Histol Histopathol (1998) 13: 415-424

Histology and Histopathology

Synaptophysin immunoreactivity in the cat cochlear nuclei

P. Gil-Loyzaga[1], M.V. Bartolomé[1] and A. Ibáñez[2]

[1]Department of Surgery II (ORL), Faculty of Medicine and Center of Cell Culture (CAI-UCM) and

[2]Department of Cell Biology, Faculty of Medicine, Complutense University of Madrid, Madrid, Spain

Summary. The presence of synaptophysin, a presynaptic Ca^{2+} binding glycoprotein, has been analyzed in the cochlear nucleus complex of the adult cat using an anti-synaptophysin monoclonal antibody. Synaptophysin immunoreactivity was differently distributed between regions of cochlear nuclei. Terminal boutons contacting directly with neurons (cell bodies and dendrites) or in the neuropil of ventral and dorsal cochlear nuclei appeared immunostained. In the ventral cochlear nucleus, synaptophysin-labelled boutons were clearly defined in contact with spherical, globular, multipolar, octopus and cochlear-nerve root neurons. However, the dorsal cochlear nucleus showed a dense immunostained network of synaptophysin-labelled puncta, inside which some neuron cell bodies were observed. The present findings show a differential distribution of synaptophysin-immunostained boutons between the ventral and the dorsal cochlear nuclei of the cat. The present description of the different synaptophysin-labelled terminal boutons in the cochlear nuclei complex and their pattern of distribution, will be useful for further studies on development, degeneration or regeneration of the peripheral auditory pathway.

Key words: Cochlear nuclei, Synaptophysin, Auditory pathway, Cat

Introduction

The cochlear nucleus (CN) complex has been divided into three main subnuclei; the anterior ventral (AVCN); the posterior ventral (PVCN); and the dorsal (DCN) cochlear nuclei. This general division is based on the presence and distribution of several types of neurons and nerve fascicle (see for review; Ramón y Cajal, 1911; Osen, 1969; Brawer et al., 1974; Lorente de Nó, 1981; Hackney et al., 1990). The neurons of the CN complex exhibit similar morphology and pattern of distribution in several mammalian species, including rat (Harrison and Irving, 1965, 1966), mouse (Webster and Trune, 1982), hamster (Schweitzer, 1990; Collinge and Schweitzer, 1991), guinea-pig (Hackney et al., 1990), rabbit (Perry and Webster, 1981), chinchilla (Morest et al., 1990) and cat (Ramón y Cajal, 1911; Osen, 1969; Brawer et al., 1974; Lorente de Nó, 1981). However, the distribution of synaptic vesicle proteins of afferent and efferent innervation has never been used to define particular regions within the CN complex.

Neurons of the CN complex receive synaptic contacts form primary afferent cochlear fibers (Kiang et al., 1982; Fekete et al., 1984; Liberman and Oliver, 1984; Rouiller et al., 1986; Ryugo and Rouiller, 1988; Hackney et al., 1990; Ostapoff and Morest, 1991), from descending auditory pathways (Conlee and Kane, 1982; Brown et al., 1988; Winter et al., 1989; Benson and Brown, 1990; Hackney et al., 1990; Ryan et al., 1990; Ostapoff and Morest, 1991), and from internuclear connections (Cant and Gaston, 1982; Ostapoff and Morest, 1991). The synaptic boutons of the CN complex have been analyzed using light and electron microscopy, classified at first as endbulbs of Held and round small synapses (Ryugo and Fekete, 1982). In addition, synaptic endings on cochlear nucleus neurons can be classified in terms of their neuronal origin (see review in Ostapoff and Morest, 1991). Synaptic vesicle proteins could provide additional information on the characteristics of neuronal contacts within the CN, since these proteins have been involved in synaptic physiology, in particular in the release of neurotransmitters (Südhof and Jahn, 1991; Südhof, 1995; McMahon et al., 1996).

Synaptophysin (SY) is one of the major Ca^{2+} binding glycoproteins located in the presynaptic membrane and the synaptic vesicles (Wiedenmann and Franke, 1985) in virtually all types of nerve terminals (Rubinstein et al., 1993; Südhof, 1995; McMahon et al., 1996). This synaptic protein seems to form tetrameric homopolymers (Trimble and Scheller, 1988; Johnston et al., 1989; Johnston and Südhof, 1990) and does not form heteromultimers (McMahon et al., 1996). It is involved in: 1) channel-forming processes, contributing to the uptake of low molecular weight compounds; 2) the vesicle-plasma membrane fusion; and 3) the release of

Offprint requests to: Prof. P. Gil-Loyzaga MD., PhD., Apartado de Correos 60075, 28080 Madrid, Spain

neurotransmitters (Jahn et al., 1985; Rehm et al., 1986; Wiedemann and Franke, 1985; Rehm et al., 1986; Thomas et al., 1988; Korematsu et al., 1993; Südoff, 1995). However, SY function could be replaced by another system or protein, since knockout SY-deficient mice exhibited a normal synaptic transmission and plasticity (McMahon et al., 1996), even though this glycoprotein appears at early developmental stages (Gil-Loyzaga and Pujol, 1988; Lajister and Kachele, 1989; Bergmann et al., 1993; Ovstacharoff et al., 1993). Immunolabelling at electron microscopic level confirmed that SY is located in clusters of synaptic vesicles, near to or forming part of the presynaptic membrane (Gil-Loyzaga and Pujol, 1988; Walaas et al., 1988; De Camilli and Takei, 1996). It is a large protein component of the membrane of the small round or flat synaptic vesicles in a wide variety of neurons (Jahn et al., 1985; Gil-Loyzaga and Pujol, 1988; Masliah et al., 1990; Tixier-Vidal et al., 1992; McMahon et al., 1996). Also, it has been described as a membrane constituent of large dense-core vesicles (Navone et al., 1986; Leclerc et al., 1989; De Camilli and Jahn, 1990; Masliah et al., 1990; McMahon et al., 1996).

The aim of the present study was to analyze the distribution pattern of SY-labelled terminal boutons within the CN and its correspondence to the synaptic distribution in the adult cat, a species widely used in CN research (Ramón y Cajal, 1911; Osen, 1969; Brawer et al., 1974; Cant and Morest, 1979a,b; Lorente de Nó, 1981). In some preliminary communications, we have identified the presence of the SY in the CN complex of several mammalian species (Gil-Loyzaga et al., 1991; Merchán-Pérez et al., 1992; Bartolomé et al., 1993), including differences between normal and early deafferented animals (Gil-Loyzaga et al., 1996).

Materials and methods

Animals and tissue preparation

Four adult pigmented cats were used in the present study. The animals, under deep general anesthesia (chloral-hydrate 300 mg/kg b.w.), were perfused for 15 min, through the ascending aorta with a fixative solution containing 98% ethanol and 2% acetic acid (Gil-Loyzaga et al., 1991; Merchán-Pérez et al., 1992; Bartolomé et al., 1993). The brainstems were rapidly removed, postfixed in the same solution for 72 hours, dehydrated and embedded in paraffin. Samples were then cut in seriated 14 μm-thick sections from frontal or sagittal planes in different specimens.

Immunocytochemical procedures

The sections were rinsed three times, for 5 min each, in 0.1M phosphate-buffered saline (PBS) at pH 7.3, containing 2% bovine serum albumin (PBS-BSA). Preincubation was carried out for 30 min in a PBS-BSA solution containing 0.1% Triton X-100 (Sigma) and 30% horse serum. The sections were then incubated overnight at 4 °C in a solution containing 2.5 μg/ml of an anti-SY monoclonal antibody (Gil-Loyzaga and Pujol, 1988) (Boehringer Mannheim, clone SY38). After three washes (5 min each) in PBS-BSA, the sections were incubated for 1 hour in biotinylated horse anti-mouse IgG (Vectastain, Vector Laboratories Inc) 1/200 in PBS-BSA. Antigen-antibody immunoreaction was revealed by using the avidin-biotin-peroxidase method (Vectastain, Vector) as previously described (Bartolomé et al., 1993; Merchán-Pérez et al., 1993). Negative controls were obtained with the same procedure described above but with the omission of the primary antibody.

Results

A sagittal section of the CN complex allowed all the cochlear subnuclei to be analyzed, on the same view, (AVCN, PVCN and DCN) (Fig. 1). A detailed study of each region required parallel coronal sections, as shown in Fig. 2, carried out through out the straight lines indicated in Fig. 1.

In panoramic views of the CN complex subnuclei, AVCN, PVCN and DCN exhibited different SY-like (SY-LI) immunoreactive patterns observed in sagittal (Fig. 1) and coronal (Fig. 2) sections. AVCN and PVCN showed a medium-density SY-LI labelling with completely devoid of immunoreaction, which could clearly correspond to the neuropil (Figs. 1, 2A-E). DCN exhibited a high-density SY-LI immunoreaction (Figs. 1, 2F,G). While in the AVCN and PVNC neuronal cell bodies could be suggested after SY-LI immunoreaction, in the DCN the observation of neuronal cell bodies was impeded by the higher density of immunolabelling (Figs. 1, 2).

It was necessary to use high magnifications on the CN areas in the panoramic sagittal (Fig. 1) and coronal (Fig. 2) views of the SY-LI immunolabelling in order to clearly define the location of the immunoreaction.

The region of the cochlear nerve root lacked any other immunoreactivity than that found around the nerve root neurons (Fig. 3A).

High magnification of sagittal or coronal sections of the AVCN and PVCN (Figs. 3, 4) showed cell bodies of several neuron types. Neurons of the AVCN were surrounded by SY-LI fibers, that could correspond to boutons projecting to these neurons (Fig. 3B-D). These neurons could correspond to spherical (Fig. 3B,C) and globular (Fig. 3D) neurons, because of their particular location. The spherical neurons (Fig. 3B,C) appeared densely and evenly distributed in the rostral ventral part of AVCN, while the globular neurons (Fig. 3D) were confined adjacent to the cochlear nerve root (Fig. 3A), and also within the anterior and medial ventral region of the AVCN.

Even though the majority of the neurons of the AVCN appeared with SY-immunolabelling surrounding the cell body (Fig. 3B-D), some other neurons, of the most rostral part, exhibited a discontinuous immuno-

labelling around the cell body (Fig. 3E). The SY-LY patches were found in the neuropil of the AVCN (Fig. 3B-E).

In the PVCN, the SY-immunolabelling appeared around the cell bodies of all three types of neurons: multipolar (Fig. 3F-H), octopus (Fig. 4A-D), and globular neurons (similar to globular neurons of the AVCN, see Fig. 3D). Also, the neuropil of the PVCN exhibited SY-LI fibers (Fig. 3F-H). Two types of multipolar neurons, large and small, exhibited SY-immunolabelled boutons around the soma and the proximal dendritic area (Fig. 3F-H). Large multipolar neurons (Fig. 3F,G) were numerous in the posterior part of the nerve root, while small multipolar neurons (Fig. 3H) were mainly located in the lateral margins of PVNC. The octopus neurons (Fig. 4A-C), found at the posterior part of PVCN or on the intermedia caustic estria (Fig. 4D), were easily identified by their characteristic ovoid shape and dendritic trees. The SY-immunolabelling was found around the cell bodies and dendrites of octopus neurons (Fig. 4A-C). Magnifications of these neurons showed a continuous layer of SY-immunolabelled boutons around the cell body and a part of the dendritic tree (Fig. 4C). In contrast, in other octopus neurons SY-immunolabelled boutons appeared restricted to the cell body (Fig. 4D).

In the DCN, SY-immunolabelling appeared as a dense granular network in the molecular layer, inside which fusiform neurons were observed (Fig. 4E). In the DCN deep layer (Fig. 4F) and central core (Fig. 4G), SY-immunolabelling was less dense than in the molecular layer. Some neuronal cell bodies appeared contacted by SY-LY fibers (Fig. 4F,G).

In the negative control sections, in which the primary antibody was absent, neither immunostaining nor any recognizable background staining was observed under working conditions.

Discussion

The distribution of SY in the CN complex was detected using a monoclonal antibody against the SY-38 antigen. The SY-immunostaining was observed as patches contacting with cell bodies and dendrites of CN neurons and inside the neuropil. Immunoreactive patches, corresponding to the terminal boutons reaching cell bodies and fibers, appeared on all of the cell types of the CN complex. A differential distribution of SY-labelled boutons was observed within the CN complex. In the AVCN and PVCN, the majority of SY-LI boutons were found around cell bodies and dendrites, and less in the neuropil, while the DCN exhibited a dense network of immunostained patches within the neuropil, which

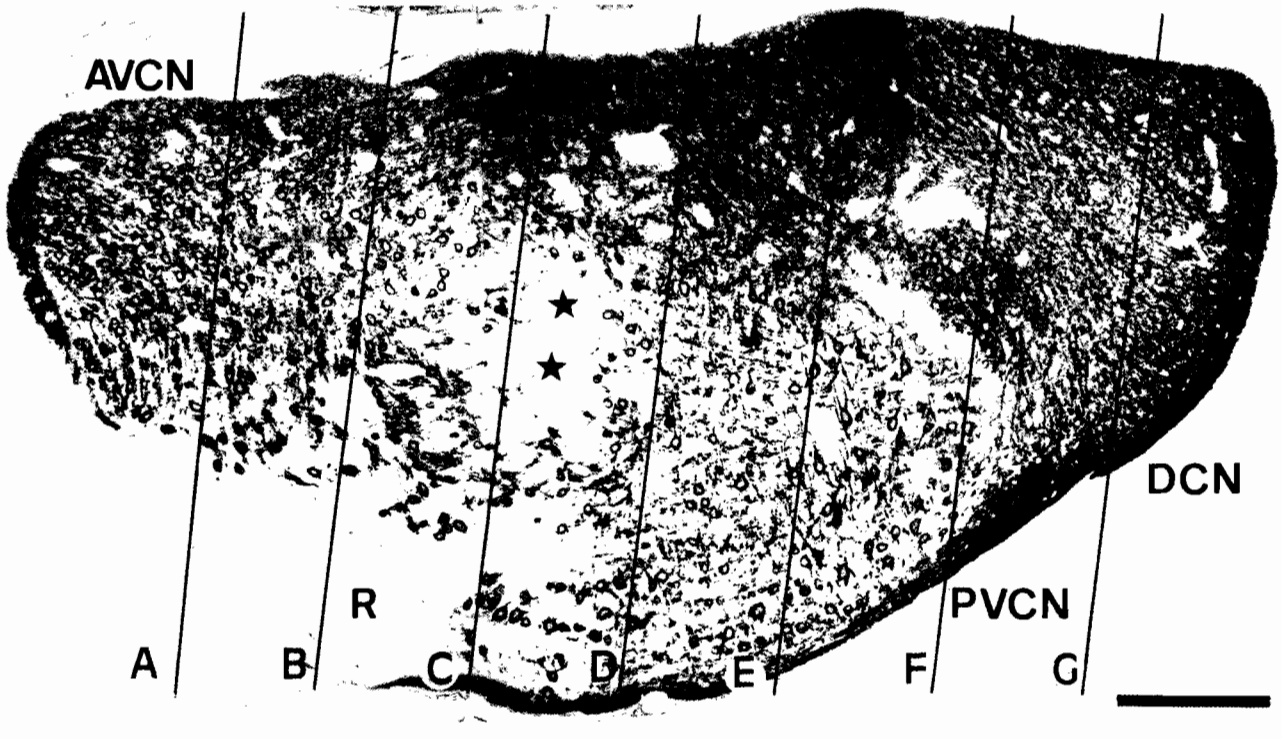


Fig. 1. Immunocytochemical detection of SY in a sagittal section of the cochlear nucleus (CN) of the cat. SY distribution can be recognized as a dark immunoreactive reaction in the three subnuclei: anterior ventral (AVCN); posterior ventral (PVCN); and dorsal cochlear subnucleus (DCN). SY-immunoplabelling appears mainly around the neuron cell bodies in both the AVCN and the PVCN. In the ventral part of the AVCN, the cochlear-nerve root (R) neurons are surrounded by abundant SY-immunolabelled boutons. The border region between both subnuclei (AVCN and PVCN) show a less dense SY-immunoreaction (Fig. 1, stars). In the DCN, SY-immunolabelling appears as a dense layer. Division lines (A-G) represent the approximate level of the different frontal sections shown in Fig. 2. Scale bar: 400 μm.


A
B
C
R
D
E
F
G

Fig. 2. Frontal sections corresponding to the levels shown in Fig. 1. SY-immunolabelling allows the recognition of AVCN neurons **(A-C)**. Thin arrows indicate the distribution of the SY-immunoreactivity in the cochlear-nerve root neurons **(B, C)**. In the PVCN, SY-immunostaining (**D,E**, asterisks) appear surrounding all neuron types, and also forming a network among them. The most superficial layer of the DCN is indicated by the open arrowheads **(C-G)**. Within the DCN, SY-immunoreactivity is particularly dense (**E**, star). Scale bar: 400 μm.

also appeared surrounding the neuronal cell bodies.

In the cat AVCN, spherical neurons have been classified into two main subtypes by the morphology of synaptic terminals contacting their neuronal somata (Cant, 1981; Oberdorfer et al., 1988; Ostappof and Morest, 1991; Ryugo and Sento, 1991). The present findings fit well with these previous reports, since SY-labelling on spherical neurons has revealed two different synaptic connection patterns. Most of the spherical neurons were found to be contacted by abundant SY-labelled boutons (Fig. 3C), while some neurons from the most rostral part of the AVCN were contacted by a small population of immunostained boutons (Fig. 3E).

Type I afferents reached mammalian CN by the endbulbs of Held (Ramón y Cajal, 1911; Brawer and Morest, 1975; Lorente de Nó, 1981; Ryugo and Fekete, 1992; Oberdorfer et al., 1988; Ostapoff and Morest, 1991). These endbulbs of Held have been classified into small, medium and large complex endings (Rouiller et al., 1986), following a decreasing gradient of size in the ventral-dorsal axis of the CN. However, the SY-immunolabelling does not allow the classification of terminal boutons with respect to their size or shape. However, the largest SY-immunolabelled boutons, reaching spherical and cochlear nerve root neurons, that could correspond to endbulbs of Held, were highly reduced in the CN after cochlea removal in the adult rat (Gil-Loyzaga et al., 1996). This is in agreement with previous ablation studies which showed that the endbulbs of Held correspond to the primary afferent terminals of the auditory nerve (Gentschev and Sotelo, 1973; Cant and Morest, 1979b; Hashisaki and Rubel, 1989).

In the cat PVCN subnucleus, two different types of multipolar neurons have been previously described based on shape and size (Cant, 1981; More, 1986) and on their own physiology (Smith and Rode, 1989). SY-immunostaining distribution did not reveal differences between the two types of large and small multipolar neurons earlier described (Cant, 1981; Moore, 1986). Further research could help to establish relationships between physiological and morphological findings. Although four morphological types of octopus neurons have been described (Kane, 1973), the SY-immunolabelled terminal boutons did not show significant differences between octopus neurons. Although some differences in SY-immunolabelling could be observed, this was mainly restricted to the octopus cell body.

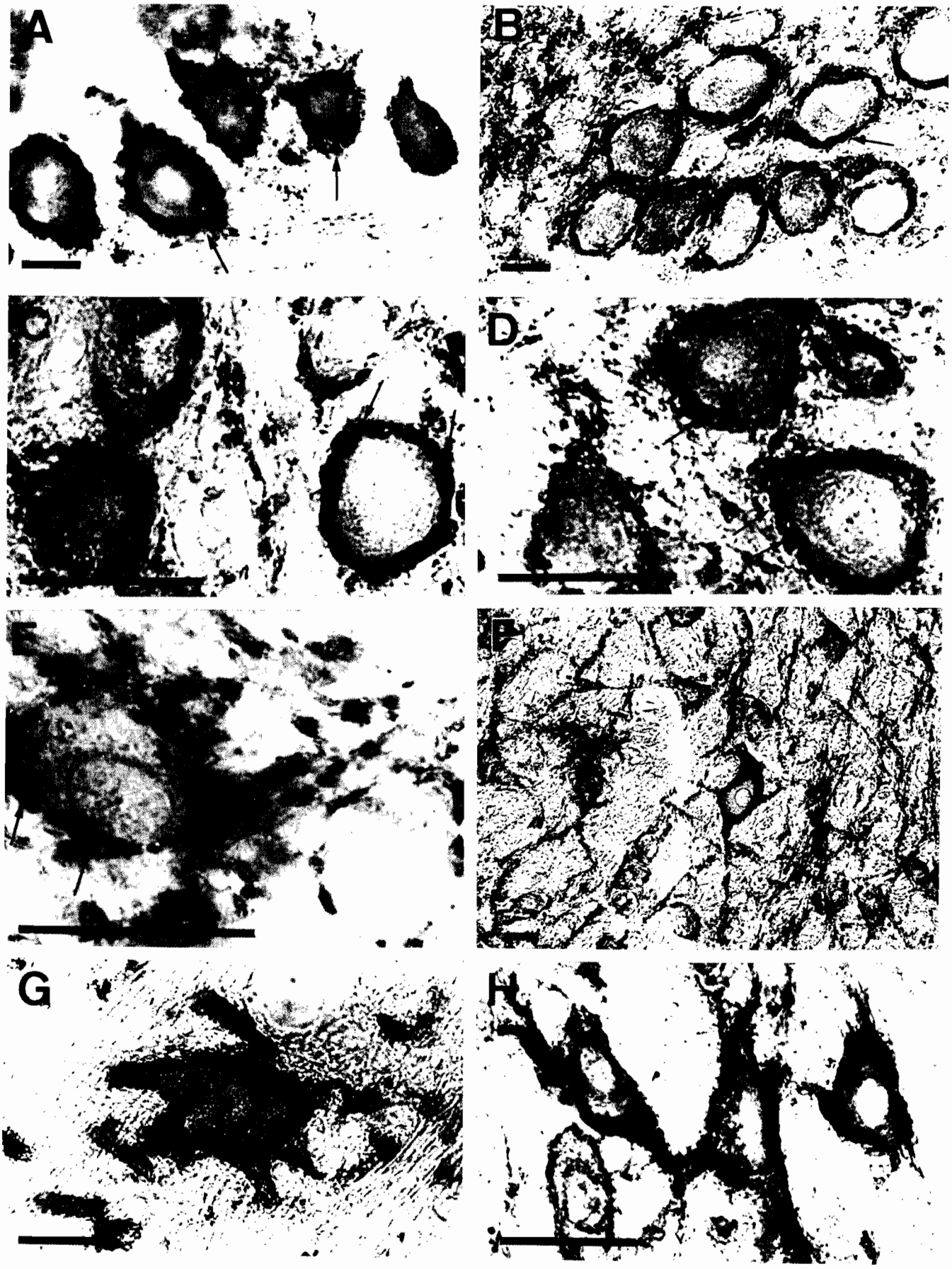
In the DCN, SY-immunolabelling was found particularly at the molecular layer, so strong that it was difficult to distinguish boutons or fibers. These results correspond to previous morphological findings (Rouiller et al., 1986), which indicated that DCN contains a more homogeneous population of primary endings than AVCN or PVCN. A distribution of SY-immunolabelling such as that observed at the DCN molecular layer was concordant with that observed in the cerebellar and cerebral cortex (Leclerc et al., 1989; Masliah et al., 1990), indicating a high connectivity at these central nervous system areas.

It has been hypothesized that SY could play a major role in the neurosecretion process in the majority of neurons, and also in endocrine cells (Navone et al., 1986; Wiedenmann et al., 1986; Leube, 1994). In act, the increase in SY phosphorylation, linked to depolarization, is dependent on Ca^{2+} (Rubenstein et al., 1993) and is involved in the control of neurotransmission. It has been hypothesized that SY could have a role as a component of the exocytic fusion pore during synaptic physiology (Südhof and Jahn, 1991; Rubenstein et al., 1993). Therefore, the recognition of synaptic vesicle proteins is an important step towards the knowledge of neurotransmitter release processes (Windenmann and Franke, 1985; Korematsu et al., 1993), as well as in the synaptic vesicle exocytosis process in the nerve terminal (Südoff, 1995; McMahon et al., 1996).

A heterogeneous population of SY-labelled small and intermediate size boutons, here described for the cat, could correspond to the distribution of excitatory and inhibitory neurotransmitters. Thus, the distribution of glutamate (Hakney et al., 1996), glutamic acid decarboxylase (Roberts and Ribak, 1987; Saint Marie et al., 1989; Vater et al., 1992), GABA (Peyret et al., 1986; Wenthold et al., 1986; Kolston et al., 1992), and glycine (Wenthold et al., 1987; Saint Marie et al., 1991; Kolston et al., 1992) clearly corresponded to SY distribution in the CN complex.

The description of the SY-labelling pattern in the CN complex of the adult normal cat may be useful for further studies on the synaptic development or degeneration process. Further investigations, including electron microscopy could clarify as to whether all the synaptic boutons in the CN express SY, as well as the

Fig. 3. High magnification of several neuron types of the AVCN and PVCN showing SY-immunolabelled terminal boutons. Some small SY-immunoreactive puncta appear in the neuropil between the different types of neurons. The SY-immunolabelled terminal boutons are observed surrounding the cell body of cochlear-nerve root neurons (**A**, arrows) and the spherical neurons **(B)**. A high magnification of the spherical neurons has allowed the observation of SY-immunolabelled terminal boutons around the neuron cell bodies (**C**, arrows). Terminal boutons are also found surrounding the neuron cell bodies of the globular neurons (**D**, arrows). The distribution of terminal boutons on some spherical neurons constituted a discontinuous SY-immunolabelled sheath (**E**, arrows). Photomicrographs show the distribution of SY-immunoreaction as small dots surrounding the soma and the dendritic tree of different types of multipolar neurons of the PVCN **(F-H)**. Scale bar: 25 μm.


A
B
C
D
G
H

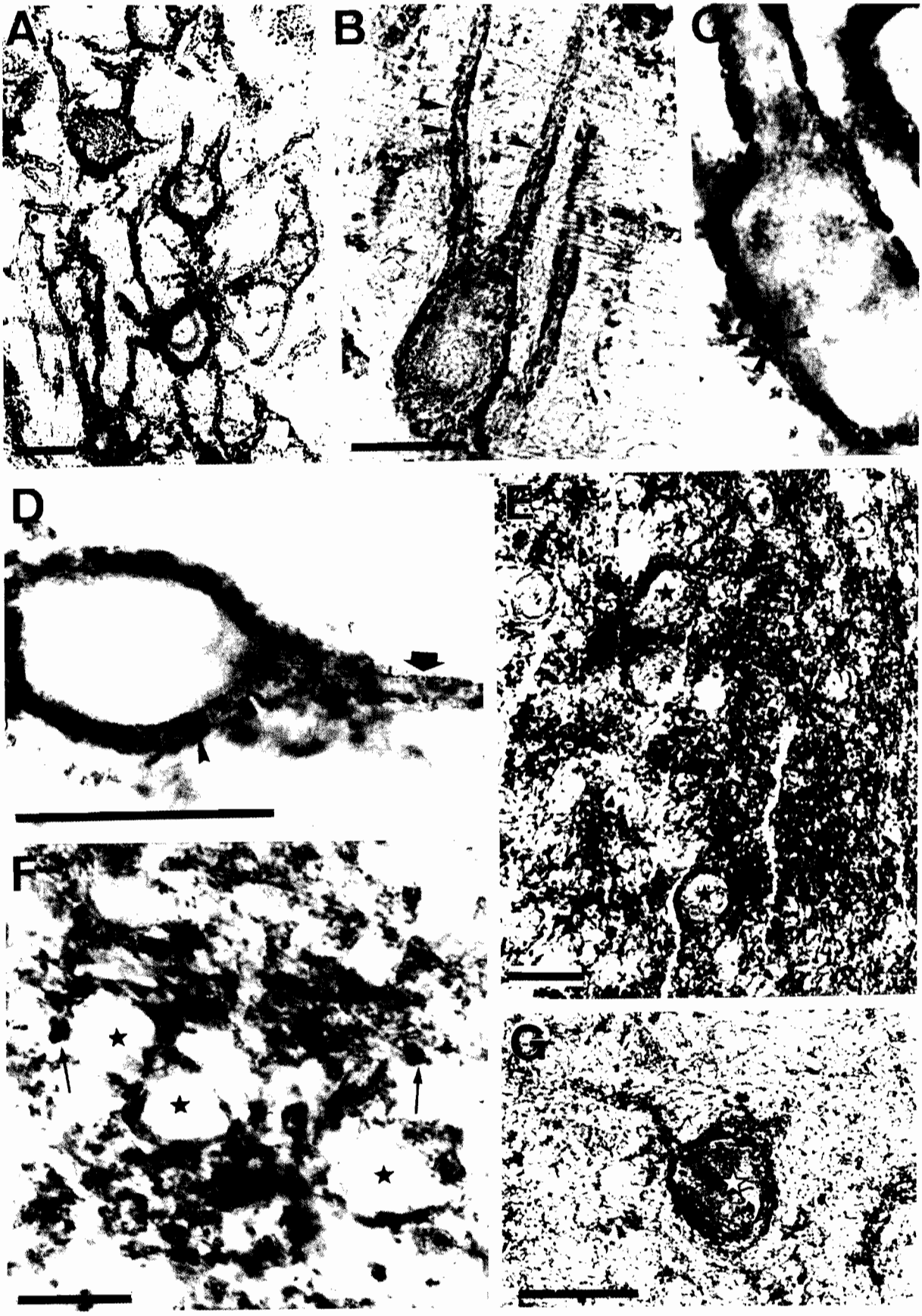

A
B
C
D
E
F
G

Fig. 4. High magnifications of several neuron types of the PVCN and DCN, which show SY-immunolabelled terminal boutons (PVCN) and a dense network of multiple small immunoreactive dots (DCN). Immunocytochemical detection of SY on the octopus neurons **(A-D)**. Octopus neurons show SY-immunolabelled boutons mainly surrounding the soma (CD) and the dendritic tree (**B**, arrowheads). In a second type of octopus neurons, the SY-immunoreactive boutons are observed restricted mainly to the neuronal cell body (**D**, arrowhead); the dendrites are only covered in a very small and proximal part (**D**, thick arrow). SY-immunoreactivity in the three main layers of the DCN: superficial **(E)**, deep **(F)** and central **(G)** areas. The neuropil presents a granular staining pattern **(E)**. Numerous scattered small SY-immunostained terminal boutons surround the neuron cell bodies (**E**, asterisk). The single larger boutons (**F**, arrows) are strongly immunoreactive in the deep region of the DCN. Stars show some neuron cell bodies, located in the deep layer **(F)**. In the central area of the DCN the SY-immunoreactive terminal boutons surround the neuron cell body (**G**, empty star) and the dendritic tree of a giant neuron. Scale bar: 25 μm.

relationship between SY-immunoreactive synapses and the tonotopic distribution in the CN.

Acknwoledgements. The authors are grateful to Mrs. T. Rodríguez for technical assistance, and Mr. D. Doig for correction of the English version manuscript. This work has been supported by the Spanish DGICYT 92/0228 and Human Capital and Mobility ERBCHRXCT940659.

References

Bartolomé M.V., Ibáñez M.A., López-Sánchez J.G., Merchán-Pérez A. and Gil-Loyzaga P. (1993). Synaptophysin immunoreactivity in the cochlear nuclei of mammals: a comparative study. J. Otorhinolaryngol. Relat. Speciality (ORL) 55, 317-321.

Benson T.E. and Brown M.C. (1990). Synapses formed by olivocochlear axon branches in the mouse cochlear nulcues. J. Comp. Neurol. 295, 52-70.

Bergmann M., Schuster Th., Grabs D., Marquèze-Pouey B., Betz H., Trauring H., Mayerhofer A. and Gratzl M. (1993). Synaptophysin and synaptoporin expression in the developing rat olfactory system. Dev. Brain Res. 74, 235-244.

Brawer J.R. and Morest D.K. (1975). Relations between auditory nerve endings and cell types in the cat's anteroventral cochlear nucleus seen with the Golgi method and Nomarski optics. J. Comp. Neurol. 160, 491-506.

Brawer J.R., Morest D.K. and Kane E.C. (1974). The neuronal architecture of the cochlear nucleus of the cat. J. Comp. Neurol. 155, 251-300.

Brown M.C., Liberman M.C., Benson T.E. and Ryugo D.K. (1988). Brainstem branches from olivocochlear axons in cats and rodents. J. Comp. Neurol. 278, 591-603.

Cant N.B. (1981). The fine structure of two types of strellate cells in the anterior division of the anteroventral cochlear nucleus of the cat. Neuroscience 6, 2643-2655.

Cant N.B. and Gaston K.C. (1982). Pathways connecting the right and left cochlear nuclei. J. Comp. Neurol. 212, 313-326.

Cant N.B. and Morest D.K. (1979a). Organization of the neurons in the anterior division of the anteroventral cochlear nucleus of the cat, Light-microscopic observations. Neuroscience 4, 1909-1923.

Cant N.B. and Morest D.K. (1979b). The bushy cells in the anteroventral cochlear nucleus of the cat. A study with the electron microscope. Neuroscience 4, 1925-1945.

Collinge C. and Schweitzer L. (1991). Details of the central projections of the cochlear nerve in the hamster revealed by the fluorescent tracer DiI. Hearing Res. 53, 159-172.

Conlee J.W. and Kane E.S. (1982). Descending projections from the interior colliculus to the dorsal cochlear nucleus in the cat: an autoradiographic study. Neuroscience 7, 161-178.

De Camilli P. and Jahn R. (1990). Pathways to regulated exocytosis in neurons. Annu. Rev. Physiol. 52, 625-645.

De Camilli P. and Takei K. (1996). Molecular in synaptic vesicle endocytosis and recycling. Neuron 16, 481-486.

Fekete D.M., Rouiller E.M., Liberman M.C. and Ryugo D.K. (1984). The central projections of intracellularly labeleld auditory nerve fibers in cats. J. Comp. Neurol. 229, 432-450.

Gentschev T. and Sotelo C. (1973). Degenerative patterns in the ventral cochlear nucleus of the rat after primary deafferentation. An ultrastructural study. Brain Res. 62, 37-60.

Gil-Loyzaga P. and Pujol R. (1988). Synaptophysin in the developing cochlea. Int. J. Dev. Neurosci. 6, 155-160.

Gil-Loyzaga P., López-Sánchez J.G. and Merchán-Pérez A. (1991). Synaptophysin immunoreactivity in the cochlear nuclei of the rat. In: The mammalian cochlear nuclei. Organization and function. Nato Advanced Res. Workshop. Salamanca. Spain. pp 114-115.

Gil-Loyzaga P., Bartolomé M.V. and Hernández E. (1996). Effect of unilateral cochlea removal on synaptophysin expression in the rat cochlear nuclei. IEB 33rd Workshop Utrech. p 62 (Abstract).

Hackney C.M., Osen K.K. and Kolston J. (1990). Anatomy of the cochlear nuclear complex of guinea pig. Anat. Embryol. 182, 123-149.

Hackney C.M., Osen K.K., Ottersen O.P., Storm-Mathisen J. and Manjaly G. (1996). Immunocytochemical evidence that glutamate is a neurotransmitter in the cochlear nerve: a quantitative study in the guinea-pig anteroventral cochlear nucleus. Eur. J. Neurosci. 8, 79-91.

Harrison J.M. and Irving R. (1965). The anterior ventral cochlear nucleus. J. Comp. Neurol. 124, 15-42.

Harrison J.M. and Irving R. (1966). The organization of the posterior ventral cohlear nucleus in the rat. J. Comp. Neurol. 126, 391-402.

Hashisaki G.T. and Rubel E. (1989). Effects of unilateral cohlea removal on anteroventral cochlear nucleus neurons in developing gerbils. J. Comp. Neurol. 283, 465-473.

Jahn R., Schiebler W., Ouimet C. and Greengard P. (1985). A 38,000-dalton membrane protein (p38) present in synaptic vesicles. Proc. Natl. Acad. Sci. USA 82, 4137-4141.

Johnston P.A. and Südhof T.C. (1990). The multisubunit structure of synaptophysin. J. Biol. Chem. 2654, 8869-8873.

Johnston P.A., Jhan R. and Südhof T.C. (1989). Transmembrane topography and evolutionary conservation of synaptophysin. J. Biol. Chem. 264, 1268-1273.

Kane E.C. (1973). Octopus cells in the cochlear nucleus of the cat: heterotypic synapses upon homeotypic neurons. Int. J. Neurosci. 5, 251-279.

Kiang N.Y.S., Rho J.M., Northrup C.C., Liberman M.C. and Ryugo D.K. (1982). Hair-cell innervation by spiral ganglion cells in adult cats. Science 217, 175-177.

Kolston J., Osen K.K., Hackeney C.M., Ottersen O.P. and Strom-Mathisen J. (1992). An atlas of glycine- and GABA-like immunoreactivity and colocalization in the cochlear nuclear complex of the guinea pig. Anat. Embryol. 186, 443-465.

Korematsu K., Goto S., Nagahiro S. and Ushio Y. (1993). Changes of immunoreactivity for synaptophysin («protein p38») following a transient cerebral ischemia in the rat striatum. Brain Res. 616, 320-324.

Lasiter P.S. and Kachele D.L. (1989). Postnatal development of protein P-38 («synaptophysin») immunoreactivity in pontine and medullary gustatory zones of rat. Dev. Brain Res. 48, 27-33.

Leclerc N., Bessly P.W., Brown I., Colonnier M., Gurd J.W., Paladino T. and Hawkes R. (1989). Synaptophysin expression during synaptogenesis in the rat cerebellar cortex. J. Comp. Neurol. 280, 197-212.

Leube R.E. (1994). Expression of the synaptophysin gene family is not restricted to neuronal and neuroendocrine differentiation in rat and human. Differentiation 56, 163-171.

Liberman M.C. and Oliver M.E. (1984). Morphometry of intracellularly labelled neurons of the auditory nerve: correlations with functional properties. J. Comp. Neurol. 223, 163-176.

Lorente de Nó R. (1981). The primary acoustic nuclei. Raven Press. New York.

Masliah E., Terry R.D., Alford M. and DeTeresa R. (1990). Quantitative immunohistochemistry of synaptophysin in human neocortex: an alternative method to estimate density of presynaptic terminals in paraffin sections. J. Histochem. Cytochem. 38, 837-844.

McMahon H.T., Bolshakov V.Y., Janz R., Hammer R.E., Siegelbaum S.A. and Südhof T.C. (1996). Synaptophysin, a major synaptic vesicle protein, is not essential for neurotransmitter release. Proc. Natl. Acad. Sci. USA 93, 4760-4764.

Merchán-Pérez A., López-Sánchez J.G., Valderrama F., Ibáñez M.A., Bartolomé M.V. and Gil-Loyzaga P. (1992). Effects of perinatal deafness on synaptophysin expression in the cohlear nuclei of the rat. 5th Internaitonal Congress on Cell Biology. Madrid. Spain.

Merchán-Pérez A., Gil-Loyzaga P., López-Sánchez J.G., Eybalin M. and Valderrama F.J. (1993). Ontogeny of gamma-aminobutyric acid in efferent fibers to the rat cochlea. Dev. Brain Res. 76, 33-41.

Moore J.K. (1986). Cochlear nuclei: relationship to the auditory nerve. In: Neurobiology of hearing the cochlea. Altschuler R.A., Hoffman D.W. and Bobbin R.P. (eds). Raven Press. New York. pp 283-301.

Morest D.K., Hutson K.A. and Kwok S. (1990). Cytoarchitectonic atlas of the cochlear nucleus of the chinchilla, *Chinchilla laniger*. J. Comp. Neurol. 300, 230-248.

Navone F., Jahn R., DiGioia G., Strukenbrok H., Greengard P. and De Camilli P. (1986). Protein p38: an integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. J. Cell Biol. 103, 2511-2527.

Oberdorfer M.D., Parakkal M.H., Altschuler R.A. and Wenthold R.J. (1988). Ultrastructural localization of GABA-immunoreactive terminals in the anteroventral cochlear nucleus of the guinea pig. Hearing Res. 33, 229-238.

Osen K.K. (1969). Cytoarchitecture of the cochlear nuclei in the cat. J. Comp. Neurol. 136, 453-482.

Ostapoff E.M. and Morest D.K. (1991). Synaptic organization of globular bushy cells in the ventral cochlear nucleus of the cat: a quantitative study. J. Comp. Neurol. 314, 598-613.

Ovscharoff W., Bergmann M., Marquèze-Pouey B., Knaus P., Betz H., Grabs D., Reisert I. and Gratzl M. (1993). Ontogeny of synaptophysin and synaptoporin in the central nervous system: differential expression in striatal neurons and their afferents during development. Dev. Brain Res. 72, 219-225.

Perry D.R. and Webster W.R. (1981). Neuronal organization of the rabbit cochlear nucleus: some anatomical and electrophysiological observations. J. Comp. Neurol. 197, 623-628.

Peyret D., Geffard M. and Aran J.M. (1986). GABA immunoreactivity in the primary nuclei of the auditory central nervous system. Hearing Res. 23, 115-121.

Ramón y Cajal S. (1911). Histologie du systeme nerveux de l'Homme et des Vertébrés. Maloine. París.

Rehm H., Wiedenmann B. and Betz H. (1986). Molecular characterization of synaptophysin, a major calcium-binding protein of the synaptic vesicle membrane. EMBO J. 5, 535-541.

Roberts R.C. and Ribak C. (1987). GABAergic neurons and axon terminals in the brainstem auditory nuclei of the gerbil. J. Comp. Neurol. 258, 267-280.

Rouiller E.M., Cronin-Schreiber R., Fekete D.M. and Ryugo D.K. (1986). The central projections of intracellularly labelled auditory nerve fibres in cats: an analysis of terminal morphology. J. Comp. Neurol. 249, 261-278.

Rubenstein J.L., Greengard P. and Czernik A.J. (1993). Calcium-dependent serine phosphoroylation of synaptophysin. Synapse 13, 161-172.

Ryan A.F., Keithley E.M., Wang Z.X. and Schwartz I.R. (1990). Collaterals from lateral and medial olivococlear efferent neurons innervate different regions of the cochlear nucleus and adjacent brainstem. J. Comp. Neurol. 300, 572-582.

Ryugo D.K. and Fekete D.M. (1982). Morphology of primary axomatic endings in the anteroventral cochlear nucleus of the cat: a study of the endbulbs of Held. J. Comp. Neurol. 210, 239-257.

Ryugo D.K. and Rouiller E.M. (1988). Central projections of intracellularly labelled auditory nerve fibers in cats: morphometric correlations with physiological properties. J. Comp. Neurol. 271, 130-142.

Ryugo D.K. and Sento S. (1991). Synaptic connections of the auditory nerve in cats: relationship betwen endbulbs of Held and spherical bushy cells. J. Comp. Neurol. 305, 35-48.

Sainte Marie R.L., Morest D.K. and Brandon C.J. (1989). The form and distribution of GABAergic synapses on the principal cell types of the ventral cochlear nucleus of the cat. Hearing Res. 42, 97-112.

Sainte Marie R.L., Benson C.G., Ostapoff E.M. and Morest D.K. (1991). Glycine immunoreactive projections from the dosrsal to the anteroventral cochlear nucleus. Hearing Res. 51, 11-28.

Schweitzer L. (1990). Differentiation of apical, basal and mixed dendrites of fusiform cells in the cochlear nucleus. Dev. Brain Res. 56, 19-27.

Smith P.H. and Rhode W.S. (1989). Structural and functional properties distinguish two types of multipolar cells in the ventral cochlear nucleus. J. Comp. Neurol. 282, 595-616.

Südhof T.C. (1995). The synaptic vesicle cycle: a cascade of protein-protein interacitons. Nature 375, 645-653.

Südhof T.C. and Jahn R. (1991). Proteins of synaptic vesicles involved in exocytosis and membrane recycling. Neuron 6, 665-677.

Thomas L., Hartung K., Langosh D., Rehm H., Bamberg E., Franke W.W. and Betz H. (1988). Identification of synaptophysin as a hexameric channel protein of the synaptic vesicle membrane. Science 242, 1050-1053.

Tixier-Vidal A., Barret A., Faivre-Bauman A., Huttner W. and

Widenmann B. (1992). Differential expression and subcellular lcoalization of secretogranin II and synaptophysin during early development of mouse hypothalamic neurons in culture. Neuroscience 47, 967-978.

Trimble W.S. and Schller R.H. (1988). Molecular biology of synaptic vesicle-associated proteins. Trends Neurosci. 11, 241-242.

Vater M., Kössl M. and Horn A. (1992). GAD- and GABA-immuno-reactivity in the ascending auditory pathway of horseshoe and mustached bats. J. Comp. Neurol. 325, 183-206.

Walaas S.I., Jahn R. and Greengard P. (1988). Quantification fo nerve terminal populations: synaptic vesicle-associated proteins as markers for synaptic density in the rat neostriatum. Synapse 2, 516-520.

Webster D.B. and Trune D.R. (1982). Cochlear nuclear complex of mice. Am. J. Anat. 163, 103-130.

Wenthold R.J., Zembel J.M., Parakkal M.H., Reeks K.A. and Altschuler R.A. (1986). Immunocytochemical localization of GABA in the cochlear nucleus of the guinea pig. Brain Res. 380, 7-18.

Wenthold R.J., Huie D., Atschuler R.A. and Reeks K.A. (1987). Glycine immunoreactivity localized in the cochlear nucleus and superior olivary complex. Neuroscience 22, 897-912.

Wiedenmann B. and Franke W.W. (1985). Identification and localization of synaptophysin, an integral membrane glycoprotien of Mr 38,000 characteristic of presynaptic vesicles. Cell 41, 1017-1028.

Wiedenmann B., Franke W.W., Kuhn C., Moll R. and Gould V.E. (1986). Synaptophysin: a marker protein for endocrine cells and neoplasms. Proc. Natl. Acad. Sci. USA 83, 3500-3504.

Winter I.A., Robertson D. and Cole K.S. (1989). Descending projections from auditory nuclei to the cochlea and cohlear nucleus of the guinea pig. J. Comp. Neurol. 280, 143-157.

Accepted November 6, 1997