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A monoclonal antibody labels type II neurons of the spiral ganglion

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Tissue from mature rat and cat cochleas was stained with basic dyes in order to distinguish between the cell bodies of type I and II spiral ganglion neurons. This same tissue was then stained with protargol, or processed immunocytochemically using a monoclonal neurofilament antibody, RT-97. Type II neurons were intensely stained, whereas type I neurons were not. We conclude that type II neurons can be clearly distinguished by their abundant neurofilament content.

Spiral ganglion neurons in the mammalian cochlea have been classified on the basis of the morphological characteristics of their cell bodies[9,11,13] and/or the pattern of hair cell innervation[2,8,12]. Type I neurons with relatively large cell bodies innervate inner hair cells and comprise 95% of the ganglion population. Type II neurons with relatively small cell bodies innervate outer hair cells, but represent only 5% of the total ganglion cell population[6,12]. Both light and electron microscopy indicate that type II cell bodies contain an abundance of neurofilaments and a paucity of ribosomes[7,11,14]. The histological affinity of type II cell bodies for neurofilament stains[9] is consistent with the specific labelling by protargol of proteins that form neurofilaments[5]. While cytological criteria enable one to identify each ganglion cell as type I or type II[9,11], the infrequent type II neurons are intermingled with the more prevalent type I neurons and are therefore difficult to detect; consequently, cell counts and population studies are tedious[9]. Such studies would be greatly facilitated if these neurons could be selectively and distinctively labelled.

On the basis of differences in neurofilament content, we reasoned that the cell bodies of type II neurons might well be labelled by a neurofilament antibody, RT-97, whereas type I neurons might not. This particular monoclonal antibody was raised against the 200 KDa neurofilament protein[15], and was known to have selective affinity for a subpopulation of neurons in the dorsal root ganglia[10]. In the present study, we stained the same cochlear tissue with different markers and directly demonstrated that the type II neurons defined by basic dyes were in fact the same neurons defined by neurofilament labelling.

The cochleas of 6 Norway brown rats (adult females, Charles River Laboratories, Wilmington, MA) and one adult female cat were examined. Intracardiac and intralabyrinthine perfusions with Bouin's fixative produced tissue yielding the most consistent results. After perfusion, each cochlea was drilled with stone burs, decalcified in 10% EDTA (1–7 days), and cryoprotected in 30% sucrose. Cryostat sections (10–15 μm thickness) were collected on double-subbed slides. The tissue was first stained by dropping 1% Toluidine blue onto the sections and immediately washing it off. The sections were then coverslipped with 50% glycerin in distilled water and left approximately 24 h until the excess dye leached out of the tissue. Spiral ganglion cells were studied, drawn with a camera lucida, classified as type I or type II, and photographed. The coverslips were then removed, and the sections stained with protargol[3] or

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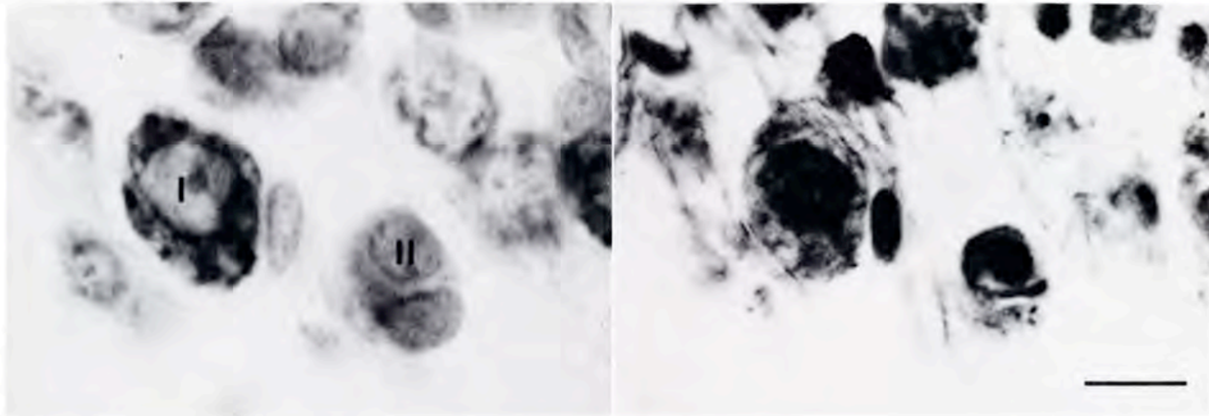

Fig. 1. Left panel: Toluidine blue staining of cochlear spiral ganglion cells in rat. The type II neuron (II) is distinguished by its small somatic size, pale cytoplasm, and darker staining nucleus. The type I neuron (I) has a larger cell body, displays prominent Nissl bodies in its cytoplasm, and has a pale staining nucleus. A satellite cell nucleus is evident against the bottom of the type II neuron and against the side of the type I neuron. Right panel: The same cells as shown on the left but stained with protargol (and at a slightly different focal plane). The type II cytoplasm and nucleus are darkly stained. This staining is unlike that of the type I neuron where only the nucleus stains darkly. Bar = 10 μm.

processed by immunocytochemical methods. For the latter, the primary antibody, RT-97, was applied for 45 min at room-temperature or overnight at 4 °C at a dilution of 1:100–200 in a solution of 0.1 M Tris-buffered saline (pH 7.5) containing 10% fetal calf serum. After washing, the secondary antibody (goat anti-mouse IgG, conjugated with peroxidase or rhodamine; Cappel, Malvern, PA) was applied for 35 min. The rhodamine-labelled sections were washed and coverslipped in glycerin. In some cases, the coverslips were later removed and the sections restained with Toluidine blue. The peroxidase sections were further reacted for 8 min with diaminobenzidine (0.05%, Sigma), dehydrated, and coverslipped with Permount. In this way, the same spiral ganglion neurons could be characterized by different staining procedures.

In basic dye preparations, the cell bodies of type II neurons were distinguished from type I neurons by their smaller size, pale staining cytoplasm, and dark staining nuclei. They were found among the more numerous type I neurons that displayed Nissl bodies in their cytoplasm and pale staining nuclei. When the same tissue was stained with protargol, the cytoplasm of type II neurons was stained, whereas that of the type I neurons was not (Fig. 1). These data revealed that type II neurons defined by basic dye criteria were the same as those distinguished with protargol.

The monoclonal antibody, RT-97, appeared highly specific for certain cochlear elements (Fig. 2). Fibers of the osseous spiral lamina, intraganglionic spiral bundle, modiolus, and auditory nerve were clearly labelled. Labelling within the ganglion itself, however, was strikingly sparse. Only a few cell bodies and their processes were stained. In most respects, labelling by RT-97 in the cochlea was consistent with ultrastructural descriptions of neurofilament distribution[4]. For example, outside the spiral ganglion where type I processes are filled with neurofilaments, they were also intensely labelled with RT-97. Efferent fibers in the intraganglionic spiral bundle and in the outer hair cell region also contain many neurofilaments and were heavily labelled by the antibody.

Within the spiral ganglion, labelling was also mostly consistent with ultrastructural descriptions of neurofilament distribution[7,11,14]. The filamentous cell bodies and processes of type II neurons were unambiguously stained (Fig. 3). The ribosome-rich, filament-poor type I cell bodies[13] were stained lightly or not at all. Type I fibers in the vicinity of their cell bodies were also unstained, a puzzling circumstance since bundles of neurofilaments have been observed there[9]. One possible explanation for the reduced antigenicity is that along the axon, there are differences in the degree to which the specific antigen is phosphorylated[1]. In any case, it seems that different seg-

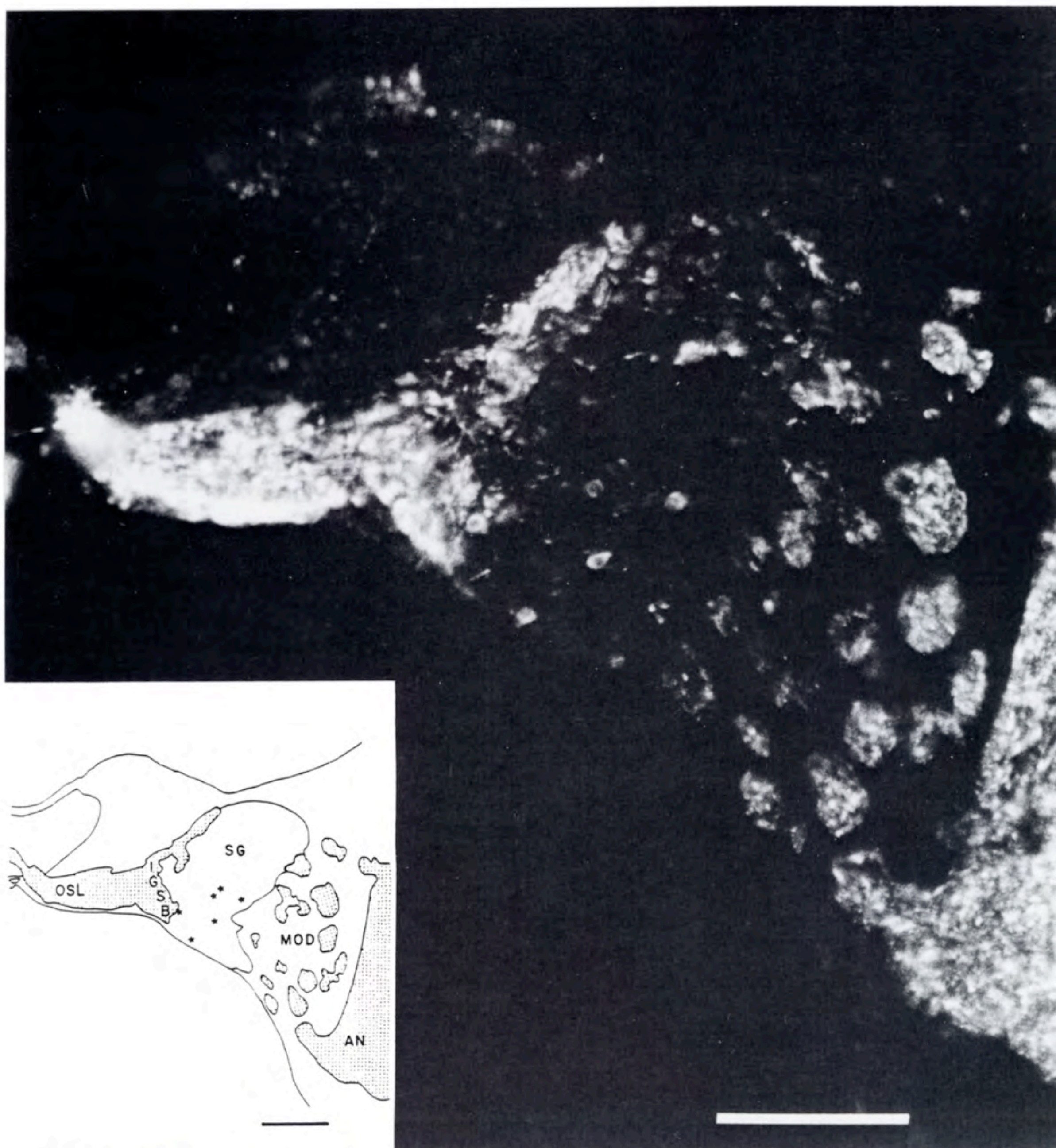

Fig. 2. Immunofluorescent micrograph of the rat cochlea labelled by rhodamine-conjugated RT-97. In the inset, labelled fibers are indicated by stippling and labelled cell bodies are indicated by asterisks. Peripheral to the spiral ganglion (SG) fibers are intensely labeled in the osseous spiral lamina (OSL) and intraganglionic spiral bundle (IGSB). Centrally, auditory nerve fibers passing through the modiolus (MOD) and into the auditory nerve (AN) also stain brightly. Labelled cell bodies are visible in the ganglion. Bar = 100 μm.

ments of the same axon can contain varying amounts or different configurations of cytoskeletal proteins.

Sections reacted sequentially with Toluidine blue and then RT-97 demonstrated that the cell bodies of type II neurons defined by basic dye criteria were the same ones labelled by RT-97 (Fig. 4). In fact, the only intensely labelled cell bodies were those of type II neurons. Type I cell bodies can show a pale reac-

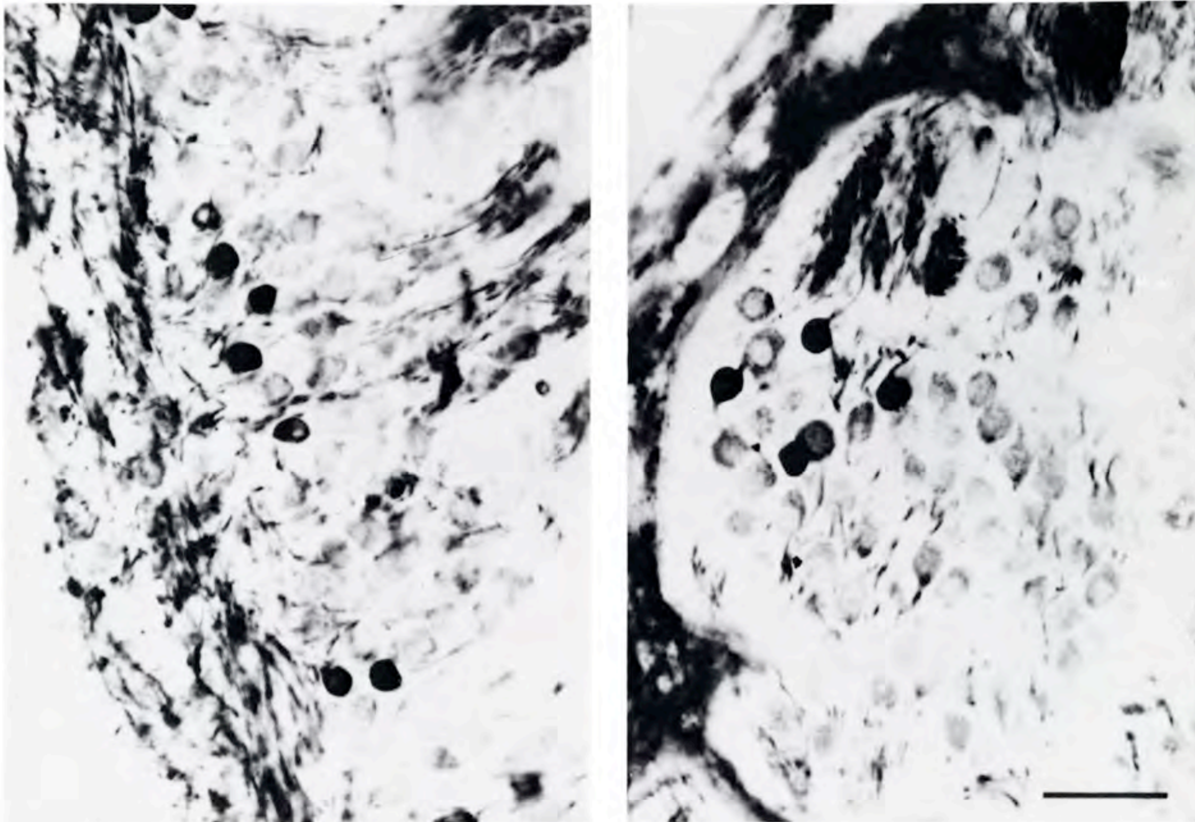

Fig. 3. Left panel: immunoperoxidase-stained section in rat labelled by RT-97. This section presents a longitudinal view of the ganglion where type II cells are darkly labelled. Right panel: immunoperoxidase-stained section in cat labelled by RT-97. This view presents a cross-section of the ganglion where type II neurons are intensely stained. Type I neurons and Schwann cells are not labelled. Other labelling indicates the presence of fibers. Bar = 50 μm.

tion product in their cytoplasm, but it was easily distinguishable from the type II response (Fig. 3). Schwann cells were not labelled with the antibody. Finally, control sections that were identically processed for immunocytochemistry, but without addition of the primary antibody, showed no cell body or fiber staining.

The positively labelled structures appeared obviously different from unlabelled structures, which was not the case for tissue stained with basic dyes or protargol. Even a fragment of a cell body was definitive; the entire cell need not be present in a section. Caution must be exercised, however, in referring to every well-stained spiral ganglion cell as a type II neuron, since the antibody appeared specific only to a cell's neurofilament content. A well-labelled neuron may occasionally indicate a degenerating cell that is neurofilament-rich. Criteria other than staining per se (e.g. somatic or fiber morphology) can provide clarification in such ambiguous cases. Under normal circumstances, studies involving counts of type II neurons, plots of their distribution within the ganglion, or morphometry will be greatly facilitated by this simple and reliable method. Moreover, the method is generalizable for other neurons of the nervous system that possess filamentous somata[10]. Finally, since the method was applicable to tissue preserved over several months, it could be utilized for a variety of comparative or pathological issues.

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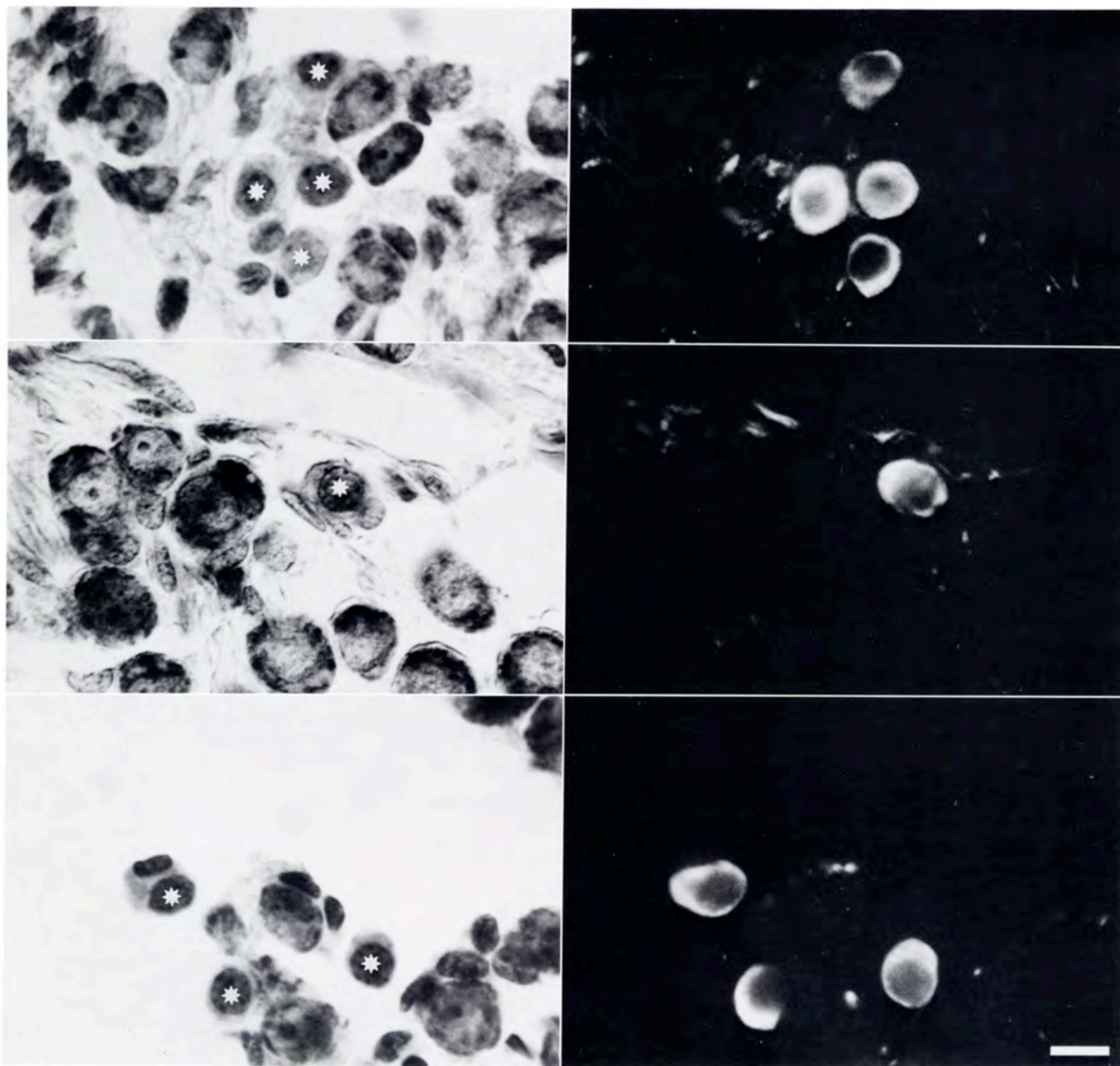

Fig. 4. Cochlear tissue from rat spiral ganglia stained with Toluidine blue (left panel) and with RT-97 (right panel). Note that the type II neurons (stars) are preferentially labelled with this antibody. Bar = 10 μm.

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