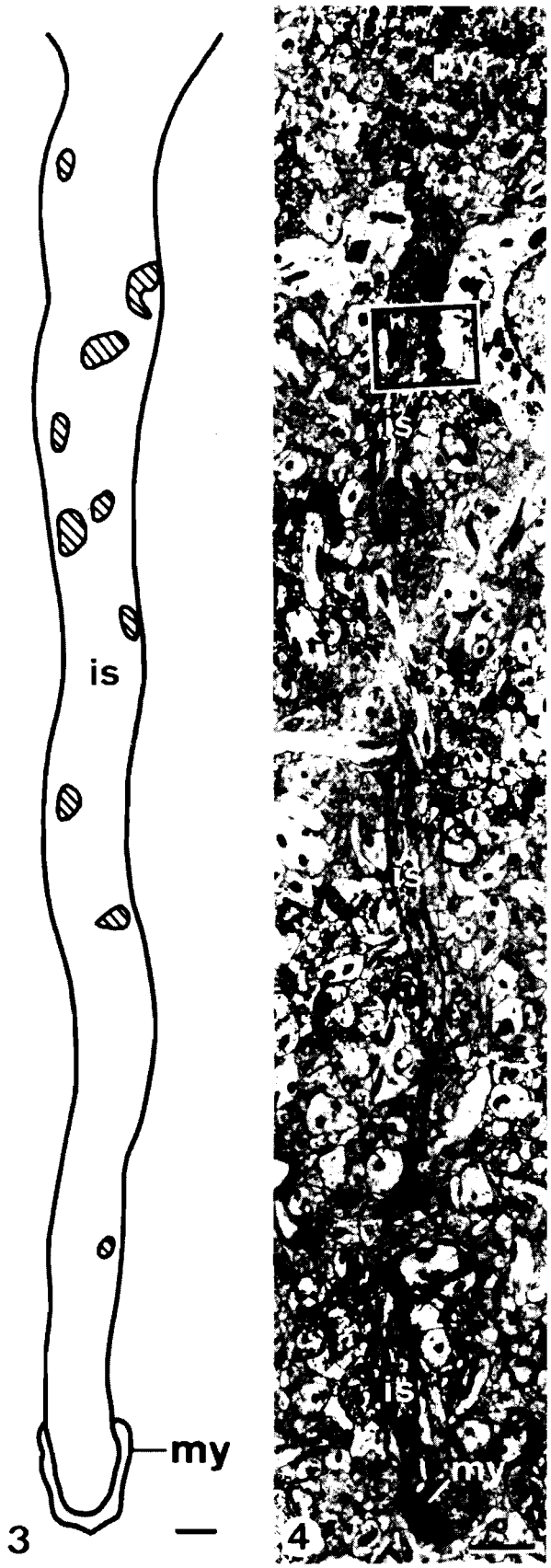

Fig. 3. Schematic two-dimensional representation of the axon initial segment (is) indicating the localization of the observed synaptic boutons. my = myelin sheat. Bar: 1 μm

Fig. 4. Photomontage showing the axon initial segement (is) from the axonal hillock to the myelin sheath (my). Note that the histochemical reaction makes the whole initial segment (is) slighly darker than the rest of the surrounding neuropil. Frame indicates an area further enlarged in figure 5. Bar: 2 μm.

were observed (Fig. 3), a fact confirming previous observations showing that the IS of pyramidal neurons in the deep layers of the cerebral cortex receive fewer synaptic contacts than pyramids in layers II-III and IV (Sloper and Powell, 1979; de Felipe et al., 1985). The highest density of synapses was found between 10 to 20 μm distance from the cell body. The axo-axonic terminals seen along the IS were elongated with the principal axis parallel to the principal one of the IS (Figs. 5-7). All terminals contained pleomorphic vesicles and the synaptic contacts were symmetrical (type II synapse in Gray's classification, 1959; Figs. 5-7). Although the observations in the other three neurons studied only refered to a part of their IS's, they seem to confirm the results mentioned above in terms of number, distribution and morphology of the synapses.

Fig. 5. Photomicrograph from a terminal bouton (T) showing a symmetrical synapse (arrowhead) in the proximal part of the axon initial segment (is). Bar: 0.5 μm

Fig. 6 and 7. Photomicrographs from two different terminal boutons (T) showing symmetrical synapses (arrowheads) on the axon initial segment (is) at 15-20 μm distance from the cell body. Bar: 0.5 μm

Discussion

The origin of the synaptic boutons on the IS of the pyramidal cells in the cerebral cortex has been widely studied, and there is a general agreement on the fact that most of these terminals are from chandelier neurons (Somogyi, 1977; Fairén and Valverde, 1980; Peters et al., 1982; Somogyi et al., 1982; de Felipe et al., 1985). Chandelier cell bodies are mostly located in layer II-III (for review see Fairén et al., 1984; Peters, 1984) though some of them, as well as their typical axonal plexuses, have also been found in the deeper layers of the cerebral cortex (Tömbol, 1978; Fairén and Valverde, 1980; Somogyi et al., 1982; Frenud et al., 1983). This fact might explain the small amount of synaptic contacts on the IS of corticocollicular neurons observed in our material as compared to pyramidal cells in more superficial layers (Sloper and Powell, 1979; Fairén and Valverde, 1980; de Felipe et al., 1985). However, in the present study no evidence showing «specific terminal portions» (stp, see Fairén and Valverde, 1980) characteristic from chandelier cells on the IS was found. Therefore, it might be that the axonal terminals observed on the IS of the corticocollicular neurons belong to non-stp of chandelier cells (Fairén and Valverde, 1980; Somogyi et al., 1982; Peters, 1984) or to other cortical interneurons such as non-spinous stellate cells (Peters and Fairén, 1978) or basket cells (Somogyi et al., 1983).

The synaptic boutons on the IS of pyramidal cells seem to be GABAergic since they show immunoreactivity with anti-GAD (Peters et al., 1982; Frenud et al., 1983; de Felipe et al., 1985) and anti-GABA sera (Somogyi et al., 1985). On the other hand, corticocollicular neurons have been shown to use excitatory aminoacid transmitters (Lund-Karlsen and Fonnum, 1978; Matute and Streit, 1985). Thus, the GABAergic inhibition (Krnjevic, 1984) at the level of the IS might have important influence in the firing of these excitatory neurons.

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