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Transitional expression of OX-2 and GAP-43 glycoproteins in developing rat cochlear nerve fibers

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Summary. The OX-2 and GAP-43 glycoproteins are two proteins involved in neuronal cell-to-cell interaction and/or growing of dendrites and axons. Therefore, for the auditory receptor the expression of these proteins could provide information on the afferent and efferent nerve fiber organization. The expression and distribution of OX-2 and GAP-43 were analyzed during the auditory receptor development and maturation (from embryonic day E13 to postnatal day P22). Both glycoproteins were early recognized in the cochleae of E13 rats. Then, they slowly but progressively disappeared, being absent when the animals reached the P22 postnatal day. At E13, a weak OX-2 expression was restricted to the perikaryon of the spiral ganglion neurons, while in the same period a strong GAP-43 immunostaining was found in both the neuronal perikaryon and the neurites. During the rat embryonic period (E13 to birth) the expression of both glycoproteins appeared progressively restricted to the neurites. During the rat postnatal period (P0 to P22), OX-2 and GAP-43 exhibited a dissimilar distribution pattern. The OX-2 glycoprotein appeared in the afferent, efferent and fibers of the auditory nerve, while the GAP-43 glycoprotein only appeared in the efferent nerve fibers. Present data suggest that OX-2 and GAP-43 could act as two complementary glycoproteins during the development, organization, and maturation of the cochlear nerve fibers. While both glycoproteins could participate in axonal growing and orientation, OX-2 could also be involved in a similar process for auditory dendrites.

Key words: OX-2, GAP-43, Development, Afferent system, Efferent system, Cochlea, Auditory system, Rat.

Introduction

The adult mammalian organ of Corti receives two kinds of innervation: afferent nerve fibers from spiral ganglion neurons, and efferent nerve fibers from the olivocochlear system (Pujol et al., 1986, Warr, 1992; Eybalin, 1993). The afferent nerve fibers originate from two different types of neurons, bipolar and pseudomonopolar, of the spiral ganglion of Corti (Pujol and Lenoir, 1986; Echteler, 1992). The efferent nerve fibers originate from neurons located at the lateral and medial olivocochlear complex of the brainstem (Pujol and Lenoir, 1986; Robertson et al., 1989; Warr, 1992; Eybalin, 1993; Gil-Loyzaga, 1995). Afferent and efferent innervation peripherally project on sensory hair cells by specific pathways. While neuronal radial processes of bipolar neurons reach the basal pole of the inner hair cells (IHCs), the corresponding spiral processes of pseudomonopolar neurons innervate the outer hair cells (OHCs). In addition, efferent fibers from olivocochlear medial system innervate the OHCs, while fibers from the olivocochlear lateral system establish synaptic connection with afferent fibers of the bipolar neurons (Warr, 1992; Eybalin, 1993). Finally, the spiral ganglion neurons project their axons to the brainstem, forming the auditory nerve (Warr, 1992).

The early development and morphogenetic maturation of the otic vesicle into two systems, vestibular and cochlear, have been largely analyzed (see review in: Pujol and Sans, 1986). Cochlear anlage, as early as the E13 rat embryonic day, showed an undifferentiated cochleo-vestibular ganglion, and also an undifferentiated and multilayer epithelium (Mbiène et al., 1989). During the embryonic and postnatal period, the afferent and efferent nerve fibers progressively reach the cochlear epithelium following a similar, gradual and polarized base-to-apex innervation process. There is a similar pattern for several mammalian species, including man (Lenoir et al., 1980; Pujol and Lavigne-Rebillard, 1985; Gil-Loyzaga et al., 1989; Mbiéne et al., 1989; Merchán-Pérez et al., 1990, 1993a, 1994). Electrophysiological studies also confirmed the functional periods of the auditory receptor (Anggard, 1965; Uziel et al., 1981).

During cochlear development, the set-up of specific connections of the auditory receptor depends on several processes. The processes include: the adhesion of cell-to-cell or to extracellular matrices (Mbiène et al., 1989; Vázquez et al., 1994; Gil-Loyzaga, 1997); the synthesis

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of neurofilaments (Romand et al., 1990; Hafidi et al., 1990, 1993); the effects of both nerve growth factor (Després et al., 1991) or neurotrophins (Després and Romand, 1994). All these processes influence axonal growth (Merchán-Pérez et al., 1993a, Knipper et al., 1995; Simmons et al., 1996); synaptogenesis (Lenoir et al., 1980; Gil-Loyzaga and Pujol, 1988); and neuronal maturation (Hafidi et al., 1990; Woolf et al., 1992).

Therefore, the expression of cell-adhesion glycoproteins clearly influences the cell-to-cell surface interactions during mammalian development of the cochlear and/or vestibular systems (Edelman, 1984; Thor et al., 1986; Richardson et al., 1987; Mbiène et al., 1989; Hafidi et al., 1990; Van Lookeren et al., 1990). Among others, some glycoproteins of the neuronal cell surface like Thy-1 (anti-Thy-1 called OX-7) (LaRocca and Wiley, 1988) have been involved in cell recognition and morphogenesis during early cochlear development (Richardson et al., 1987; Terkelsen et al., 1989).

The OX-2 is an immunoglobulin similar to Thy-1 (LaRocca and Wiley, 1988), which has been identified as being involved in cell-to-cell interactions, neuronal fiber fasciculation process and/or the development of neuronal networks (Barclay and Ward, 1982). Thy-1 expression in mouse embryo cochlea was found in perikaryon and dendrites (Terkelsen et al., 1989). The OX-2 glycoprotein is also expressed in neuronal cell bodies and processes during brain development, and could be involved in neuronal cell-to-cell interactions (Webb and Barclay, 1984; Williams, 1985), in prenatal (Barclay and Ward, 1982; Clark et al., 1985; Morris and Beech, 1987) and postnatal rat cerebellum (Webb and Barclay, 1984).

The growth association protein (also called GAP-43, B-50, neuromodulin, F1 or pp46) is a calmodulin-binding phosphoprotein located in growing axons and growth cones associated with both developing and regenerating axons (Meiri et al., 1986; Skene et al., 1986; Benowitz and Routtenberg, 1987; Benowitz et al., 1987; Gispen et al., 1991). In the central nervous system of the rat, GAP-43 is located in neuropil-rich brain areas associated with presynaptic membranes (Sorensen et al., 1981; Gispen et al., 1991). In vitro studies showed that GAP-43 protein was restricted to the axons of developing neurons (Van der Neut et al., 1990; Burry et al., 1991) and the dendrites (Goslin et al., 1988; DiFiglia et al., 1990). Moreover, GAP-43 has been previously involved in specific connective processes of the developing sensory retina epithelium (Kapfhammer et al., 1994), the olfactory epithelium (Verhaagen et al., 1989, 1990; Schwob et al., 1994) and the auditory receptor (Merchán-Pérez et al., 1993a, Knipper et al., 1995; Simmons et al., 1996).

The aim of the present study was to analyze the complementary expression of both OX-2 and GAP-43 glycoproteins, starting from the early development (E13) of the rat cochlea until their disappearance at about the time of the functional maturation (P22) of the auditory receptor. The expression of these glycoproteins during cochlear development could contribute to a better knowledge of the highly particular innervation pattern exhibited by the auditory receptor in adulthood.

Material and methods

Animals

In this study Long-Evans pigmented rats were used. The care and experimental use of all animals of this study were in strict accordance with the animal welfare guidelines of the Declaration of Helsinki

Cochlear tissue preparation.

Three rats were used for each analyzed stage (E13, E18, E20, P0, P3, P6, P9, P12, P15, P22). The day of the positive vaginal plug was considered as embryonic day 0 (E0) and as postnatal day 0 (P0) the day of the birth.

Embryos: E13 and E18

Under deep general anesthesia (chloral-hydrate 300mg/kg b.w.), pregnant rats were sacrificed, and E13 and E18 embryos were anesthetized with ether, quickly decapitated, and fixed by immersion in a 2% acetic acid in 98% ethanol solution. Samples were then dehydrated, embedded in paraffin, and cut in serial frontal, horizontal, mid-modiolar, or perpendicular plane to the mid-modiolar axis, sections of 9 µm thickness

Embryos (E20), postnatal and adulthood

The older embryos (E20), postnatal, and adult rats, were placed under deep general anesthesia, (chloral-hydrate 300mg/kg b.w.), the cochleae were rapidly removed and were quickly fixed in a 2% acetic acid in 98% ethanol solution. All cochleae samples were postfixed in the same fixative solution for about 72h. When necessary (from P9 on), the cochleae were decalcified (during 1 to 3 days) in a saline solution containing 1% of ascorbic acid (Merchán-Pérez et al., 1999). The samples were then dehydrated, embedded in paraffin, and cut in serially mid-modiolar sections of 9 µm thickness.

Immunocytochemical procedures

Similar immunocytochemical procedures were used for both OX-2 and GAP-43 antibodies. The sections were rinsed three times, for 5 min each, in 0.1M phosphate buffered saline (PBS) at pH 7.33. Pre-incubation was carried out for 30 min in a PBS solution containing 30% of horse normal serum. The sections were then incubated overnight at 4 °C in a solution containing 1/100 anti-OX-2 (MCA 44, Batch Nº 4682, Serotec, Oxford), or 1/250 anti-GAP-43 monoclonal antibodies (Oncogene Science). After three washings (5 min each one) in PBS, the sections were incubated for 1h in biotinylated horse anti-mouse IgG (Vectastin,

Vector) 1/200 in PBS. Antigen-antibody immunoreaction was revealed using avidin-biotin-peroxidase method (Vectastin, Vector) (Merchán-Pérez et al., 1993a,b; Gil-Loyzaga et al., 1997).

Negative controls were carried out in all different stages and for both antibodies by omission of the primary antibody and using the same procedure described above.

Results

Present results showed the expression of the two glycoproteins OX-2 and GAP-43 during cochlear development. The onset of expression for both glycoproteins, the following changes during the auditory receptor maturation, and the late disappearance of positive immunoreaction are summarized in Table I.

Early expression of OX-2 and GAP-43 in the cochlear anlage

At embryonic day E13, a columnar undifferentiated epithelium was the first anlage for the cochlear receptor (Fig. 1A); showing the cochlear ganglion anlage under it (Fig. 1A). A respectively weak with anti OX-2 (Fig. 1B) and strong with anti GAP-43 (Fig. 1C, 1D) positive immunoreactions appeared within the cochlear ganglion neurons and their neurites, at the basal coil.

At E18 a horizontal cochlear section (Fig. 2A) showed a strong OX-2 expression in cochlear ganglion neurons and their neurites (Fig. 2A-D). The OX-2 expression was mainly found in a peripheral bundle of the ganglion (Fig. 2A,D, thick arrows) and in the axons of the auditory nerve (Fig. 2A, arrowheads). A mid-modiolar section (Fig. 2B,C) showed a similar immunostaining pattern in different coils (Fig. 2B). At high magnification, afferent nerve fibers could be seen penetrating into the undifferentiated epithelium (Fig. 2C, arrows). Around the periphery of the cochlear ganglion, a dense net of OX-2 positives fibers was identified (Fig. 2D, thick arrows). A total absence of immunolabelling was observed in negative control sections (Fig. 2E).

From E18 on, a dramatic change was observed in GAP-43 expression within the cochlea. At embryonic day E18 the GAP-43 immunoreactive fibers were just found within the most peripheral part of the spiral ganglion at different plane sections (Fig. 3A-E, arrows). This distribution pattern is particularly evident in a perpendicular plane to the modiolar axis sections covering from the basal coil to the middle one (Fig. 3B). A mid-modiolar cochlear section showed the immunoreactive nerve fibers under the undifferentiated cochlear epithelium (Fig. 3D). These nerve fibers slightly penetrated into the undifferentiated epithelium (Fig. 3E,F, thin arrows), and they remained close to the access point of it.

At E20, the general morphology of the cochlear epithelium dramatically changed. The Reissner's membrane, the tectorial membrane and the spiral limbus were evident (Fig. 4A,B). The expression of OX-2 remained in the soma of the spiral ganglion neurons, afferent and efferent nerve fibers projecting on the developing organ of Corti (Fig. 4A), while GAP-43 expression exhibited a distribution more related to efferent nerve fibers (Fig. 4B). In a high magnification of the developing organ of Corti, GAP-43 expression appeared under the basal pole of IHCs (Fig. 4C, thick arrows), within the inner spiral bundle and, as well-defined dots located under the basal pole of OHCs (Fig. 4C, thin arrows).

Table 1. Immunocytochemical distribution of anti-OX-2 and anti-GAP-43 in cochlear spiral ganglion and organ of Corti innervation of rats, from embryonic E13 to postnatal day P22.

	OX-2	GAP 43
Ganglion neuronal perikarya	E13 - P3	E13 - E18
Onset of nerve fibers inside the undifferentiated epithelium	E18	E18
Only efferent immunostained fibers in the IGSB	P6 - P12	E18 - P12
Immunostained fibers under IHCs and OHCs	E20-P22	E20-P22

E: embryonic day; P: postnatal day; IGSB: intraganglionic spiral bundle; IHCs: inner hair cells; OHCs: outer hair cells.

Postnatal expression of OX-2 and GAP-43 in the cochlea

At birth (P0), OX-2 (Fig. 4D) and GAP-43 (Fig. 4E) expression showed a similar distribution pattern to that seen at the earlier embryonic day E20. At high magnification, the OX-2 immunostained fibers mainly projected on IHCs (Fig. 4F, arrowheads), while the expression of GAP-43 appeared under IHCs (Fig. 4G, thick arrows), as a dense packed immunostaining, and under the OHCs (Fig. 4G, thin arrows), as isolated dots.

At postnatal day P3, the OX-2 expression was observed within the perikaryon of spiral ganglion neurons, and within the afferent and efferent nerve fibers projecting to the organ of Corti (Fig. 5A). A very high GAP-43 expression was observed within the efferent nerve fibers under IHCs and OHCs (Fig. 5B). A panoramic view of the apical coil exhibited a strong GAP-43 positivity in nerve fibers projecting to the auditory receptor or in fibers of the auditory nerve (Fig. 5C).

From day P6 on, an important decrease in OX-2 expression in cell bodies of the spiral ganglion neurons was noted (Fig. 5D). However, a significant OX-2 immunolabelling was observed within the intraganglionic spiral bundle (Fig. 5D, arrowheads), and under the IHCs (Fig. 5E, thick arrows), being scarce under OHCs (Fig. 5E, thin arrows). The GAP-43 expression, at day P6, was considerable within the intraganglionic spiral bundle and in fibers reaching the auditory receptor (Fig. 5F, arrowheads). A high magnification of the same slide shows a strong

immunolabelling under IHCs (Fig. 5G, arrowheads), and OHCs (Fig. 5G, arrows).

From day P9 to day P22, the organ of Corti reached its adult shape (Fig. 6). At day P9, the rat organ of Corti showed a significant OX-2 expression located under IHCs, within the inner spiral bundle (Fig. 6A). Some scarce immunoreactive fibers were found crossing the Corti's tunnel, in a similar manner to olivocochlear medial efferent fibers (Fig. 6A, thin arrows). A very strong GAP-43 expression was observed at day P9 (Fig. 6B), in the inner spiral bundle, under IHCs and OHCs, and within some olivocochlear medial efferent fibers which crossed the Corti's tunnel (Fig. 6B, thin arrows).

At day P12, OX-2 expression was restricted to the

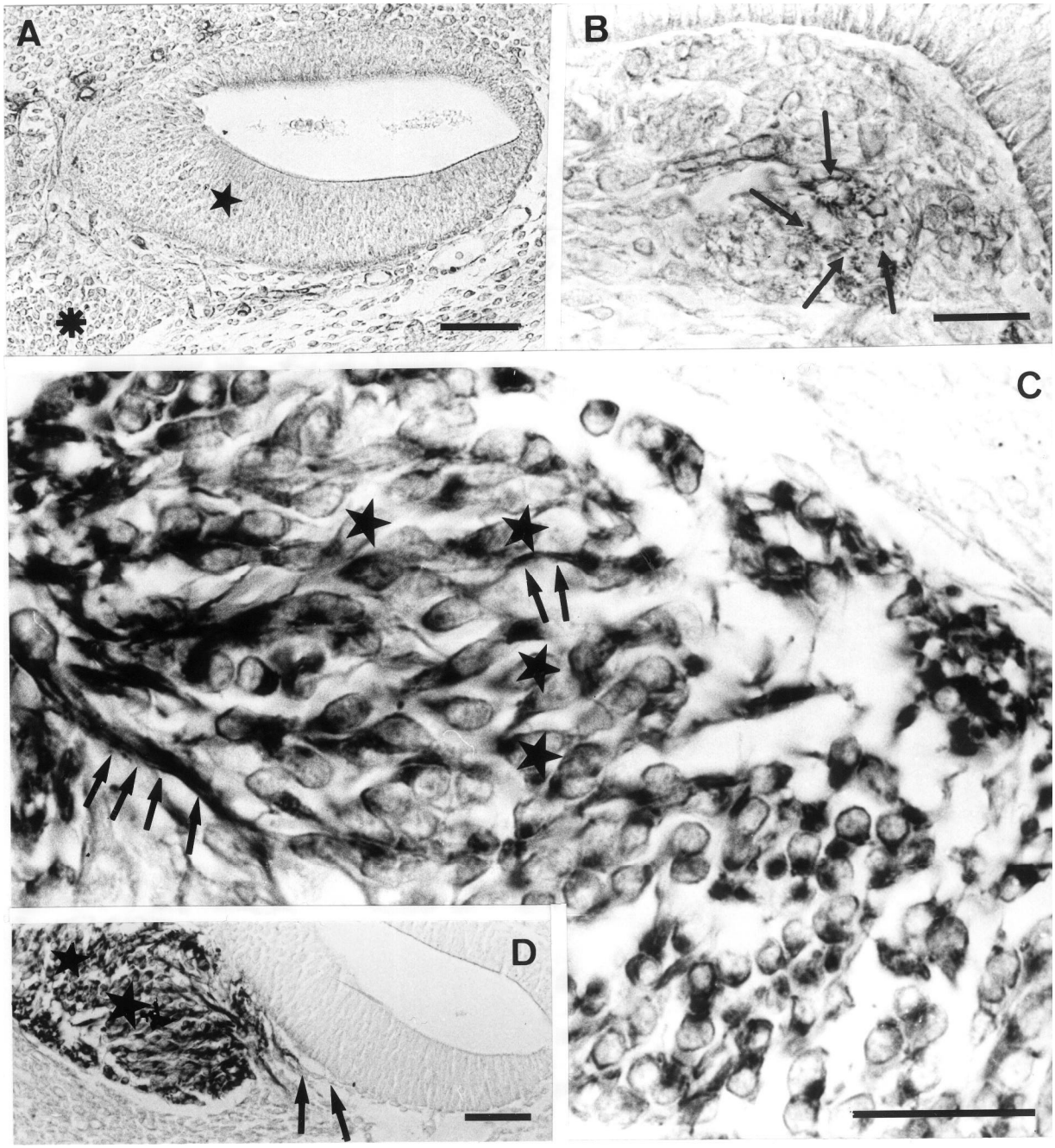


Fig. 1. Developing cochlea at embryonic day E13: the embryo head is cut on a frontal plane. The early otocyst shows a columnar epithelium (**A**, star) and a neighboring cochlear ganglion anlage (**A**, asterisk). A weak OX-2 immunoreactivity is observed in the cochlear ganglion anlage at the basal coil (**B,** arrows). GAP-43 immunoreactivity stains first afferent neurites (**C, D** arrows) and cochlear ganglion cell bodies (**C, D** stars) of the cochlear ganglion. Scale bars: A, D, 50 µm; B, C, 25 µm.

apical coil (Fig. 6C). A very slight OX-2 expression appeared around the IHCs (Fig. 6C). Also GAP-43 expression was highly reduced when compared to previous stages being restricted under and around IHCs (Fig. 6D). At day P15, the OX-2 expression was very scarce around IHCs (Fig. 6E). Lastly, at day P22 the expression of both OX-2 (Fig. 6F) and GAP-43 (Fig. 6G) was irrelevant.

Discussion

Present results have demostrated the transitional expression of two neural glycoproteins (OX-2 and GAP-43), that have been involved in neuronal differentiation and fasciculation. At the earlier stages, day E13, both the OX-2 and GAP-43 glycoproteins were found within the cell body and neurites of spiral ganglion neurons. During

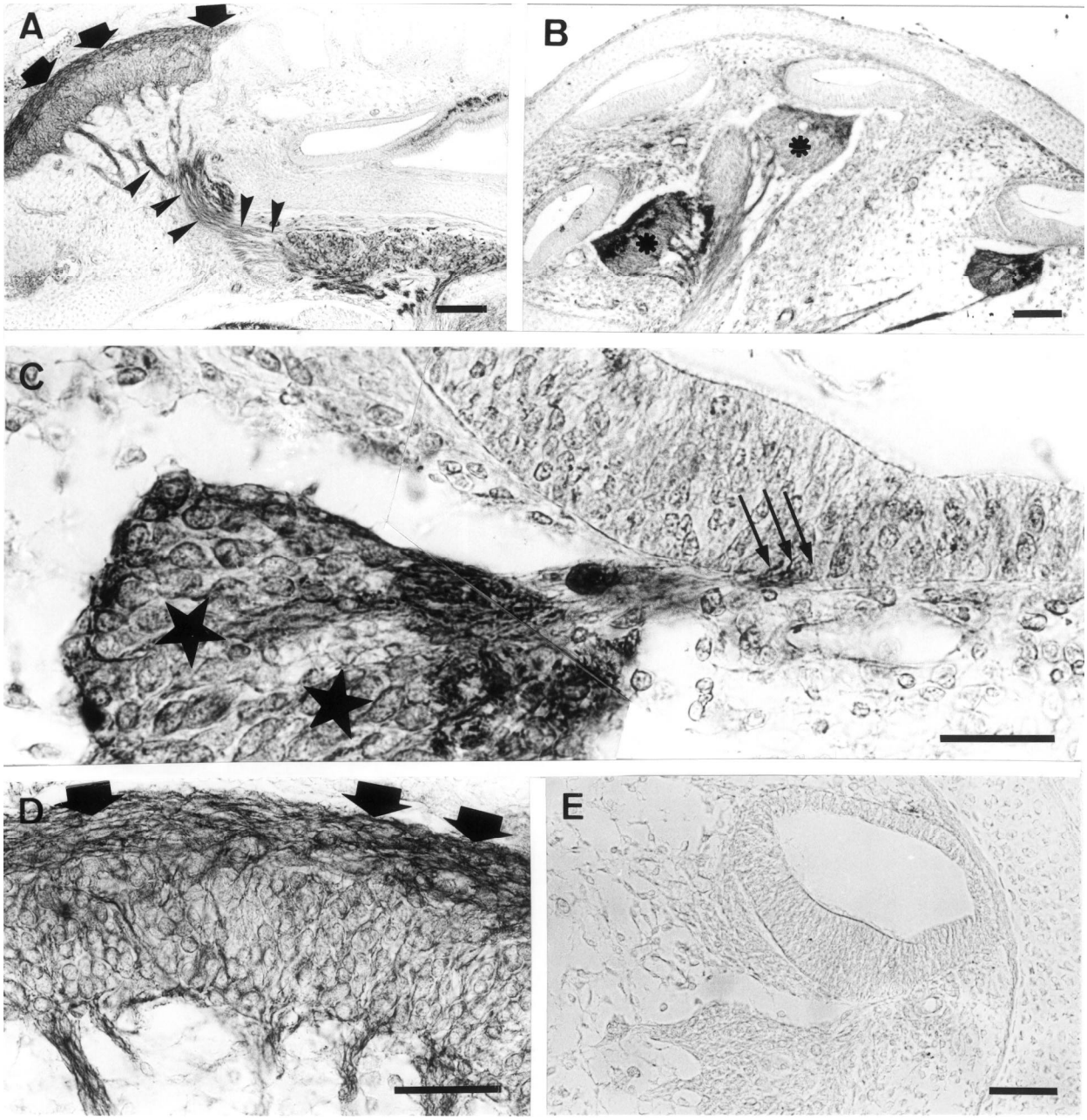


Fig. 2. The OX-2 immunoreactivity in the cochleae of E18 embryonic rat. Horizontal sections **(A-D)**, exhibit a positive OX-2 immunolabelling in efferent fibers innervation (**A, D**, thick arrows), and auditory nerve (**A**, arrowhead). Mid-modiolar sections show the OX-2 immunoreactivity mainly located in the spiral ganglions at different coils (**B**, asterisk). The cochlear basal coil **(C)** shows a strong OX-2 immunolabelling in neuronal cell bodies (**C**, stars) and their processes reaching the epithelium (**C**, arrows). A control section shows a total absence of immunolabelling **(E)**. Scale bars: A, B, 100 µm; C, 25 µm; D, E, 50 µm.

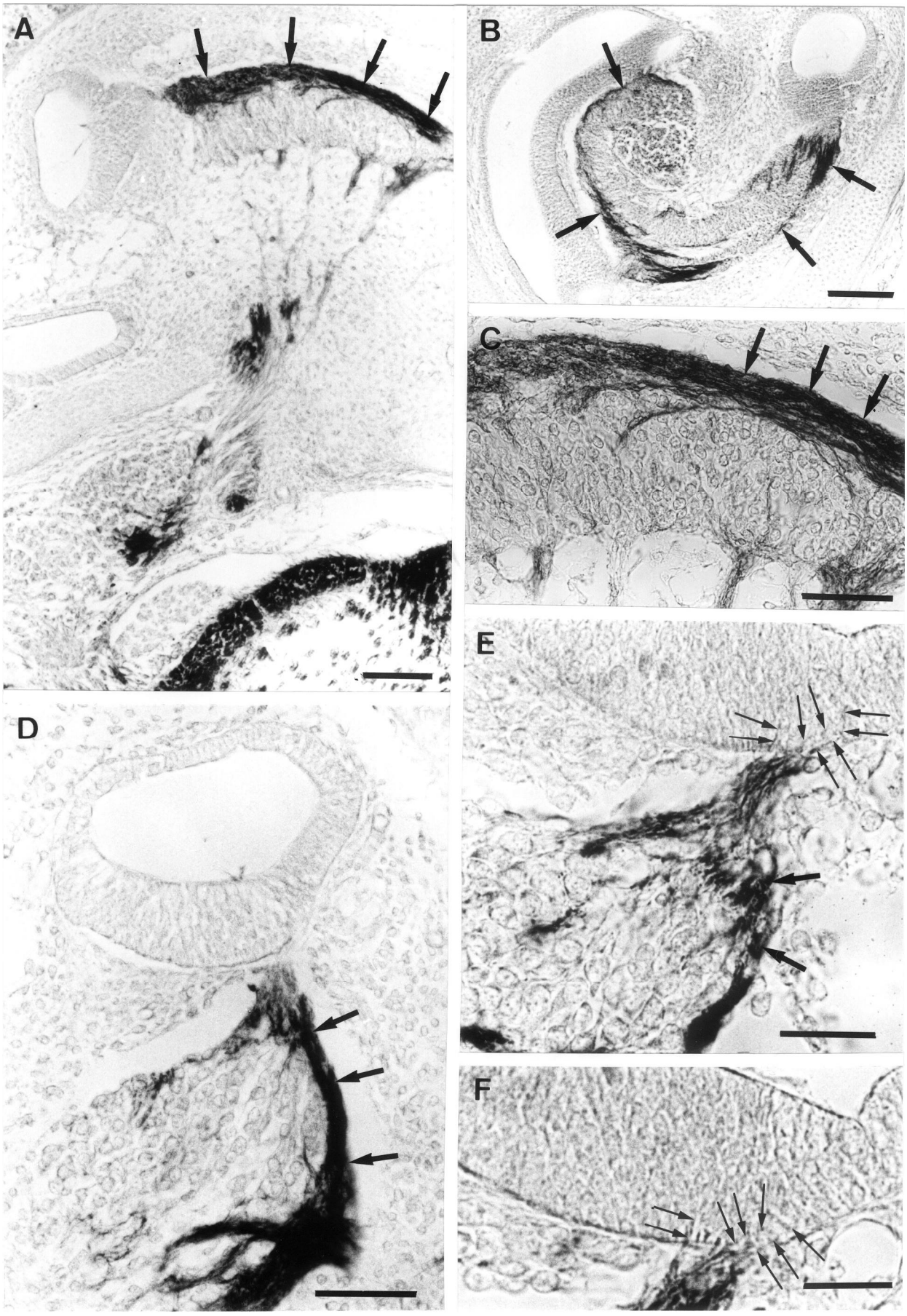


Fig. 3. The cochleae of embryonic day E18 show a strong GAP-43 immuno-labelling efferent fibers surround the peripheral region of the spiral ganglion and the intra-ganglionic spiral bundle. The distribution pattern of these fibers is observed in horizontal (**A, C**, arrows), perpendicular to the modiolar axis (**B,** arrows), and mid-modiolar sections of the middle coil (**D-F**, arrows). Also GAP-43 positive efferent fibers innervation are present into the cochlear un-differentiated epithelium (**E, F**, thin arrows). Scale bars: A, B, 100 µm; C, D, 50 µm; E, F, 25 µm.

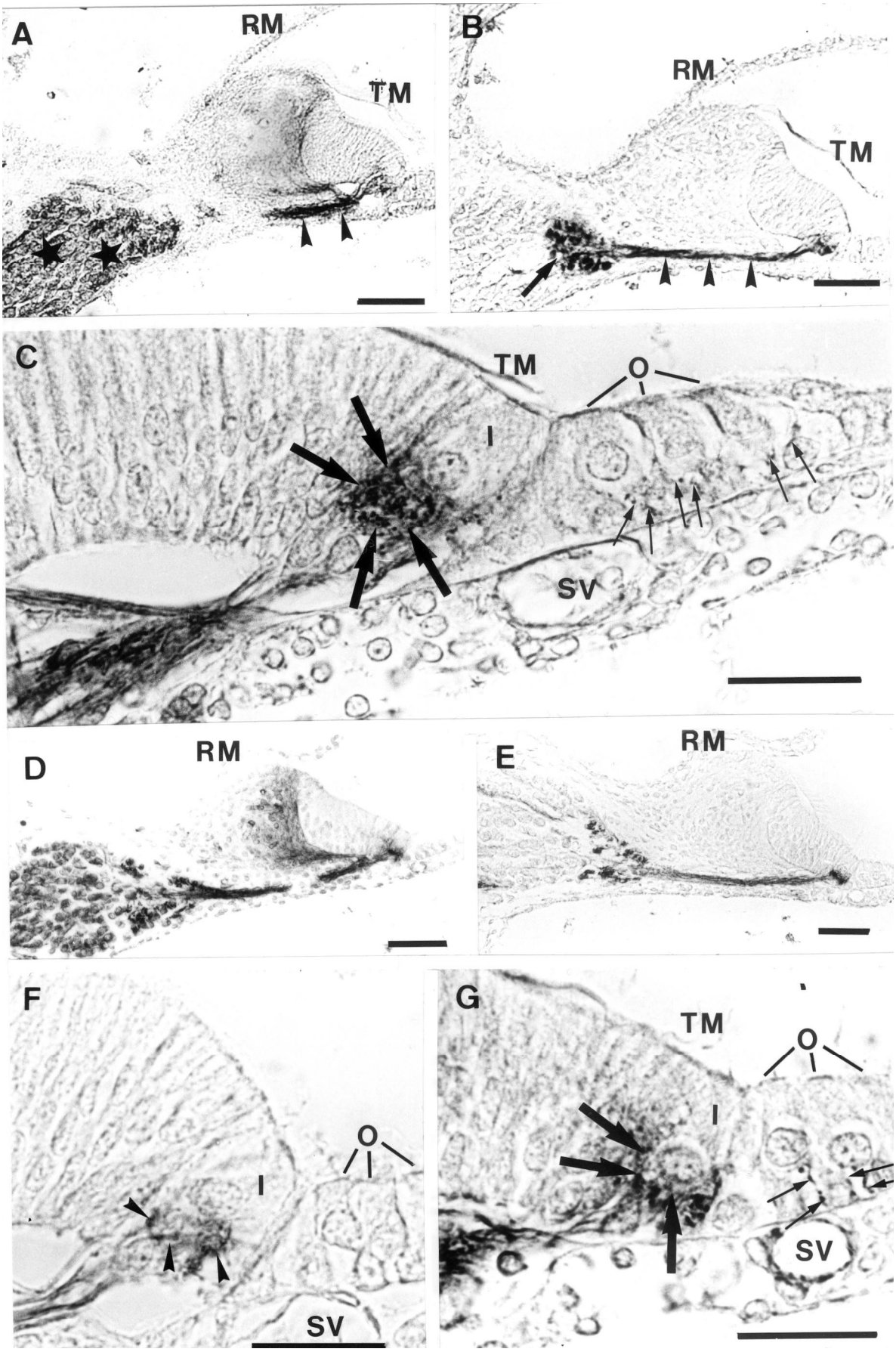


Fig. 4. Mid-modiolar sections of basal coil cochlea at the embryonic day E20. OX-2 immunolabelling is observed in the perikaryon of spiral ganglion neurons (**A**, stars) and the fibers reaching the auditory receptor (**A**, arrowheads). At the same embryonic day, the GAP-43 immunoreactivity appears within the intraganglionic spiral bundle (**B**, arrow) and the efferent nerve fibers reaching the cochlear epithelium (**B**, arrowheads). At high magnification, the GAP-43 immunoreactive fibers are observed under the basal pole of inner (I) hair cells (**C**, thick arrows) and outer (0) hair cells (**C**, thin arrows). At birth (P0) the OX-2 **(D, F)** and the GAP-43 **(E, G)** distribution pattern are similar to the previous developmental stages **(A, C)**. At high magnification, the OX-2 (**F**, arrowheads) and the GAP-43 (**G**, arrows) immunoreactive fibers surround the basal pole of inner (I) hair cells. Also, GAP-43 immunolabelling is noticed under the outer (0) hair cells (**G**, thin arrows). Scale bars: A, B, E, 50 µm; C, F, G, 25 µm.

the embryonic development (E18-E20), the most peripheral part of growing nerve fibers (filopodes) expressed both glycoproteins. This fact allowed the identification of the early arrival of embryonic nerve fibers to the undifferentiated auditory receptor to be made. During the postnatal development, the organization of the innervation pattern of the auditory receptor was identified by OX-2 and GAP-43 expression. At adulthood both glycoproteins finally disappeared.

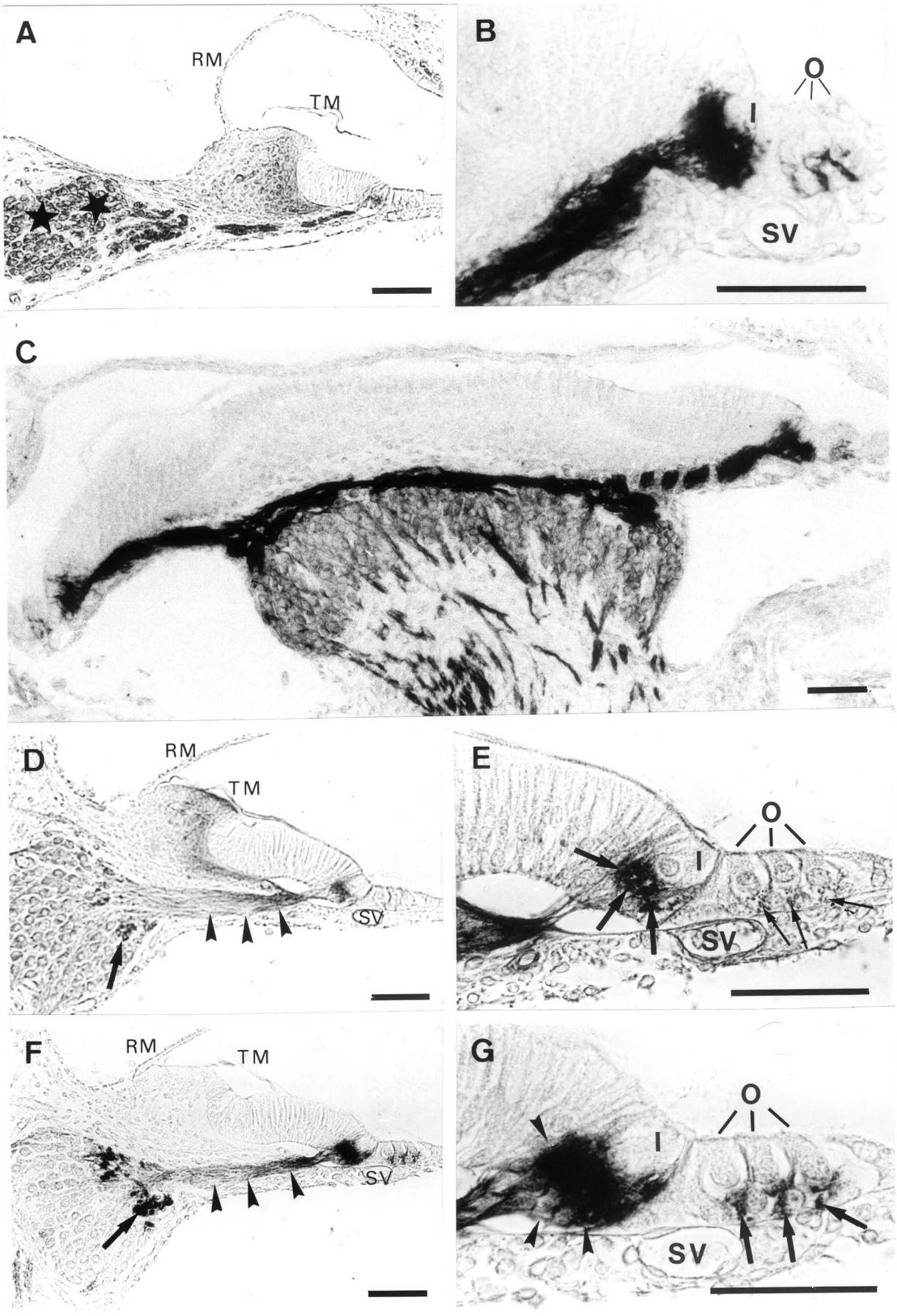
The cochlear innervation shows a base-to-apex pattern of maturation (Pujol and Sans, 1986) that has now been confirmed by the progressive base-to-apex expression and disappearance of OX-2 and GAP-43 glycoproteins. Also, another gradient has been pointed out for afferent to efferent nerve fibers. The afferent system starts to mature first, followed a little later by the efferent system (Lenoir et al., 1980; Pujol and Sans, 1986). This was also suggested by the analysis of the sensitivity to glutamate-agonists (Gil-Loyzaga and Pujol, 1990), by the synaptophysin expression (Gil-Loyzaga and Pujol, 1988; Knipper et al., 1995; Simmons et al., 1996), by the neurotransmitter expression (Merchán-Pérez et al., 1990, 1993b, 1994), and by the expression of neuroactive substances (e.g. CGRP, GABA, or ACh) (Gil-Loyzaga et al., 1989; Mérchan-Pérez et al., 1990; 1993b, 1994) in auditory nerve fibers and the electrophysiological maturation (Uziel et al., 1981; Puel and Uziel, 1987).

The OX-2 is a cell surface glycoprotein present in neuronal cell bodies and processes of the developing brain (Barclay and Ward, 1982; Webb and Barclay, 1984; Williams, 1985; Morris and Beech, 1987). Present findings also noted the OX-2 expression in the neuronal cell bodies and afferent dendrites during the embryonic and early postnatal period. However, during this period it can not be excluded that the olivocochlear efferent fibers also expressed this OX-2 glycoprotein. Conversely, from P6 on, OX-2 immunoreactivity was restricted to efferent nerve fibers, following a similar pattern to that largely reported for the mature olivocochlear efferent system. These findings could suggest that OX-2 glycoprotein early appeared on cell bodies and afferent fibers of spiral ganglion neurons, even though they could be expressed by efferent nerve fibers until adulthood. This progression could correspond to that previously reported as a gradient of maturation from afferent to efferent auditory nerve fibers (Lenoir et al., 1980; Pujol and Sans, 1986).

GAP-43 is a calmodulin-binding phosphoprotein classically found in neuronal growth cones after the determination of neuronal polarity (Skene et al., 1986, 1989; Benowitz and Routtenberg, 1987). This glycoprotein is inserted into the cytoplasmic face of the cell membrane in association with the membrane cytoskeletal components (Goslin et al., 1988; Burry et al., 1991; Ohno et al., 1994). It was suggested that GAP-43 glycoprotein is restricted to axons (Van der Neut et al., 1990; Burry et al., 1991) and dendrites (Goslin et al., 1988; DiFiglia et al., 1990) of developing neurons. In the auditory receptor the GAP-43 expression was found within efferent projections, specially during postnatal development (Merchán-Pérez et al., 1993a; Knipper et al., 1995; Simmons et al., 1996). However, the present report, which also focused on early GAP-43 expression, noted this expression in spiral ganglion neurons, including perikaryon and all neuronal projections, in particular at the earliest embryonic stages (before E18). These results fit well with the GAP-43 expression found in the whole cell body and projections of the developing neurons of retina and olfactory receptor (Verhaagen et al., 1989, 1990; Schwob et al., 1994; Kapfhammer et al., 1994). In developing neurons, GAP-43 has been involved in nerve fiber outgrowth, synapse formation and the onset of neurotransmitter release (Dekker et al., 1989; Kapfhammer et al., 1994), being down-regulated in the mature nervous system (Kapfhammer et al., 1994). The expression of OX-2 and GAP-43 in the auditory receptor (present results), which appeared at the beginning of the neuronal development of fasciculation, still remained during the synaptogenesis period (the onset was identified by synaptophysin expression) (Gil-Loyzaga and Pujol, 1988; Knipper et al., 1995; Simmons et al., 1996), and the early expression of neuroactive substances (e.g., CGRP, GABA, or ACh) (Gil-Loyzaga et al., 1989; Mérchan-Pérez et al., 1990, 1993b, 1994). In addition, our results have suggested that in late embryonic and postnatal stages confined the GAP-43 expression was restricted to efferent fibers. In addition GAP-43 expression in efferent fibers of the apical coil was previously reported in late maturation stages (P12) (Sobkowicz and Slapnick, 1994; Knipper et al., 1995). Lastly, our results confirm that OX-2 and GAP-43 expression markedly declined when the neurons reached the adult maturation.

The expression of OX-2 and GAP-43 was found previously to the myelinization process (Romand and Romand, 1990; Hafidi et al., 1990; Woolf et al., 1992; Toesca, 1996). In addition, it has been reported that GAP-43 immunoreactivity was strictly confined to the unmyelinated axons (Curtis et al., 1992; Risling et al., 1994). In the damaged rat retina unmyelinated or lightly

Fig. 5. At postnatal day P3, OX-2 immunolabelling is noted in cell bodies and nerve fibers of the cochlear ganglion neurons (**A**, stars). A strong GAP-43 immunolabelling is observed under the inner (I) and outer (O) hair cells along the basal **(B)** to apical **(C)** cochlear coils. At postnatal day P6, the OX-2 immunolabelling **(D, E)** appears within the intraganglionic spiral bundle (**D**, arrow) and nerve fibers reaching the cochlear epithelium (**D**, arrowheads). At high magnification, the OX-2 immunoreactive fibers are present under the basal pole of inner (I) hair cells (**E**, thick arrows) and outer (0) hair cells (**E**, thin arrows). At postnatal day P6, **(F, G)**, GAP-43 immunoreactive nerve fibers are noticed within the intraganglionic spiral bundle (**F**, arrow) and GAP-43 nerve fibers (**F**, arrowheads) reaching the inner (I) hair cells (**G**, arrowhead) and outer (0) hair cells (**G**, arrows). Scale bar: 50 µm. I: inner hair cells; O: outer hair cells; RM: Reissner membrane; TM: tectorial membrane; SV: spiral vessel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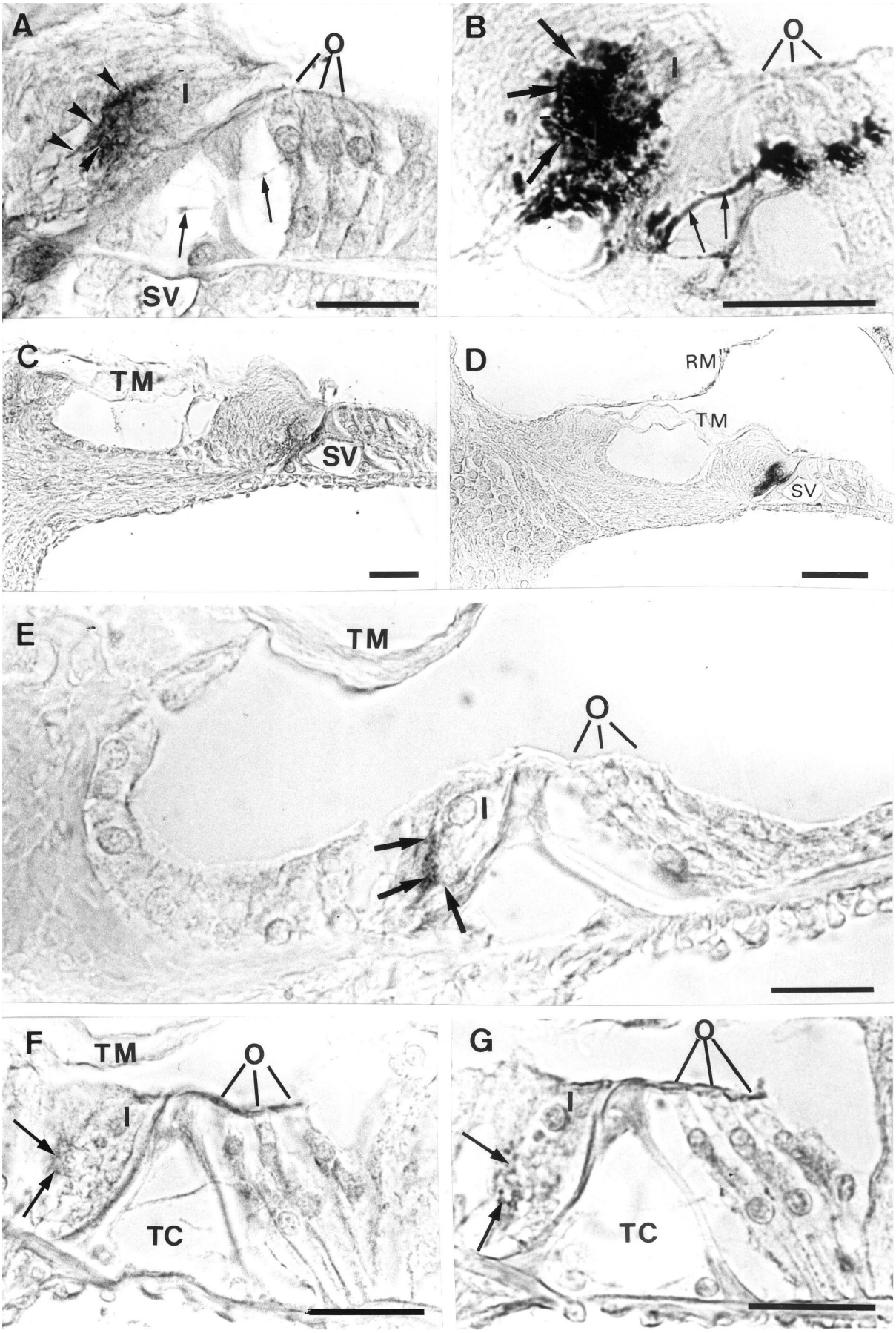

Fig. 6. In mid-modiolar sections a decrease of OX-2 and GAP-43 immunolabelling is observed from P9 to P22. At postnatal day P9, in the middle cochlear coil, the OX-2 expression is present under inner (I) hair cells (**A**, arrowhead) and some efferent OX-2-positive fibers (**A**, thin arrows) cross the tunnel of Corti reaching the outer (O) hair cells. GAP-43 shows a very strong immunoreactivity under inner (I), and some GAP-43-positive efferent fibers (**B**, thin arrows) cross the tunnel of Corti and reaching the outer (O) hair cells **(B)**. At postnatal day P12, in the apical coil, OX-2 **(C)** and GAP-43 **(D)** immuno-rreactivities appear restricted to the inner (I) hair cells. At high magnification of a medial cochlear coil section at postnatal day P15, the OX-2 shows immunoreactive terminals under inner (I) hair cells (**E**, arrows). In the basal coil of adult rat cochlea (P22) a quite negligible OX-2 **(F)** and GAP-43 **(G)** immunoreactivity is observed. Scale bars: A, B, E, F, G, 25 µm; C, D, 50 µm. I: inner hair cells; O: outer hair cells; RM: Reissner membrane; TC: tunnel of Corti; TM: tectorial membrane; SV: spiral vessel.

myelinated areas expressed high levels of GAP-43 (Coggeshall et al., 1991; Kapfhammer and Schwab, 1994; Kapfhammer et al., 1994). When the myelinization process is achieved (Woolf et al., 1992) the auditory electrophysiological potentials reach their characteristics and shape (Uziel et al., 1981; Puel and Uziel, 1987), period which correspond to the final decline of GAP-43 and OX-2 expression (present results).

Even though the function of both OX-2 and GAP-43 glycoproteins during neuronal maturation still remains unclear (DiFiglia et al., 1990; Curtis et al., 1992; Kapfhammer et al., 1994). However, these glycoproteins have been involved in developing neuronal interactions, nerve fasciculation, axonal growth and neuronal regeneration (Barclay and Ward, 1982; Webb and Barclay, 1984; Skene et al., 1986; Meiri et al., 1986; Morris and Beech, 1987; Benowitz and Routtenberg, 1987; Benowitz et al., 1987; Skene and Virág, 1989, Gispen et al., 1991; Burry et al., 1992).

Our present findings fit well with these reports, indicating that both molecules are involved in the axogenesis and/or dendritogenesis until the final neuron maturation, in the auditory receptor. The onset of OX-2 and GAP-43 glycoproteins clearly correspond to the beginning of fasciculation. The functional maturation, the neurotransmitter release and the stabilization of neuronal connections, could also depend on the progressive restriction of the OX-2 and GAP-43 expression, which evidently correspond to the inhibition of the neurite growth.

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