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RESEARCH NOTE

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Calcitonin gene-related peptide immunoreactivity selectively labels accessory optic nuclei and pathways of the rat visual system

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Abstract The present study shows the distribution of calcitonin gene-related peptide (CGRP)-immunolabeled neuronal somata and fibers in the accessory optic system of adult rats. CGRP-immunoreactive cell bodies were small to medium-sized and mostly fusiform or oval-shaped. Both immunolabeled somata and fibers were found in the dorsal and lateral terminal nuclei as well as in the interstitial nucleus of the superior fasciculus (posterior fibers); whereas only immunoreactive fibers were found in the ventral division of the medial terminal nucleus, particularly its rostral portion. These results indicate that CGRP-containing neurons are present in all nuclear components of the accessory optic system and suggest that this neuropeptide may play a neuromodulative role in eye movements.

Key words Calcitonin gene-related peptide · Accessory optic nuclei · Lateral terminal nucleus · Medial terminal nucleus · Dorsal terminal nucleus

Introduction

Previous studies have analyzed the distribution of calcitonin gene-related peptide (CGRP) in the mammalian central nervous system with immunocytochemical methods. These studies showed that CGRP neurons and fibers are present in many brain regions (Rosenfeld et al. 1983; Kawai et al. 1985; Skofitsch and Jacobowitz 1985a; Kruger et al. 1988a). Also, they stressed the importance of finding CGRP-immunolabeled axons in specific parts of the somatosensory pathway and in portions of the gustatory and olfactory systems. These investigators speculated that CGRP may play a role in nociception or chemosensory systems. One sensory system that appears to lack any evidence of CGRP-immunolabeled axons and somata in these studies is the visual system.

The early studies of Kawai et al. (1985) and Skofitsch and Jacobowitz (1985a) revealed CGRP-immunopositive neurons in regions bordering on the medial geniculate nucleus. However, they did not recognize that these somata may be a part of the visual system, specifically its accessory optic system (AOS). Several of the nuclei of the AOS reside in this region and they receive direct visual projections from the retina (Hayhow et al. 1960; Giolli 1961).

The AOS of mammals consists of four pairs of terminal accessory optic nuclei, designated as the dorsal, lateral, and medial terminal nuclei (DTN, LTN, MTN; Hayhow et al. 1960) and the interstitial nucleus of the superior fasciculus, posterior fibers (inSFp; Giolli et al. 1984; Simpson et al. 1988a). The MTN is further divisible into ventral and dorsal sectors based upon cytoarchitectural criteria and nerve fiber connectivity (Hayhow et al. 1960; Giolli et al. 1984).

Currently, the nuclei of the AOS are receiving much attention, because they are important for gaze control and visual-vestibular interaction (Simpson et al. 1988a; Grasse and Cynader 1991; Cohen et al. 1992). The present study was designed to analyze the distribution of CGRP-labeled somata and fibers in the nuclei of the AOS.

Materials and methods

Animals and tissue preparation

Four adult male albino rats (Sprague-Dawley) weighing 300–350 g were used in this study. The animals were deeply anesthetized with pentobarbital sodium and perfused transcardially with 0.12 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in phosphate buffer (PB, pH 7.4). The brains were removed and postfixed in the perfusion solution for 4–8 h at 4°C, and then stored in cold PBS for several hours to a few days. Blocks containing the brain and spinal cord were sectioned in either the coronal ($n=2$) or sagittal ($n=2$) planes at 50 μm with a vibratome, and alternate sections were collected in PBS and processed for immunocytochemistry and Nissl staining (cresyl violet).

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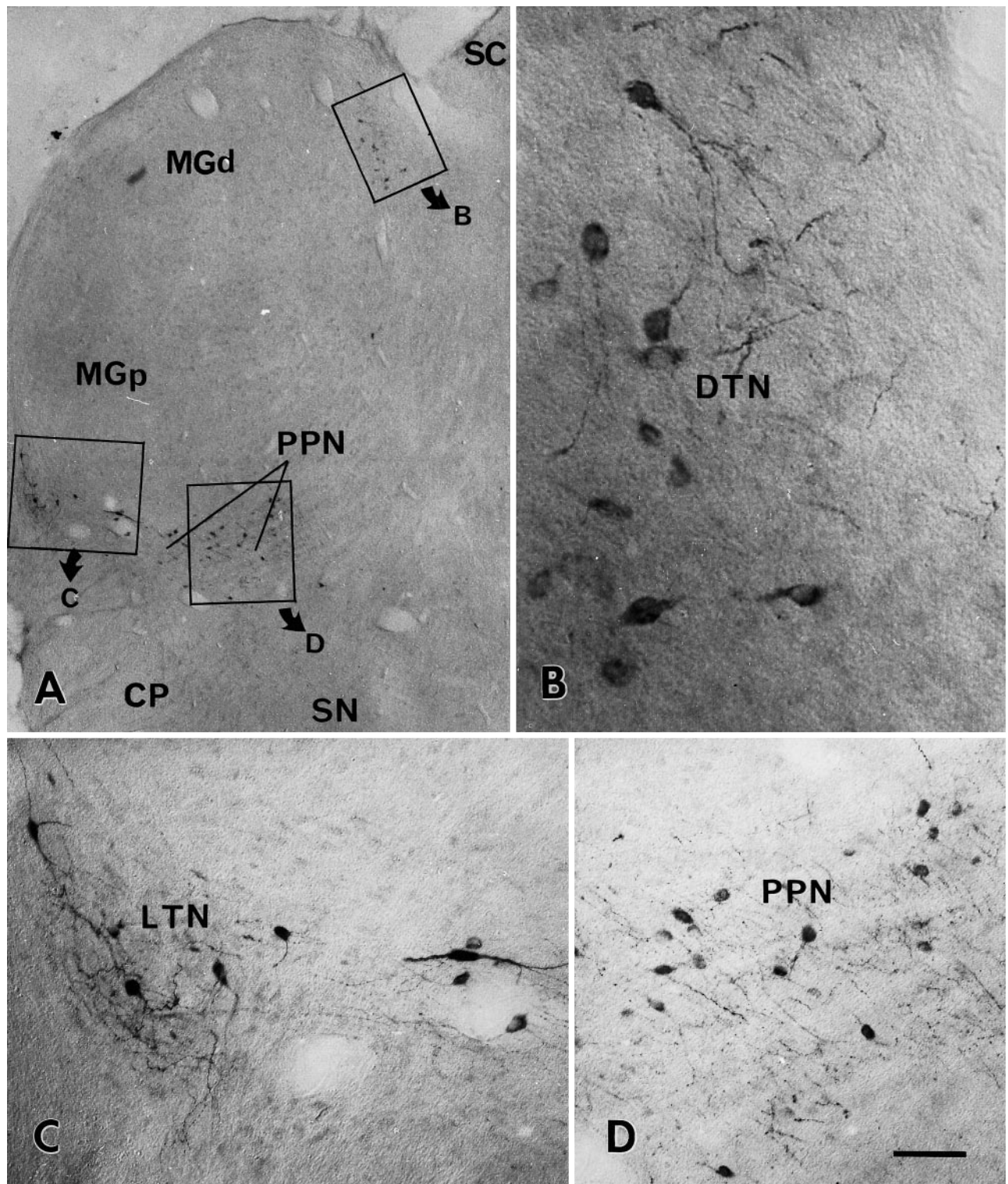


Fig. 1A–D Photomicrographs of a coronal section through the mesencephalic-diencephalic junction at the level of the superior colliculus (*SC*) and principal nucleus of the medial geniculate body (*MGp*). **A** shows CGRP-containing somata and fibers in the dorsal (*DTN*) and lateral (*LTN*) terminal nuclei of the accessory optic system (boxes labeled **B** and **C**, respectively) and the peripeduncular nucleus (*PPN*). The upper boxed region in **A** between the dorsal nucleus of the medial geniculate body (*MGd*) and the *SC* corresponds

to the *DTN* and is enlarged in **B**, in which several somata and processes are immunolabeled. The lower left box in **A** is enlarged in **C** and shows the intensely immunostained CGRP neurons and fibers in the *LTN* located ventrolateral to the *MGp*. The *PPN* is medial to the *LTN* and is also enriched with CGRP-immunoreactive somata and processes, as shown in the enlargement in **D**. Scale bars: 200 μ m **A**; 20 μ m **B**; 50 μ m **C,D** (*CP* cerebral peduncle, *SN* substantia nigra)

Immunocytochemistry

Free-floating sections were stained for CGRP using the standard avidin-biotin complex (ABC) method. Briefly, endogenous peroxidase activity was eliminated with a 20-min incubation in 0.1% H₂O₂. Background staining was blocked by a 2-h incubation in 5% normal goat serum (NGS) at room temperature, after which the sections were incubated in a PBS solution containing 5% NGS, 0.3% Triton X-100, and a rabbit anti-CGRP primary antibody at a concentration of 1:10 000 overnight at 4°C and with agitation. After several rinses with PBS, the tissue was further incubated in 1% goat anti-rabbit IgG with 0.5% NGS in PBS for 1 h at room temperature, followed by an incubation in 1% ABC solution for another hour. The immunoreaction products were visualized with 0.05% DAB and 0.005% H₂O₂. Three 10-min rinses with PBS were used between all incubations.

The CGRP antibody was obtained from Sigma Chemical Company (St. Louis, Mo.). Specific staining of tissue with this antibody is inhibited by preincubation of the antiserum with 10 µM CGRP (rat). Immunolabeling of neuronal profiles was obtained with 1:2 000 to 1:20 000 dilutions of this antiserum. Other immunocytochemical reagents were obtained from Vector Laboratories. Processing the sections with the primary antibody omitted, or replaced by normal rabbit serum, yielded no specific immunolabeling.

Immunostained sections through the thalamus and midbrain were examined under both bright- and darkfield illumination with a Zeiss Axioplan photomicroscope, with particular attention being paid to the AOS, including its MTN, DTN, and LTN, and its superior fasciculus of the posterior fibers. Bright- or dark-field photographs were obtained from these nuclei and the AOS pathway.

Results

The present data were obtained from sections studied in coronal and sagittal planes to visualize both the extent and distribution of the CGRP-immunolabeled fibers and the morphology of CGRP-immunolabeled somata and dendrites. Sections containing the spinal cord displayed the previously described distribution of CGRP-labeled fibers in the dorsal horn and immunolabeled motoneurons (Skofitsch and Jacobowitz 1985a). Thus, the specificity of the anti-CGRP serum was confirmed with this result as well as the labeling of other structures in the forebrain that were previously described, including the ventromedial nucleus of the thalamus (Kruger et al. 1988b).

The AOS showed CGRP-labeled somata and fibers in three of the four nuclei that comprise this system. The DTN is located within the pretectal complex in the angle between the medial geniculate nucleus and the superior colliculus, as observed in coronal sections (Fig. 1). CGRP-labeled neurons were found within this nucleus. The labeled somata were round or oval in shape and often

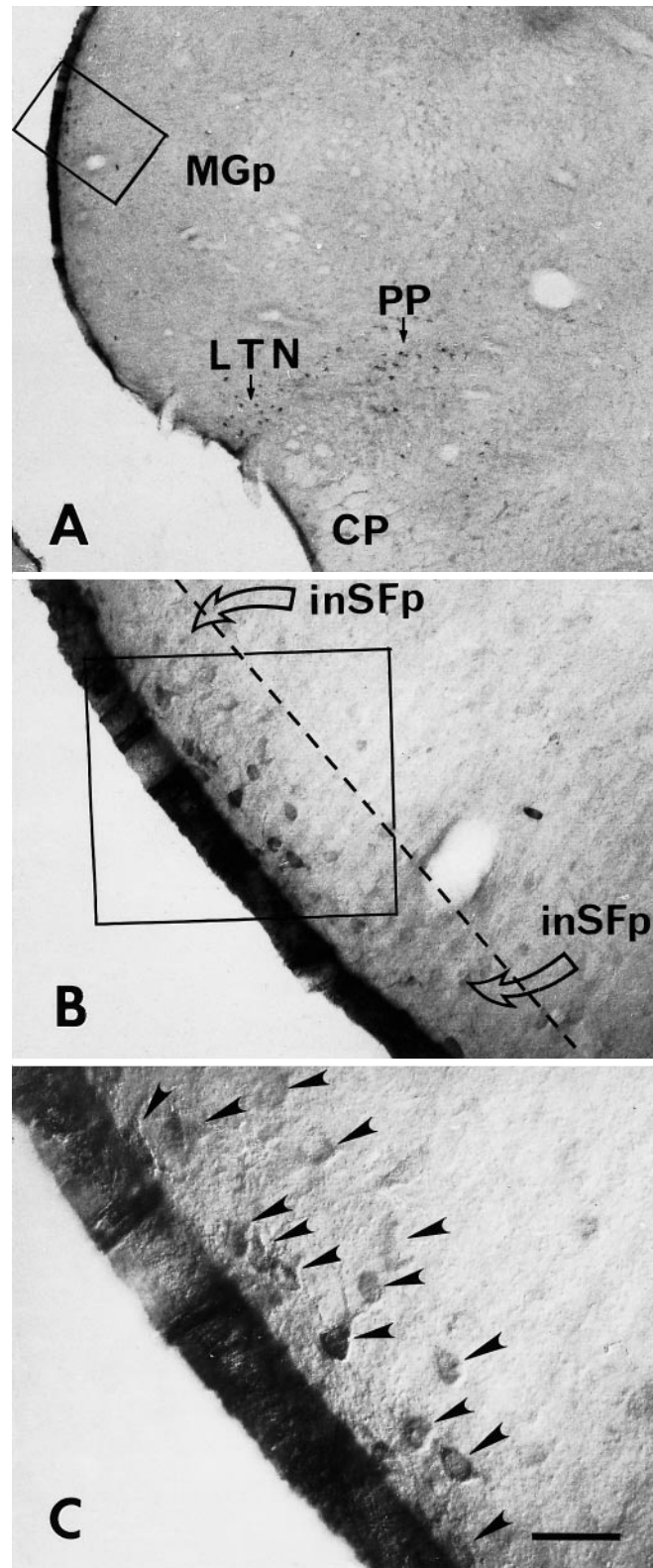
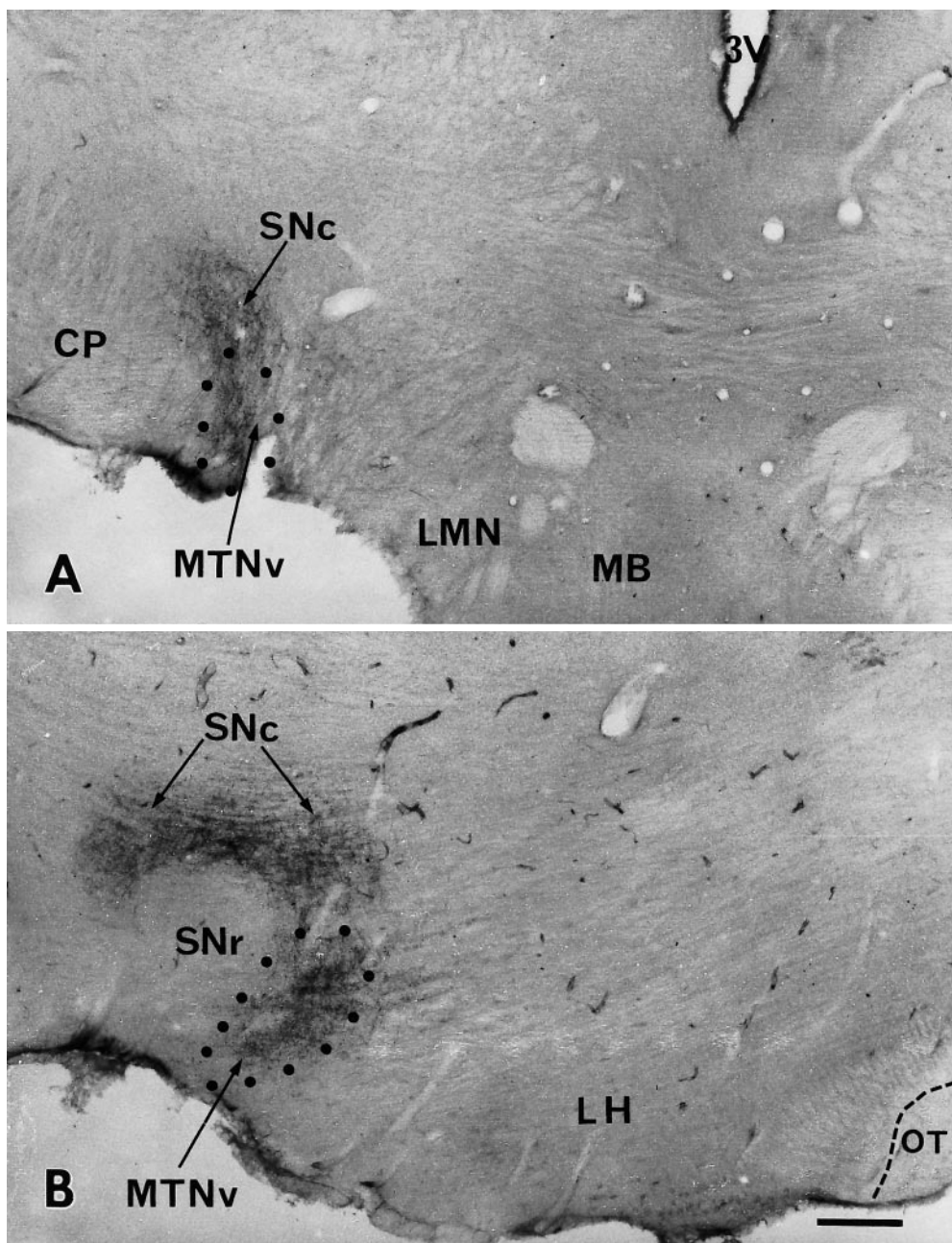


Fig. 2 **A** Is a photomicrograph from a coronal section through the mesencephalic-diencephalic junction at a similar level to that in Fig. 1. Note again the labeling of CGRP-immunoreactive neurons in the lateral terminal nucleus (LTN) and peripeduncular nucleus (PPN). The box contains the interstitial nucleus of the superior fasciculus, posterior fibers (*inSFp*), and it is enlarged in **B** to show several labeled somata in this accessory optic nucleus (*arrowheads*). **C** Is an enlargement of the box in **B** to show the details of the labeled cells (*arrowheads*). Broken line shows the appropriate border between the *inSFp* and the MGp. Scale bars: 200 µm **A**; 50 µm **B**; 20 µm **C** (CP cerebral peduncle)

Fig. 3A, B Photomicrographs of coronal (A) and sagittal (B) sections through the brainstem and diencephalon to show CGRP immunoreactivity in the ventral division of the medial terminal nucleus (*MTN_v*) that is outlined with *solid dots*. This structure of the accessory optic system contains exclusively immunolabeled fine fibers but not somata. The neighboring pars compacta of the substantia nigra (*SNc*) also contains densely packed CGRP-labeled fibers. *Scale bar: 200 μm* (*CP* cerebral peduncle, *LH* lateral hypothalamus, *LMN* lateral mammillary nucleus, *MB* mammillary body, *OT* optic tract, *3V* third ventricle)



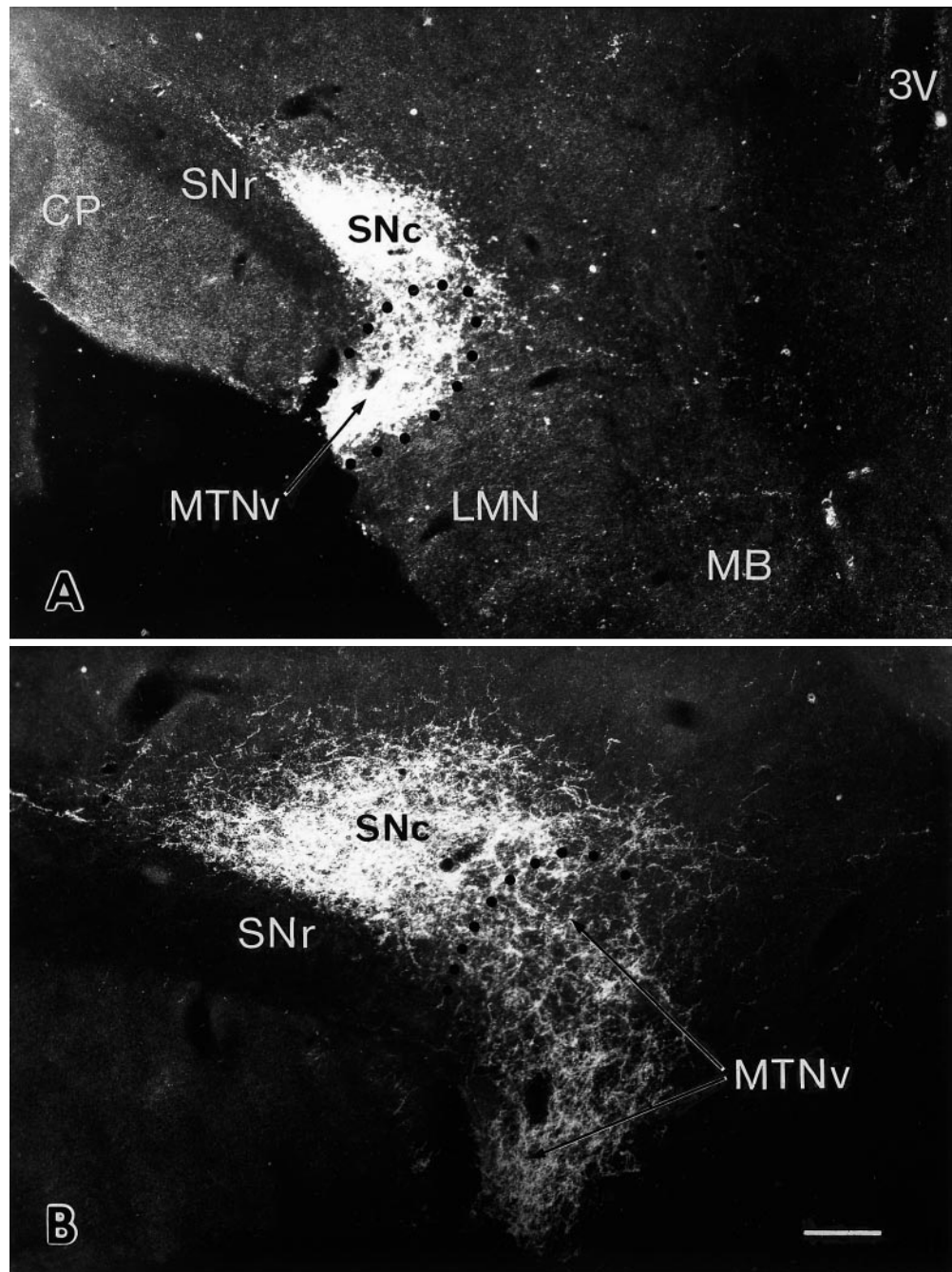
showed labeled dendrites extending from them (Fig. 1B). Many of these neurons are fusiform cells. In addition to the labeled processes that were continuous with the somata in the DTN, there were several isolated, CGRP-immunolabeled processes that had features of dendrites, but some of them could also be immunolabeled fibers. Labeled somata and dendrites were also observed in sagittal sections containing the DTN.

The inSFp also showed CGRP-labeled somata. This nucleus is intercalated within the posterior fibers of the superior fasciculus of the AOS, and most of the neuronal somata are compressed between these fibers and the medial geniculate nucleus (Fig. 2), and, more ventrally, the cerebral peduncle. As a result, the CGRP-labeled somata

in the inSFp are fusiform to cuboidal in shape. Only a few dendrites were observed to arise from these somata and they were mainly of a fine caliber (Fig. 2C). Small bundles of CGRP-labeled fibers were observed coursing ventrally within the inSFp.

CGRP-labeled somata were observed in one other nucleus of the AOS, the LTN. This nucleus is located in the angle ventral to the medial geniculate nucleus but dorsal to the cerebral peduncle and lateral to the peripeduncular nucleus (PPN; Fig. 1). The labeled somata in the LTN were larger than those found in either the DTN or inSFp, and they had large dendrites that were confined within the borders of the nucleus. The labeled somata in the LTN appeared to be contig-

Fig. 4A, B Dark-field photomicrographs of a coronal section through the midbrain showing the details of the CGRP-immunolabeled processes in the ventral division of the medial terminal nucleus (*MTN_v*) and pars compacta of the substantia nigra (*SN_c*). Both regions are packed with axonal processes, but no immunolabeled somata appear to be present. Scale bars: 200 μ m **A**; 50 μ m **B** (*SN_r* pars reticulata of the substantia nigra)



uous with the previously described CGRP-labeled neurons in the PPN (Fig. 1A,D). Several bundles of labeled fibers passed through the LTN and PPN. Some may represent axons that arise from neurons of these nuclei.

The remaining nucleus of the AOS that shows immunolabeling for CGRP is the MTN. This nucleus resides within the ventral portion of the midbrain between nuclei comprising the ventral tegmental area and the medial part of the substantia nigra (Fig. 3). Only CGRP-labeled fibers appear in this region and most of them lie within the medial pars compacta of the substantia nigra. However, im-

munolabeled fibers that ramify in the MTN are confined to the rostral part of its ventral subdivision (Figs. 3, 4). Here, these fibers appear to outline somata and dendrites of immunonegative neurons.

Discussion

The present findings show the presence of CGRP-immunostained somata and fibers in the DTN, LTN and inSFP, and CGRP-immunostained fibers in the rostral portion of the ventral division of the MTN. Although a few CGRP-

labeled neurons were mapped in the vicinity of the medial geniculate nucleus by Kawai et al. (1985), the present description is the first to identify these CGRP-containing profiles as neurons of the AOS nuclei. These CGRP-labeled neurons located peripheral to the medial geniculate nucleus form a continuum that connects the four nuclear components of the AOS extending dorsally from the DTN in the pretectum to the ventral MTN in the midbrain tegmentum. The CGRP labeling of the AOS nuclei is quite selective, with the neighboring nuclei of the thalamus and pretectum being immunonegative for CGRP. However, the CGRP-immunolabeled neurons in the medial portion of the LTN appear to form a continuum with the more medially lying PPN that also contains a large number of CGRP-immunolabeled neurons, as shown in the present as well as in earlier studies (Kruger et al. 1988a). This continuous distribution of CGRP neurons in the LTN and PPN might have been one of the reasons that previous studies failed to identify CGRP labeling within AOS neurons. CGRP-labeled somata and fibers are present in three of the four AOS nuclei, and the remaining nucleus, the MTN, contains only immunolabeled fibers. The fine fibers in the ventral portion of the MTN are suggested to be axon terminals of CGRP-immunostained somata noted within the DTN, LTN, and dorsal stretch of the inSFp, based upon the observation of rich interconnections between AOS nuclei (Giolli et al. 1984, 1992).

Immunocytochemical studies have demonstrated that all four AOS nuclei contain high proportions of GABAergic neurons (Ottersen and Storm-Mathisen 1984; Giolli et al. 1985; Mugnaini and Oertel 1985), and many of these GABAergic neurons are involved in interconnections between AOS nuclei as well as to the nucleus of the optic tract (NOT). For example, GABAergic neurons in the MTN (both ventral and dorsal subdivisions) project strongly to the DTN, inSFp, and NOT (van der Togt et al. 1991; Giolli et al. 1992; van der Want et al. 1992). Conversely, NOT neurons in the rat project strongly to the MTN exclusively via GABAergic neurons (van der Togt et al. 1991, 1993). Other studies have reported a significant number of somata and axon terminals in the AOS nuclei that contain glutamate (Nunes Cardozo et al. 1991) and neuropeptides such as substance P (Brecha et al. 1987) and somatostatin (Laemle and Feldman 1985). In addition, certain opioid receptors, specifically the μ -subtype, are present in exceedingly high concentrations throughout each of the rat AOS nuclei and NOT (Giolli et al. 1990). These findings suggest that both classic transmitters and neuropeptides are involved in the regulation of the activity of AOS neurons.

Electrophysiological studies have established that the AOS is a visual system designed in vestibular coordinates (Simpson et al. 1979, 1988a). The DTN, LTN, and MTN consist of neurons that are primarily responsive to selective movement, directed, respectively, in horizontal (Grasse and Cynader 1982) or vertical (up-down: Grasse and Cynader 1982; Mustari and Fuchs 1989; or down-up: Benassi et al. 1989; Simpson et al. 1988b; Lui et al.

1990) planes. The neurons forming the inSFp seem to be directionally tuned according to their proximity to the DTN, LTN, or MTN (Benassi et al. 1989).

CGRP has been found in particularly high concentrations in several primary afferent systems. For example, the dorsal root ganglion neurons and their axon terminals within laminae I and II of the spinal cord are highly reactive for CGRP (Gibson et al. 1984; Gulbenkian et al. 1986; Carlton et al. 1987). Similarly, the trigeminal input to the brainstem is also strongly labeled for CGRP (Sugimoto et al. 1997). CGRP-immunoreactive neurons and axon terminals are also enriched in the tractus solitarius of the rat (Morishima et al. 1985) and some regions of the olfactory system (Kawai et al. 1985; Kruger et al. 1985a). In these primary sensory systems, CGRP is often found to be colocalized with substance P (Lee et al. 1985; Skofitsch and Jacobowitz 1985b; Gulbenkian et al. 1986). Our present finding of CGRP in the AOS of the visual system provides additional data to indicate that CGRP is localized to multimodal sensory neuronal elements. The biological significance of CGRP within this primitive part of the visual system may be somewhat analogous in function to the role played by CGRP-containing neurons in the somatosensory, gustatory, and olfactory pathways.

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