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Vestibular efferents contain peripherin

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Abstract

Vestibular efferents have a common origin with the motoneurons of the facial nerve. In adults they share a number of common features, such as the same transmitter. Here we show using retrograde transport and immunohistochemistry, that the vestibular efferents, like facial motoneurons, contain peripherin. This supports the suggestion that peripherin-positive fibers at the apex of the cristae ampullaris are efferents.

Keywords: Intermediate filament; Rhombomere; Branchiomeric; Neurofilament

Otic efferents share a common developmental origin with the branchiomeric motoneurons of the facial nerve in rhombomere 4 [2]. The various groups, e.g., motoneurons, vestibular efferents and auditory efferents separate during ensuing development, but the axonal trajectories are very similar. One might expect to find other similarities. Indeed, vestibular efferents and facial motoneurons are cholinergic and contain CGRP [1,3,9,17–19,25,26,29–33].

The locations of vestibular efferent cell bodies have been described in several mammalian species [3,4,8–10,12,16,19,27,32]. In gerbils, two populations of efferents have been identified based on retrograde transport of horseradish peroxidase (HRP) combined with acetylcholinesterase staining [19]. The majority of cells are found in a tight group between the genu of the 7th cranial nerve and the fourth ventricle. This group, termed the 'e' group, appears to be quite consistent across different species [8,10,12,16,27]. The neurons lateral to the abducens nucleus in the cat [4,9,23] are probably comparable. The second population of efferents in gerbils is found at similar anterior–posterior levels and consists of more scattered cells ventral to the genu and exiting portion of the facial nerve [19]. This population is also fairly consistently described [4,8–10,16]. Dechesne et al. [4] described retrogradely-labeled cells on both sides of the descending portion of 7 close to the genu, but considered them to be a single population. In some animals additional groups of efferents are described (e.g., [8,12,16]). It is unclear if these are genuine species differences or represent differences in methods.

The maximum number of retrogradely-labeled cells in a single gerbil, both ipsilateral and contralateral to the injected ear, was 218 [19]. These injections were all very small; therefore, this is almost certainly an underestimate. Larger, but still relatively small, numbers have been found in different species [1,4,10]. In these studies, efferents labeled from one ear are found on both sides of the brainstem, and some neurons were double labeled when distinct tracers were injected into each ear [4]. These suggest that there must be extensive branching of any individual efferent neuron.

Peripherin is a 58 kDa intermediate filament protein found in some neurons [5,21,28]. The name reflects the fact that it was first found in neurons of the peripheral nervous system and central neurons with an axon extending into the periphery [5,21,28]. In addition to facial motoneurons [21,28], peripherin is found in some sensory neurons including bouton-only vestibular afferents [13–15]. However, peripherin-postive fibers are found in the central regions of the cristae in gerbils [6,7] and rats [20] where there should be no bouton-only afferents. This paradox would be resolved if peripherin is also found in vestibular efferents. The expression of peripherin in vestibular efferents was retested in the present study, using as a positive

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control peripherin expression in additional brain areas (i.e., branchiomeric motoneurons and axons) as the metric upon which the staining was judged [24].

Gerbils (*Meriones unguiculatus*) ($n > 20$) of both sexes were anesthetized with a large overdose of sodium pentobarbital (i.p.) and perfused through the heart with normal saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). One rat was perfused the same way. The large number of gerbils is a reflection of several different experiments where the brainstems were stained for peripherin and the fact that we used the established staining of branchiomeric motor nuclei, including the facial nucleus ([5,20] and references therein) as a positive control and to assess each new lot of anti-peripherin antibody (rabbit polyclonal, Chemicon, CA, Cat #AB1530, various lots, immunogen: trp-E peripherin fusion protein containing all but the last 4 N-terminal amino acids of rat peripherin. Company data indicates that it stains a single band at the appropriate molecular weight, and specifically denies binding to closely related intermediate filament proteins). The brains were removed and placed in fixative containing 20% sucrose overnight. Transverse sections through the brainstem were cut on a freezing microtome at 40 μm (gerbils) or 25 μm (rat), and processed for immunohistochemistry as free-floating sections. After treatment with triton X-100 and blocking serum the sections were exposed to the anti-peripherin at a concentration of 1:2000 in phosphate buffered saline (PBS) at 4 °C for 24–48 h. The primary antibody was detected using Vectastain Elite kits (Vector Lab, CA) and a nickel enhanced diaminobenzidine (Ni-DAB) reaction. The Vectastain kits contain all of the reagents except the primary antibody and Ni-DAB. The sections were then rinsed and mounted on gelatin-subbed slides.

In another group of nine animals, we used various fluorescent tracers to retrogradely label the efferents. Multiple injections were made in both the horizontal and anterior semicircular canal cristae using a glass pipette with a tip diameter of 30–40 μm and a picospritzer (General Valve, CA). No effort was made to prevent spread to the utricle. While the best evidence comes from birds, it is thought that efferent axons branch to innervate several cristae [4,9]. Therefore, we felt that, even if our injections did not label every efferent, they would give us an adequate sample. There was no spread of tracers into middle ear structures, nor were cochlear efferents labeled. Our best results were obtained with 3% fluorogold (Flurochrome, CO) as the retrograde tracer. The animals were anesthetized with sodium pentobarbital (35 mg/kg, i.p.), supplemented with ketamine (25 mg/kg, i.m.) and aseptic procedures were used. Following an 8–10 day survival the animals were reanesthetized and perfused as described above. In these animals, the peripherin antibody was detected using avidin coupled to tetramethylrhodamine (Molecular Probes, OR) following the biotinylated secondary antibody. The tissue was examined using an epiflourescence microscope with appropriate filters. The vestibular ganglion served as a control for fluorescence bleed-through. Since we did not attempt to fill all of the afferents, we expected some ganglion cells to be labeled with flurogold, but not anti-peripherin, and some cells to be peripherin-positive but flurogold-negative. In these two populations, the fluorescent marker was only visible with the appropriate filter combination. As anticipated, a population of small ganglion cells was double-labeled.

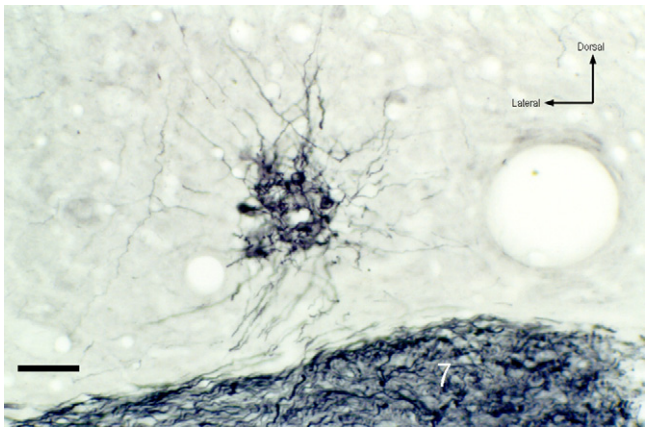

Fig. 1. Vestibular efferents contain peripherin. A photomicrograph showing a portion of the genu of the seventh nerve (7) and the 'e group' of efferents both labeled with the antibody to peripherin. Dorsal and lateral directions are indicated. Scale bar = 50 μm.

The procedures were approved by the UTMB Institutional Animal Care and Use Committee.

As anticipated, several groups of neurons in the brainstem were peripherin positive. These included the branchiomeric motoneurons of cranial nerves 7, 9 and 10 as well as their exiting axons as they coursed through the brainstem. Afferents in the descending trigeminal tract were also labeled. Between the internal genu of the 7th nerve and the IVth ventricle, there was a cluster of peripherin-positive neurons (Fig. 1). A cluster of cells is labeled by the peripherin antibody in the same location in the rat (Fig. 2). The cells are not as heavily labeled in the rat, but in this animal the labeling in the facial nerve is also not as heavy. In gerbils the intensity of the staining in these neurons parallels the intensity in seven. The rat sections were divided and reacted with several different batches of peripherin antibody. Here also the intensity of staining in the two structures was parallel. The 10 μm diameter cell bodies were

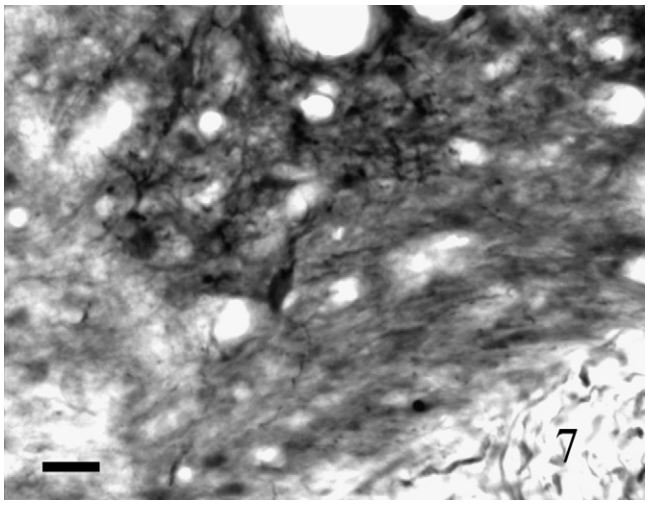

Fig. 2. A photomicrograph of the 'e' group and the genu of the seventh nerve in a rat. The orientation is the same as in Fig. 1. Scale bar = 20 μm.