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Tonotopic organization of the cochlear nucleus of the mustache bat, *Pteronotus parnellii*

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Summary. The tonotopic organization of the cochlear nucleus (CN) of the mustache bat, *Pteronotus parnellii* was studied by injecting horseradish peroxidase (HRP) in physiologically characterized CN-sites known to respond to a certain frequency. The tracer was transported by the branched fibers of the auditory nerve and bands of labeled terminals were found in each of the CN-subdivisions. Low to high frequencies are orderly represented in rostrocaudal direction in the anteroventral CN (AV) and in ventrodorsal direction in the posteroventral (PV) and dorsal CN (DCN). In all 3 subnuclei a vast overpresentation of the frequency band between 54–66 kHz, which includes the dominant second harmonic of the echolocation calls, is superimposed on this basic mammalian pattern of frequency representation. A deviation from the standard mammalian scheme of tonotopic arrangement is found in the cytoarchitecturally distinct 'marginal cell group' (MA). This cell group extends in rostrocaudal direction along the medial margin of the AV, and the frequency representation in its most rostral 2 thirds is biased towards the low frequency range of the faint first harmonic of the echolocation call (24–32 kHz). Consequently the low frequencies lie adjacent to the regular slab organization of the range of higher frequencies represented in the AV. The temporal response patterns of MA neurons to tone stimuli are predominantly phasic or onset-types, in contrast to the 'primary like' response type which prevails in the AV. The frequency representation and the physiological characteristics of the MA group can be relevant in the context of target range determination by echolocation.

Key words: HRP – Echolocation – Cochlear nucleus

Abbreviations. *AV* anteroventral cochlear nucleus; *AVa* anterior part of AV; *AVp* posterior part of AV; *BF* best frequency; *CF* constant frequency component of echolocation calls; *CN* cochlear nucleus; *DAB* diaminobenzidine, *DCN* dorsal cochlear nucleus; *EP* evoked potential; *FM* frequency modulated component of echolocation calls; *HRP* horseradish peroxidase; *LSO* lateral superior olive; *IC* inferior colliculus; *MA* marginal cell group; *MAl* lateral part of MA; *MAm* medial part of MA; *OAE* otoacoustic emission; *PV* posteroventral cochlear nucleus; *PVl* lateral part of PV; *PVm* medial part of PV; *PVc* caudal part of PV (octopus cell region); *RF* resting frequency; *SEOAE* synchronous evoked OAE; *TMB* tetramethylbenzidine; *VIII* eight nerve.

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Introduction

The auditory system of the mustache bat, *Pteronotus parnellii*, is specialized to process the species characteristic echolocation signal (Pollak et al. 1972; Suga et al. 1975; Suga 1984). The echolocation call is composed of 4 harmonics, each of which includes a long constant frequency component (CF_{1-4}) followed by a fast downward frequency modulation (FM_{1-4}) (Schnitzler 1970; Suga 1984).

Two aspects of signal processing are of particular interest. The dominant CF_2-component at 61 kHz carries information about target velocity and is useful for detecting fluttering prey in echocluttered habitats (Goldman and Henson 1977). A narrow band around this frequency is overrepresented in central auditory nuclei (e.g. Zook et al. 1985). The expanded mapping pattern and the exquisite sharp tuning found in neurons in this frequency range can be traced back to specialized features of the cochlea (Pollak et al. 1972; Suga et al. 1975; Suga and Jen 1977; Leake and Zook 1985; Kössl and Vater 1985b).

The first harmonic FM_1-component in combination with higher harmonics plays an important role in determination of target range by delay-sensitive cortical neurons (O'Neill and Suga 1979, 1982). So far specialized representation patterns of the first harmonic frequency range have not been demonstrated at peripheral stations of the ascending auditory pathway.

The present study analyses in detail the functional organization of the CN of the mustache bat. The CN is the first central synaptic relay in the ascending audito-

ry system. It receives an orderly pattern of tonotopically organized projections from frequency specific regions of the cochlea. Since the CN is composed of 3 distinct subdivisions (anteroventral, posteroventral, and dorsal CN), each of which is supplied by separate terminal fields of the branching auditory nerve fibers, a triple representation of the hearing range is created (Lorente de No 1933a, b; Rose et al. 1959; Evans and Nelson 1973; Evans 1975; Bourk et al. 1981; Feng and Vater 1985).

With neurophysiological recordings and by tracing the frequency specific termination patterns of auditory nerve fibers with horseradish peroxidase (HRP), we investigated the tonotopic organization of the CN-subdivisions with particular emphasis on the echolocation frequencies. In particular, it was determined how the expanded cochlear representation of the CF_2-frequency range is fitted into the basic mammalian organization of the CN-subdivisions. Furthermore, we characterized a distinct representation of the frequency range of the first harmonic in relation to cytoarchitectural specializations of the CN and investigated in detail the temporal response patterns of neurons tuned to this frequency range. We conclude that the CN of the mustache bat contains a module specifically designed to process information on the onset of the first harmonic call components which is an important prerequisite for the determination of target range by delay sensitive neurons at higher auditory centers.

Materials and methods

Thirteen mustache bats, *Pteronotus parnellii*, from Jamaica were used for this study. Prior to each experiment the individual's CF_2-frequency was measured when the bat was not flying and therefore not lowering the emitted frequency to compensate for positive Doppler-shifts in the echoes. Measured in this way the CF_2-frequency is called resting frequency (RF). As an additional characteristic of each individual bat, the otoacoustic emissions (OAE) of the cochleae of both ears were measured with microphones in the outer ear canal (Kössl and Vater 1985a, 1990).

The methods used for neurophysiological recording, HRP-injection, and histological procedures follow those described by Feng and Vater (1985). Briefly, prior to each HRP-injection, the frequency representation in the area was thoroughly mapped. This was done by determining the frequency of lowest threshold (Best frequency: BF) of single units and multiunits encountered in closely spaced parallel penetrations of fine tipped (6–15 MΩ) glass pipettes filled with 3 *M* KCl. Then a HRP-filled recording micropipette (10% HRP in acetate buffer; impedance 10 to 20 MΩ; tip diameter 3 to 6 µm) was used to verify the BFs and to inject HRP iontophoretically (positive current of 0.5 to 1 µA delivered for 5 to 14 min). After a survival time of 24–28 h the animals were killed by an overdose of Nembutal and perfused through the heart with 0.9% saline followed by fixative (1.25% glutaraldehyde, 1% paraformaldehyde) and washing solution (2.5% sucrose in 0.05 *M* phosphate buffer). Alternating series of 54 µm thick transverse brain sections were incubated with tetramethylbenzidine (TMB; Mesulam 1978) or diaminobenzidine (DAB; Adams 1977). A summary of the locations of the injection sites and the respective frequency ranges is given in Table 1. The size of the injection sites ranged from 70 to 200 µm diameter (DAB-reacted material, Table 1, Fig. 2). The sections were counterstained with neutral red (TMB- or DAB-material) or cresyl violet (DAB-material). The BFs recorded at the injection sites are shown in Table 1. In 10 bats, a single HRP-injection was placed in the CN. One animal received 2 HRP-injections in 2 different areas of the same CN, in another animal HRP was injected into the left and the right CN. In one bat the recording site was marked with a lesioning current. The nomenclature of Zook and Casseday (1982) is used for cytoarchitectural subdivisions of the CN. Two injections were placed in the posteroventral CN, the remainder were in the anteroventral CN and/or in the marginal cell group (Table 1). HRP was transported within fibers that innervated the other subdivisions of the CN, and to cells in the spiral ganglion. The resulting patterns of HRP-label in the cochlea were described by Kössl and Vater (1985b).

Results

Morphology of the cochlear nucleus

As in other mammals, the CN of *Pteronotus* is divided into 3 main subdivisions. The cytoarchitectural details of these subdivisions have been described by Zook and Casseday (1982), and they are summarized in Fig. 1. The anteroventral CN (AV) (Fig. 1a–d) is the largest subnucleus and occupies about half of the total volume of the CN. It can be further subdivided into an anterior part (AVa) which contains mainly small spherical cells, and a posterior part (AVp) which contains a variety of cell types (Zook and Casseday 1982). The posteroventral CN (PV) (Fig. 1a–e) is composed of a lateral subdivision (PVl) that contains mainly multipolar cells, and a medial subdivision (PVm) that contains smaller cells and is characterized by a higher cell density. The caudal subdivision of the PV (PVc) contains octopus cells (not shown in Fig. 1). The dorsal CN (DCN) is only weakly laminated (Fig. 1e). In that respect it is unlike that of common laboratory animals (cat: Osen 1969) but similar to that of primates (Moore 1980). The most unusual and species specific feature in the CN of *Pteronotus* is the 'marginal cell group' (MA). As shown in detail in Fig. 1f, it contains a population of large multipolar cells located at the medial margin of the CN, which is not present in other mammals (Zook and Casseday 1982). In the present study, we have further subdivided the MA into a medial part (MAm) that lies medial to AV and PV (Fig. 1a–d), and a lateral part (MAl) that occupies the region at the common border between AV, PV and DCN (Fig. 1e). This subdivision is based upon differences in the frequency representation and the neurotransmitter content within the MA: in the MAm there is a dense network of catecholaminergic fibers restricted to this area and not extending into the MAl or adjacent AV. In addition the MAm contains dense stain for acetylcholine-esterase, compared to scarce stain in the MAl (Kössl et al. 1988).

Horseradish peroxidase transport patterns

Small HRP-injections were placed in regions of the CN shown by electrophysiological recordings to represent a particular frequency range (Fig. 2, Table 1). The tracer

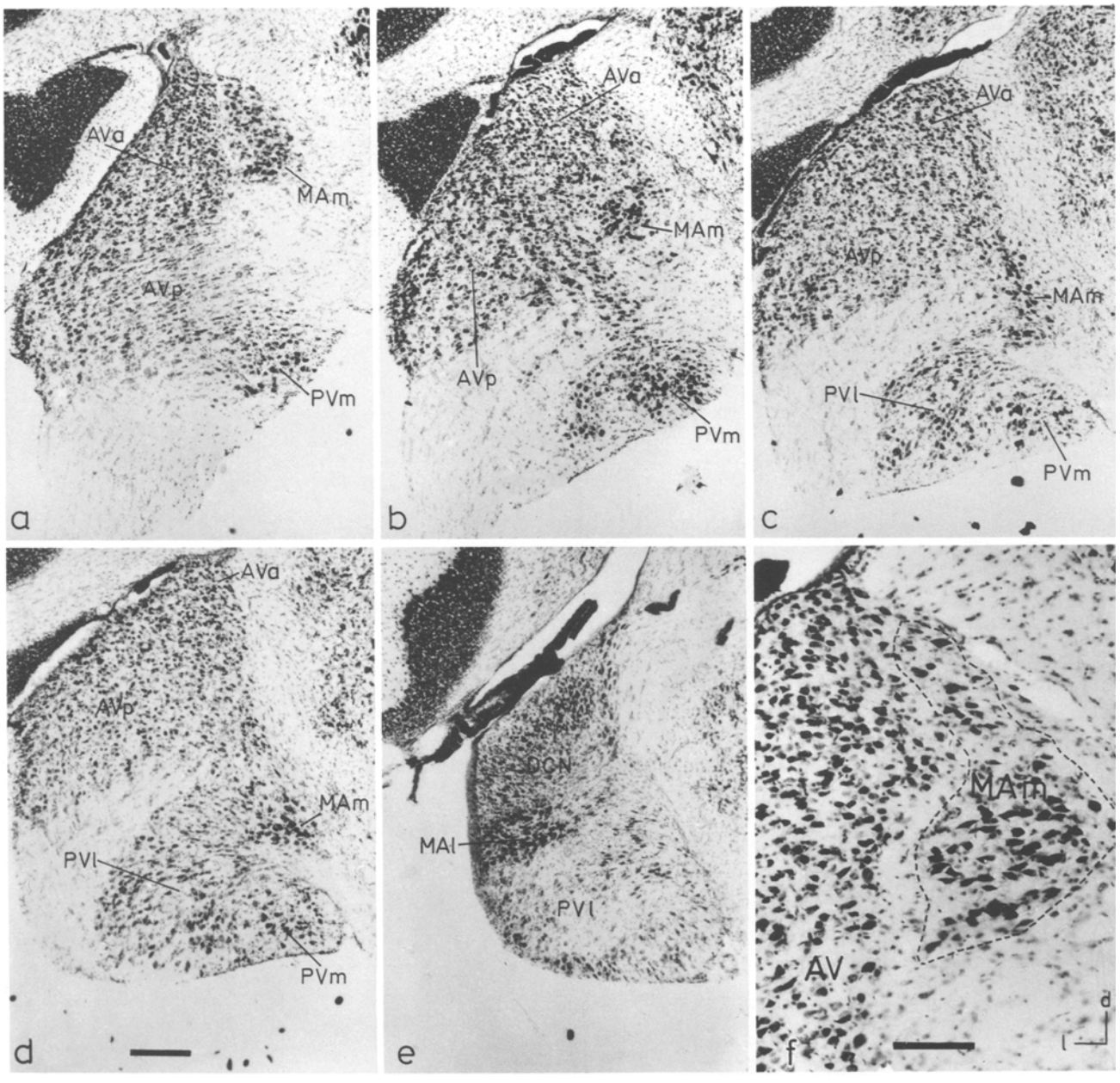


Fig. 1a–f. Cytoarchitecture of the cochlear nucleus (transversal sections, 42 μm thickness, Nissl stained). The sections **a–e** are ordered from rostral to caudal. Note the marginal cell group (MAm and MAl) composed of large multipolar neurons located at the medial edge of the anteroventral CN (MAm, shown in detail in **f**), and between dorsal and posteroventral CN (MAl); for further explanation see text; calibration bar: 200 μm **a–e**, 50 μm **f**

was taken up by terminals of auditory nerve fibers and transported back along the length of the fibers to label not only their cells of origin in the spiral ganglion, but also the ramifications of each fiber in the other subdivisions of the CN. As in other mammals, the auditory nerve fibers of *Pteronotus* bifurcate into an ascending branch that supplies the AV, and a descending branch that further ramifies to innervate the PV and DCN. Thus, a single injection in one subdivision of the CN yields information about the representation of the corresponding frequency range in the other subdivisions. Figure 3 shows labeled auditory nerve fibers derived from an injection in the AV. The tracer was transported retrogradely within the ascending branch to the bifurcation point and from there anterogradely in the descending branch towards the termination fields in the PV and DCN. To demonstrate the high spatial resolution of this technique, two injections were placed in the same AV, in regions responsive to 46 and 60.6 kHz respectively. From these injections two clearly distinct bands of label are visible in PV and DCN (Fig. 4). Labeled nerve terminals characteristically take the form of boutons in the DCN and PV (Figs. 4, 5). In the AV, endbulbs of Held were often observed in addition to boutons. In the DCN,

Table 1. HRP-Injections

Bat	RF (kHz)	OAE (kHz)	Injection site			Labeled terminals				Number of labeled cells				
			BF (kHz)	Sub-nucleus	Diameter (μm)	AV	PV	DCN	MA	AV	PV	DCN	MA	Spiral-ganglion
1	60.3	61.10	23	AV	100	++	+	++	+++	–	–	5	3	25
11	61.4	62.20	46/60.6	AV	200/190	+++	+++	+++	–	–	–	30/41	–	117/103
4	59.3	59.69	59.6	AV	70	++	++	++	–	–	–	3	–	7
5	61.7	62.15	61.8	AV(+MA)	170	+++	+++	++	++	–	–	–	11	79
6	60.6	61.55	66	AV(+MA)	130	++	++	++	++	–	–	7	–	36
10	60.8	61.23	70	AV	150	++	++	+	–	–	–	–	–	38
		61.25	85	AV	130	+	+++	++	–	–	2	10	1	50
7	59.1	60.27	74	AV	120	++	++	++	–	–	–	15	1	33
8	61.6	62.11	91	AV	170	+++	+++	+	–	–	–	21	–	39
3	60.0	61.15	54.5	PV	120	+++	+++	++	–	–	–	15	–	78
9	61.0	61.43	111	PV/MA	150	+++	++	++	+	–	–	1	–	22
2	60.5	61.10	29.3	MA	90	+	–	+++	++	–	–	5	1	4
12	61.5	61.91	31.5	MA	electric lesion									
13	61.6	62.26	29/61.4 –64	MA/AV	150	+++	++	++	+++	–	–	6/8	–	31/17

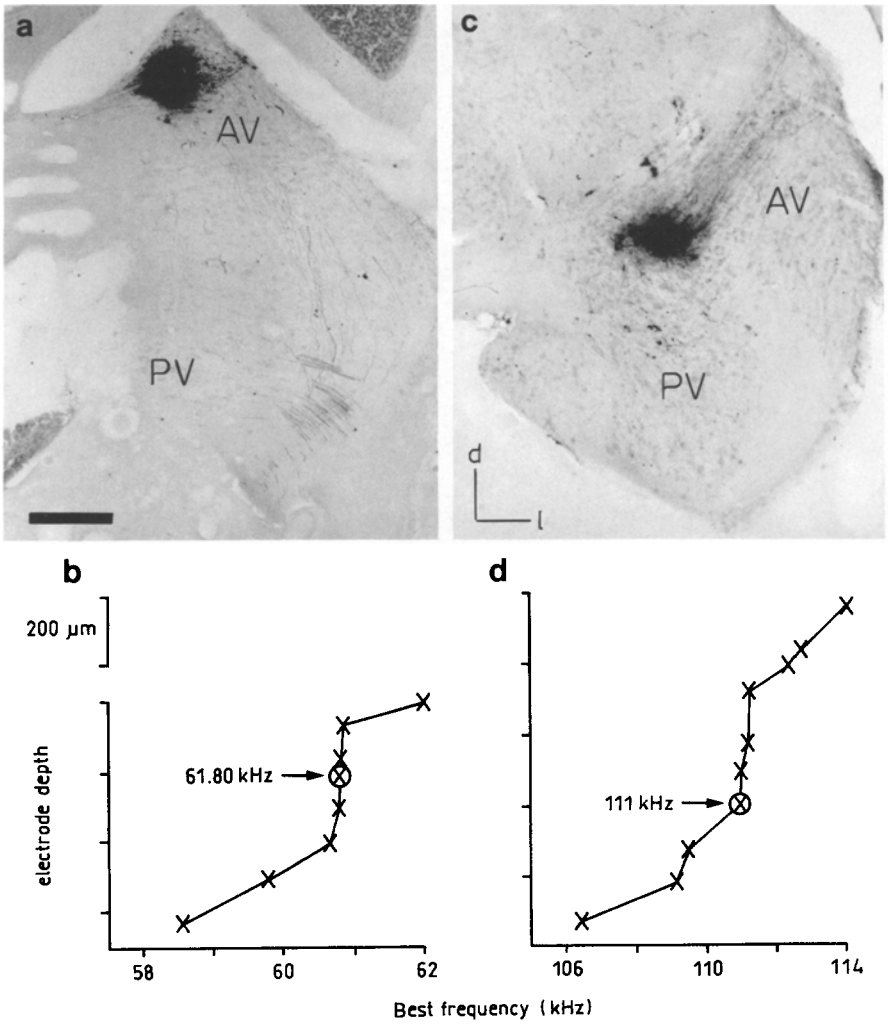


Fig. 2. a Photomicrograph of a HRP-injection site in the anteroventral CN (transversal section, DAB-reacted); **b** Corresponding plot of BFs of CN-units encountered during the penetration with the HRP-electrode; × indicates the BF at the injection site; **c** HRP-injection site in the posteroventral CN and **d** corresponding electrode tract; calibration bar: 200 μm

labeled cell bodies are often found among the labeled terminals (Fig. 4a, arrow; Table 1). These neurons project, within the CN, to the injection site in the AV or PV; the connections thus established between the subdivisions appear to be frequency specific as seen for the horseshoe bat (Feng and Vater 1985) and the mouse (Wickesberg and Oertel 1988). The frequency-specific patterns of transport in the CN can be illustrated best by the results of a few injections (Figs. 6–11) located in regions responsive to the frequency range corresponding to the different harmonics of the echolocation signal which are defined as follows: first harmonic CF_1: 30–31 kHz, FM_1: 24–30 kHz; second harmonic CF_2: 60–62 kHz, FM_2: 48–60 kHz; third harmonic CF_3: 90–93 kHz, FM_3: 72–90 kHz; fourth harmonic FM_4: 96–120 kHz. By combining these results a complete frequency map can be obtained (Fig. 12).

Injections in the range of the dominant second harmonic. A double injection in the regions of AV responsive to 46 and to 60.6 kHz, respectively included one group of neurons tuned to the lower limit of the FM_2-range and another group tuned to a frequency at the lower limit of the CF_2-frequency range (Fig. 6). In the PV and DCN two distinct slabs of labeled terminals (Figs. 4, 6) were discernible. On the basis of frequency mapping obtained by electrophysiological recordings in the respective subnuclei, the dorsal bands correspond to the frequency range of 60 kHz in DCN and PV and ventral slabs to the low frequency ranges, respectively. In the PV the more ventrally located slab (46 kHz) was confined to the medial subdivision (PVm), and the dorsal slab (60.6 kHz) was located in the lateral subdivision (PVl). In the AV there was transport along the remainders of the two isofrequency slabs containing the injections.

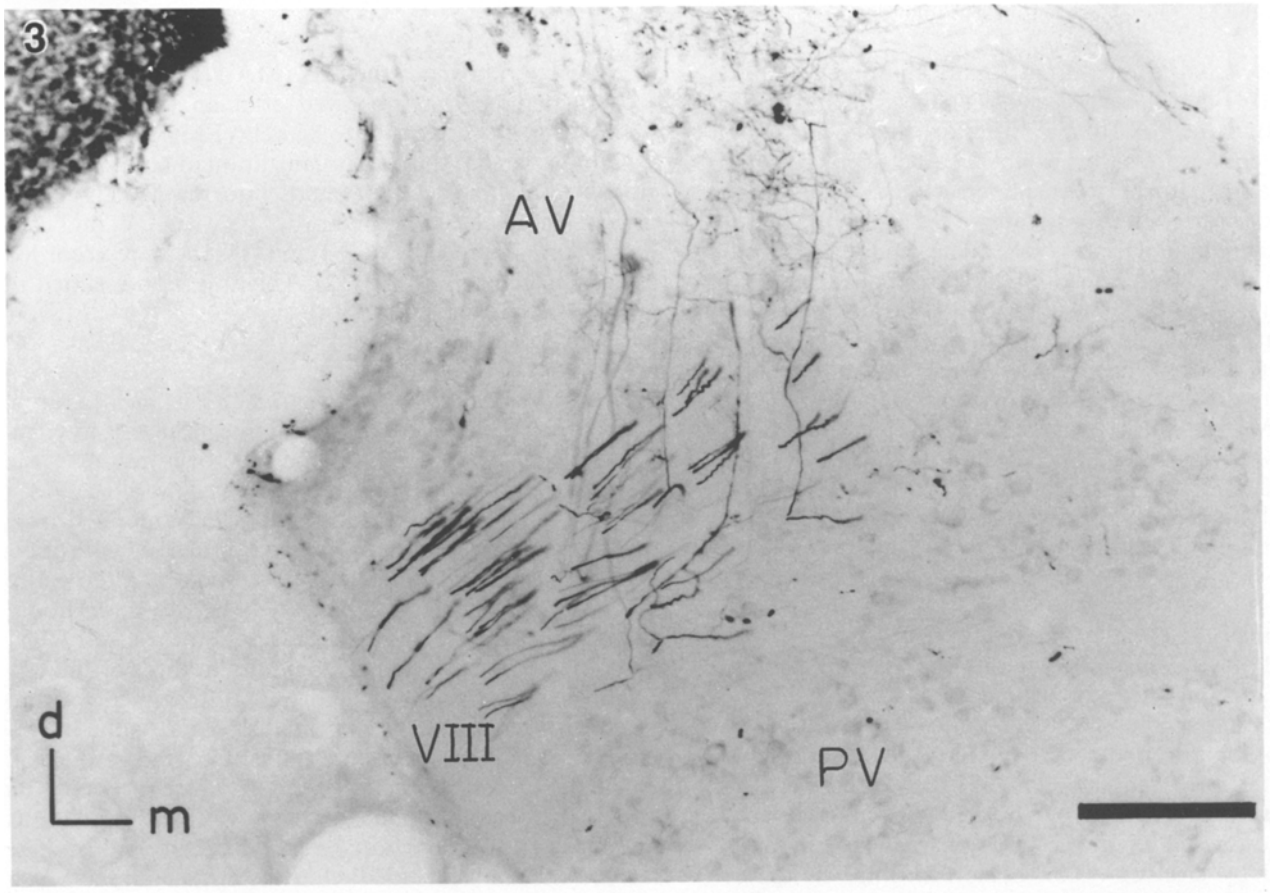
The location and orientation of an isofrequency slab in the AV is demonstrated by an HRP-injection in the PV (Fig. 7). The injection site was located at the border between the lateral and medial subdivision of the PV in a region responsive to 54.5 kHz. Labeled auditory nerve fibers and terminals were found in the rostral AV, in a continuous slab which extended dorsoventrally throughout all of AVa and AVp, but was restricted in the rostrocaudal direction to only 160 μm. The anterograde label in the AV did not extend into the adjacent MAm which may indicate a tonotopic separation of MAm and AV (see below). In the DCN a single band of transport contained both labeled cells and terminals.

Injections in the range of the third and fourth harmonics. The high frequency harmonics of the echolocation call include the frequency range between 72 and 120 kHz and are less intense than the second harmonic. Figure 8 illustrates transport from a large injection in the posterior AV, centered within the CF_3-range (91 kHz). Labeled terminals and cells were found in the dorsal part of DCN and labeled terminals in the dorsal and caudal PV (PVc). The terminals extended from the dorsal PVl and PVc into the lateral part of the MA (MAl) (Fig. 8). A similar pattern of label was observed after an injection in an area of the AV responsive to 85 kHz (FM_3-range). These data indicate that the slab organization of auditory nerve fibers supplying the PV extends into the MAl. A third injection was placed at the border between PVl and caudal MAm, where cells tuned to 111 kHz were recorded (shown in scheme of Fig. 12). This injection resulted in label in the most caudal AVp.

Injections in the range of the first harmonic. Figure 9 shows the results of a very small injection in a low frequency region (23 kHz) of the rostroventral AV; the frequency represented here is the lower limit of the FM_1-range. The AV, DCN, and medial PV contained only few weakly labeled terminals. However, an extensive network of labeled terminals was observed along the entire rostrocaudal extension of the medial MA. In addition, the border region between lateral MA and ventral DCN contained labeled terminals. The orientation of the terminal field within the medial MA differs greatly from that of isofrequency slabs in the AV. Whereas the isofrequency slabs in the AV were quite restricted in the rostrocaudal direction, the label in the MAm occupied its entire rostrocaudal extent. At least two fiber populations are expected to contribute to this labeling pattern. The labeled terminals included relatively large boutons which may correspond to auditory nerve fiber endings and small boutons and varicosities which possibly derive from descending pathways like the catecholaminergic system which was shown to form a dense terminal plexus within the rostrocaudal extent of the MAm group (Kössl et al. 1988). To test whether the MAm group, despite the fact that its location parallels the complete rostrocaudal extension of the AV, is indeed responsive to the low frequencies, physiological recordings and additional injections were made in this area. Figures 10 and 11 show an injection at 29.3 kHz, considerably caudal to the first injection, but centered at the MAm. There were labeled terminals throughout the entire rostrocaudal extent of the MAm, as there were after the injection in the rostral AV (Fig. 9). Close to the injection site some labeled fibers spread into the adjacent AV. In the DCN strongly labeled cells and terminals were found ventrally together with a more patchy labeling pattern dorsally. In this case no label was present in the PV, but another MAm injection (29 kHz, Fig. 14) resulted in labeled terminals in the PVm, which implies that at least part of the MAm innervation is derived from auditory nerve fibers bifurcating in a fashion similar to those destined for other CN-regions.

Frequency map

To produce a tonotopic map of the CN, data from the individual experiments were pooled and plotted in transverse, sagittal and horizontal reconstructions, using a reference atlas that was based on reconstructions from


3
AV
VIII
PV
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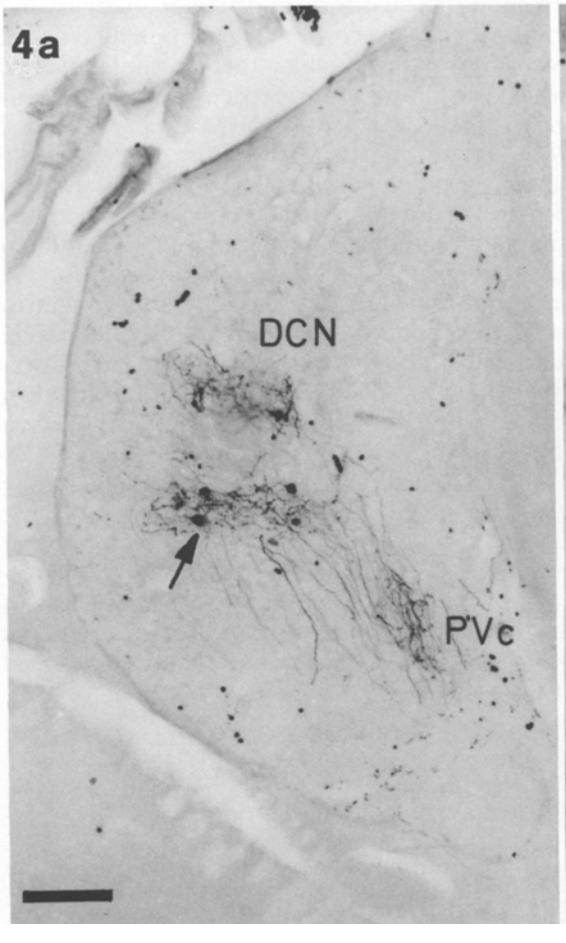
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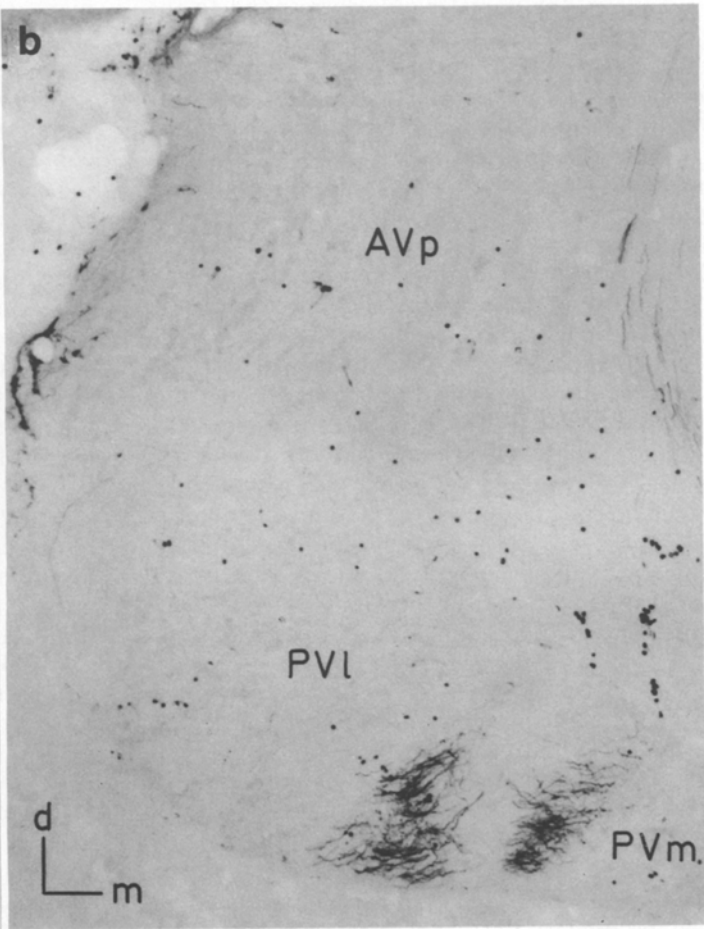
PVc


b
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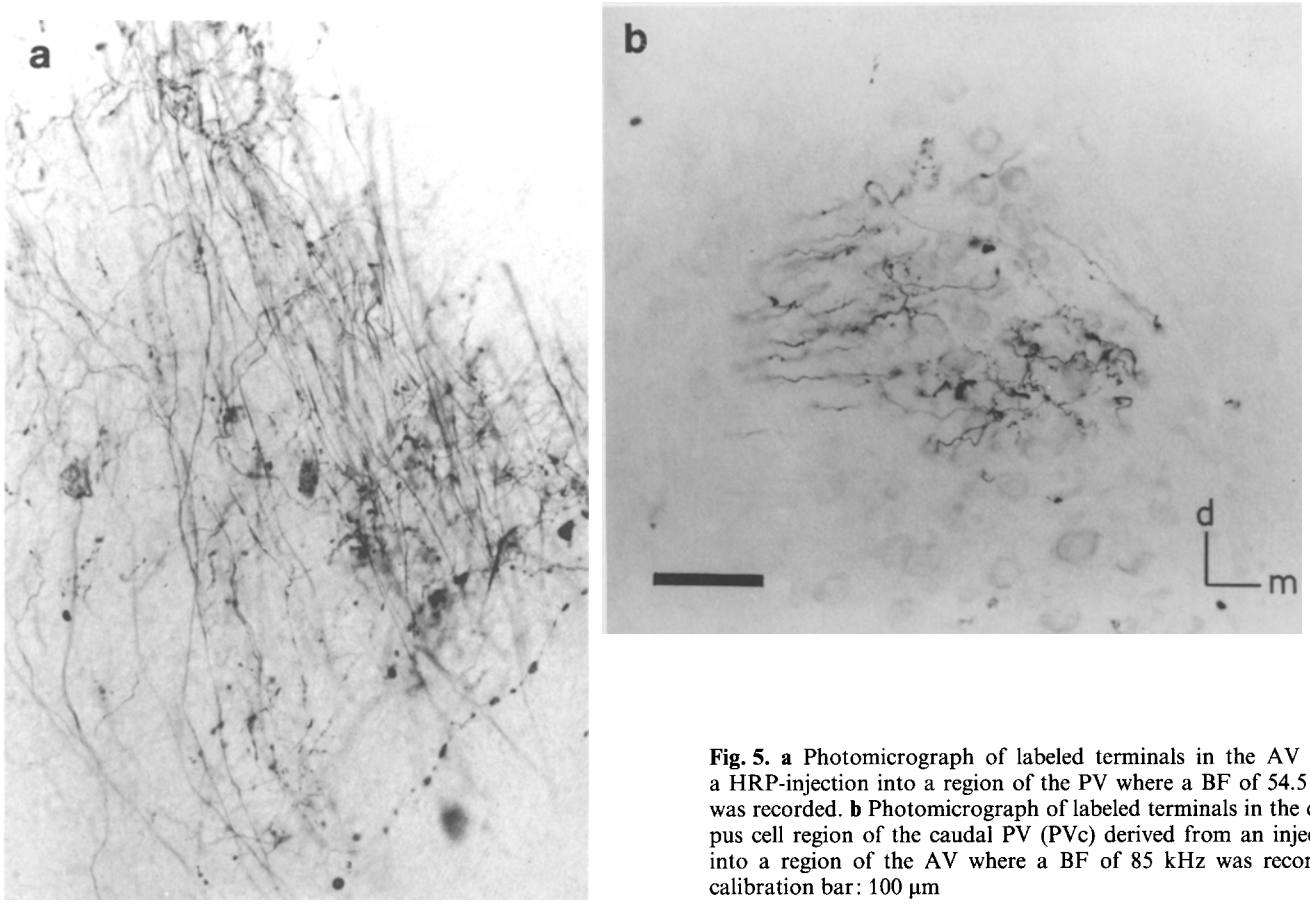

Fig. 5. a Photomicrograph of labeled terminals in the AV after a HRP-injection into a region of the PV where a BF of 54.5 kHz was recorded. **b** Photomicrograph of labeled terminals in the octopus cell region of the caudal PV (PVc) derived from an injection into a region of the AV where a BF of 85 kHz was recorded; calibration bar: 100 μm

3 experimental brains (bat #9, 11 and 12). The upper left diagram of Fig. 12 shows the transverse view of an injection in the PV with a corresponding slab of labeled terminals in the AV. The middle diagram shows two injection sites in the AV with corresponding terminal label in the AV, PV and DCN in sagittal reconstruction. The right diagram shows a horizontal reconstruction of an injection site in the MAm. The lower part of Fig. 12 schematically illustrates the tonotopic organization derived from the complete set of data. In the AV, the isofrequency planes are ordered in an increasing frequency sequence going from rostral (low) to caudal (high). In the PV and DCN, there is an orderly sequence of isofrequency planes extending from ventral (low) to dorsal (high). Each subnucleus contains a complete tonotopic range. In all 3 subdivisions the range of frequencies between 54.5 and 66 kHz is expanded; this range makes up about 40% of the total volume of the AV and PV. The overrepresented frequency range corresponds to CF_2 and upper FM_2. In addition, a slight expansion of the range between 85 and 91 kHz in comparison to adjacent frequencies is evidenced in the AV (Fig. 12, sagittal view). In the rostral AV and the ventromedial PV the frequencies below 46 kHz occupy a very restricted space. However, in the MAm, both recordings and injections demonstrate that its frequency representation is biased for the range of the first harmonic (i.e. below 32 kHz). The presentation of frequencies within the MAm group is not in register with the rostrocaudal sequence of isofrequency contours in the adjacent AV as shown by recordings and injections. Two injections and one lesion in the MAm (29 kHz, 29.3 kHz, and 31.5 kHz, respectively) were located at rostrocaudal positions which, in the adjacent AV, represent the frequency range between 60 and 85 kHz (right diagram of lower row in Fig. 12). In contrast to the MAm, the tonotopic sequence of the MAl is at least partly in register with that of the PV, since the MAl receives labeled terminals continuous with slabs of label in the PV following injections into AV regions responsive to high frequencies (Fig. 8). The MAl group also receives terminals from fibers supplying the ventral DCN (AV injection at 23 kHz).

Fig. 3. Photomicrograph of labeled bifurcating auditory nerve fibers derived from a HRP-injection into the AV; calibration bar: 100 μm

Fig. 4a, b. Photomicrograph of labeled fibers and terminals resulting from HRP-injections into two different AV-regions where BFs of 46 kHz and of 60.6 kHz, respectively were recorded. Two distinct bands of labeled terminals are found in the DCN **a** and in the PV **b**. In addition in the DCN cell bodies are retrogradely labeled (arrow); calibration bar: 100 μm

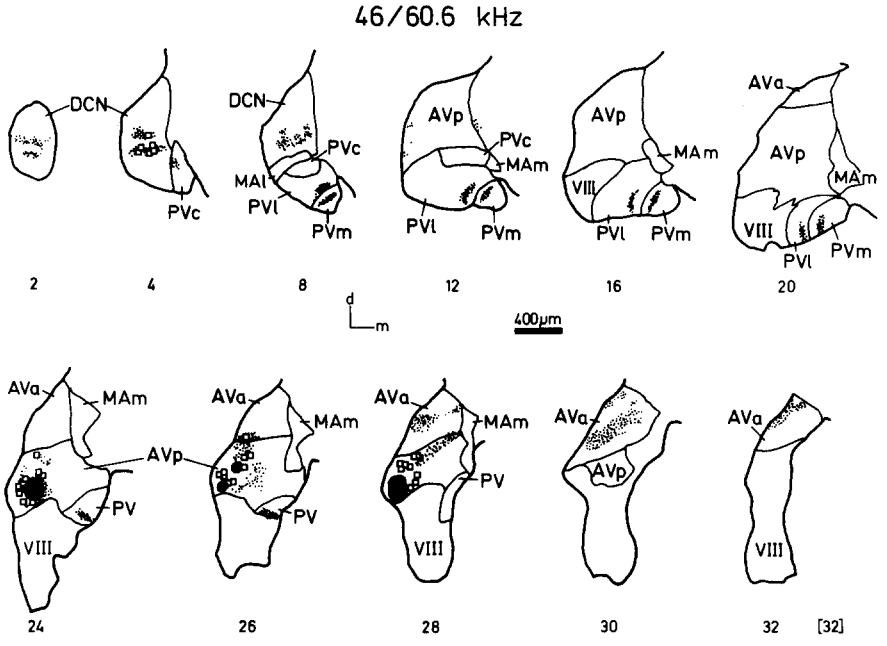

Fig. 6. Pattern of transport of HRP in the cochlear nucleus after two injections into AV-regions responsive to 46 kHz (section 28) and 60.6 kHz (section 24), respectively (black areas indicate the injection sites as defined by DAB reacted slices); two distinct stripes of HRP-label are found in the DCN (labeled terminals are indicated by dots and cell bodies by open squares) and in the PV (labeled terminals). The transverse sections are arranged from caudal to rostral; the slice number refers to the caudalmost CN section, the total number of CN-sections is given in brackets

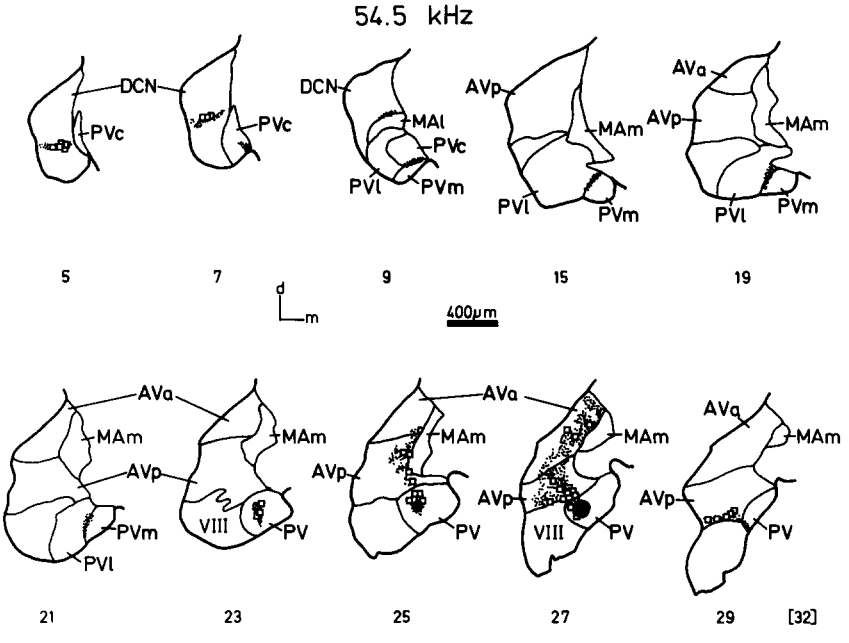

Fig. 7. Pattern of transport of HRP in the cochlear nucleus resulting from an injection into a PV-region responsive to 54.5 kHz. The labeled terminal field in the AV forms a dorsoventrally extended slab; for further explanation see Fig. 6

Specialized aspects of the marginal cell group

Frequency representation. The anatomical results indicate that the MAm mainly processes frequencies between 23 and 32 kHz. Figure 13 shows further details of the frequency representation evidenced by a series of parallel electrode penetrations spanning part of the caudal to rostral and the dorsomedial to ventrolateral extent of the AV and the MAm. In all passes, the BFs of the most dorsomedial neurons were below 33 kHz and single unit activity was always accompanied by strong evoked potentials (EPs) between 25 and 29 kHz. These pronounced EPs are probably due to highly synchronized firing activity and precise latencies of the first spikes occurring in response to the frequencies of the FM_1-component of the echolocation signal (see below). In more ventrolateral regions there is an abrupt transition to higher BFs (50–100 kHz). As the electrode was advanced further, the BFs continuously decreased. This is consistent with the tonotopic arrangement of the AV, since the oblique angle of the penetration crosses through the different isofrequency laminae. Both lesions and HRP-injections show that the dorsally located low frequency bands correspond to the MAm. The proximity

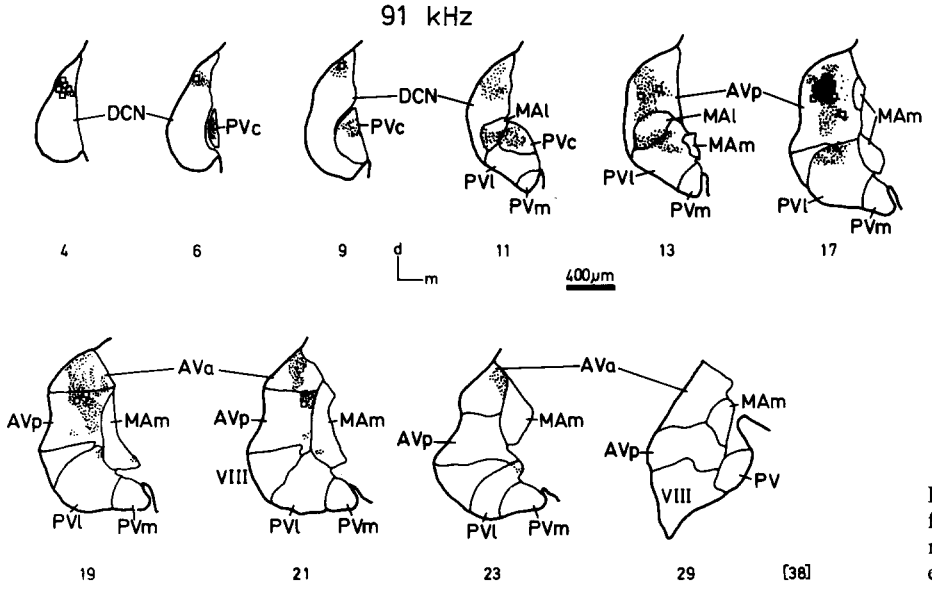

Fig. 8. Pattern of transport of HRP from an injection into an AV-region responsive to 91 kHz; for further explanation see Fig. 6

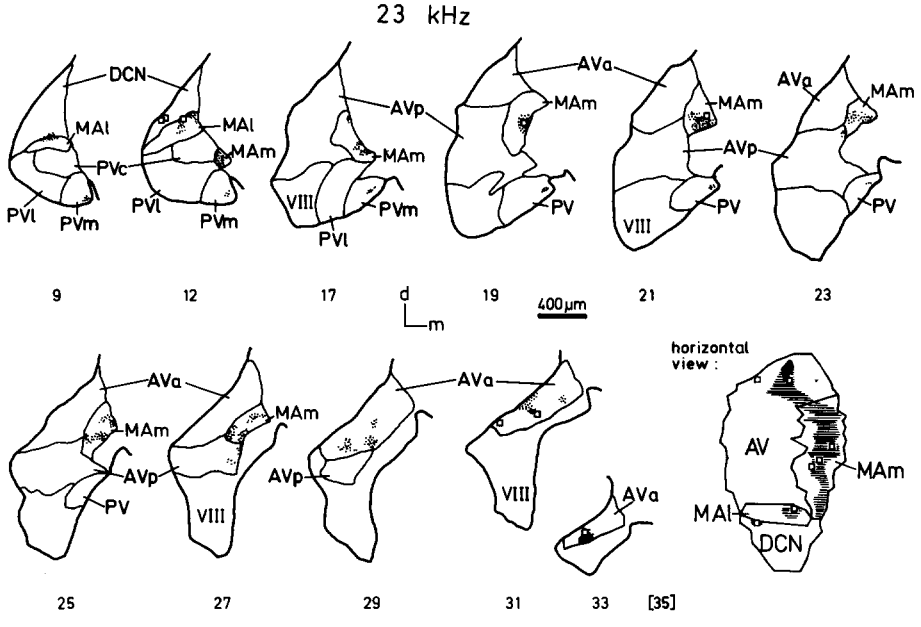

Fig. 9. Pattern of transport of HRP in the cochlear nucleus resulting from an injection into an AV-region responsive to 23 kHz. On the bottom (right) a horizontal reconstruction of the transverse sections is outlined to show the rostrocaudal extent of the field of labeled terminals (hatched area). Labeled terminals are found over the whole length of the MAm. For further explanation see Fig. 6

of neurons tuned to the frequency range of the first harmonic to neurons of much higher BF in the AV is illustrated by the injection in Fig. 14a centered in the MAm but extending into the adjacent AV as well. At the injection site a single neuron with a BF of 29 kHz was recorded. Background multiunit activity was tuned to 61.4–64 kHz, presumably originating from the nearby AV. The injection was large enough for the HRP to spread into the adjacent AV (Fig. 14a) and accordingly the tracer was taken up by auditory nerve terminals in both AV and MAm. In the cochlea (Fig. 14b), two distinct regions widely separated from one another contained labeled spiral ganglion cells providing further evidence for the discontinuity in the frequency representation of MAm and AV. About 2/3 of the labeled cells are located in the apical part of the spiral ganglion and their afferent dendrites supply the apical low frequency regions of the basilar membrane (Fig. 14b). These cells probably project to the center of the injection site in the MAm. One third of the labeled spiral ganglion cells was found within the enlarged basal turn of the cochlea and their dendrites contacted inner hair cells on the stretch of basilar membrane which is specialized on processing the frequency range around 60 kHz (Kössl and

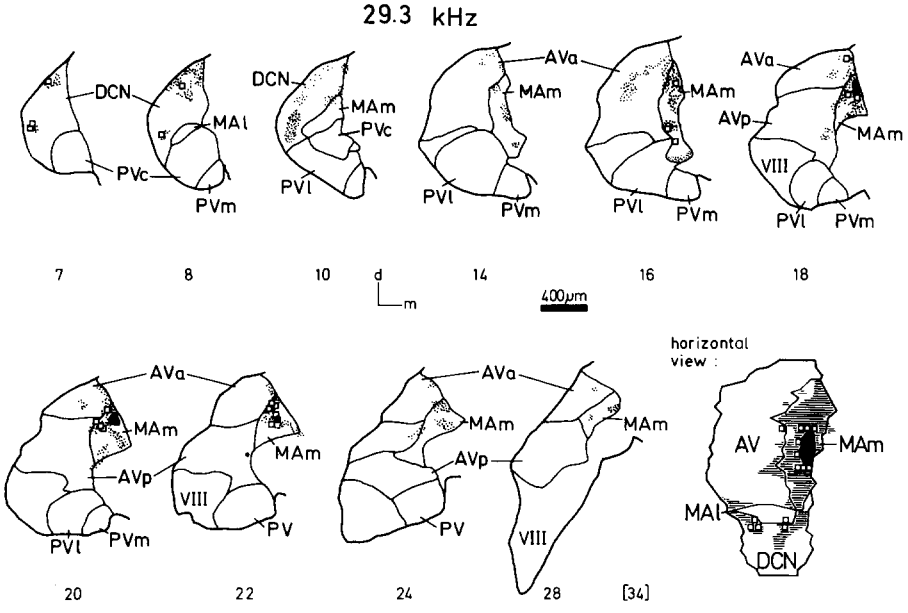

Fig. 10. Pattern of transport of HRP in the cochlear nucleus after an injection into the MAm (29.3 kHz BF). Terminals are labeled within the whole extension of the MAm but not within the MAl (see horizontal reconstruction at the bottom). For further explanation see Figs. 6, 9

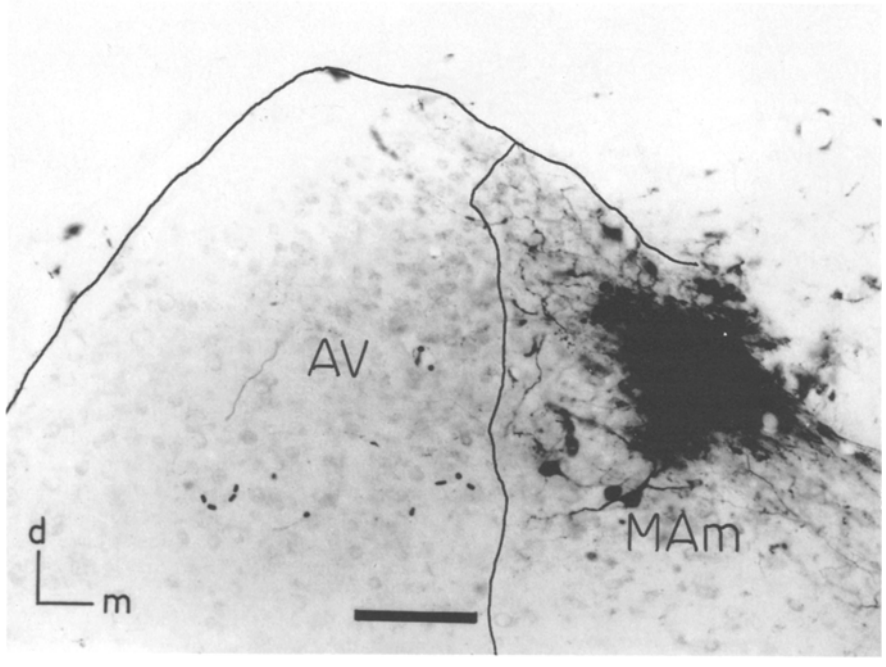

Fig. 11. Photomicrograph of a HRP-injection site in the medial MA region with labeled multipolar cells close to the center of injection (29.3 kHz; calibration bar: 100 µm)

Vater 1985b). These cells supposedly project to the adjacent AV.

Temporal response patterns. Neurons with BFs between 24 and 32 kHz were recorded mainly in the MAm and in their temporal response patterns, phasic onset components were more accentuated than in neurons responsive to higher frequencies. To substantiate this observation, we analyzed in detail the temporal response patterns of 230 single units, recorded in both AV and MAm and tuned to frequencies between 9 and 116 kHz.

Neuronal response patterns recorded at levels 20 to 30 dB above the threshold at the BF were classified according to the terminology of Kiang et al. (1965), Evans and Nelson (1973) and Feng and Vater (1985). The proportion of neurons belonging to each type is summarized in Table 2. Primary like neurons (category 1), characterized by their sharp onset component followed by tonic excitation (Fig. 15) were most common (25%). Most of these units were spontaneously active. Some neurons only showed tonic excitation (category 2, 14%). Category 3 includes pauser neurons with a wide gap in their PST-histograms or sharp notches that mainly occur after an onset component (8%). Chopper neurons (category 4, 3%) either showed only a few regulary spaced response peaks at the beginning of the stimulus or

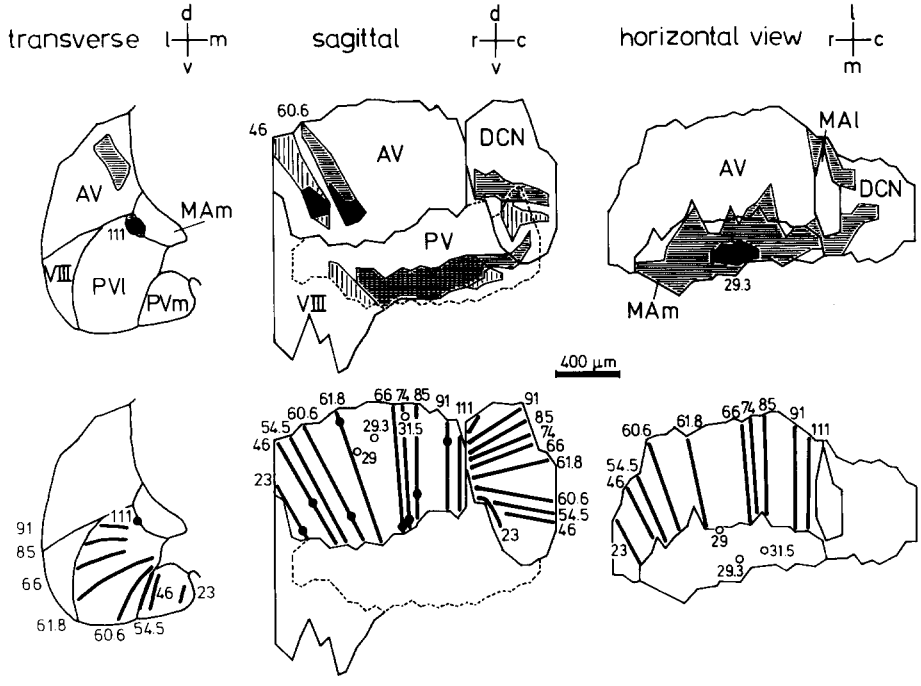

Fig. 12. Frequency map of the cochlear nucleus. *Top row:* HRP-labeled projection fields from four representative injections into the PV (left diagram, transverse reconstruction), the AV (middle diagram, sagittal reconstruction), and the MAm (right diagram, horizontal reconstruction). The BFs at the injection sites (black areas) are given by numbers; the location of the corresponding terminal fields is indicated by hatched areas. *Bottom row:* Schematic summary diagram of HRP-injection sites (circles) and projection fields (thick lines), reconstruction in three different planes

Table 2. Temporal response patterns of single units recorded in the anteroventral CN and MAm. For further explanations see text

Response	# of neurons	(total # : 230)
1. Primary like	58	(25%)
2. Tonic	32	(14%)
3. Pauser/notch	18	(8%)
4. Chopper	7	(3%)
5. Phasic primary like	53	(23%)
6. Phasic-A	30	(13%)
7. Phasic-B	14	(6%)
8. Phasic-C	18	(8%)

throughout the stimulus length. In neurons of the categories 5–8, pronounced phasic response components occurred. These phasic neurons comprised 50% of the sample. To arrange the single units in categories ranging from tonic to extremely phasic the relative strength of phasic and tonic activity was measured. In neurons of category 1 and 2 (primary like and tonic), the number of spikes counted within the first 5 ms of the response was less than 50% of the number of spikes counted within the next 15 ms. In phasic primary like neurons (category 5, 23%) the number of spikes within the first 5 ms of the PST-histogram was more than 50% and less than 100% of the counts within the following 15 ms window. In phasic-A neurons (category 6, 13%) the number of spikes in the first window lay between 100% and 300% of the number in the second window. Phasic-B neurons (category 7, 6%) were characterized by a number of spikes in the first window more than 300% higher than in the second window. Phasic-C units (category 8, 8%) were pure onset neurons which only responded with one or two spikes within the first 5 ms.

Figure 16 illustrates that for the total population of neurons as well as for neurons within BFs within the frequency ranges of the second and third harmonic of the echolocation calls (48–63, 72–94 kHz) the tonic response types prevailed. However, for neurons tuned to the frequency range of the first harmonic (24–32 kHz), and in particularly in neurons with BFs in the frequency range of the FM-part of the first harmonic (24–30 kHz), the dominant response type was phasic.

Discussion

General tonotopic arrangement

In *Pteronotus*, as in other mammals (e.g. Lorente de No 1933a, b; Rose et al. 1959; Feng and Vater 1985), each of the 3 subdivisions of the CN contains a complete representation of the hearing range. In the AV, the isofrequency planes as defined by physiological recordings and HRP-label are flat sheets oriented dorsoventrally. High frequencies are represented more caudally than low frequencies. Such an arrangement is also seen in the horseshoe bat (Feng and Vater 1985). In its essential features the orderly representation of isofrequency lamina in the AV of the two bat species, as depicted in sagittal view, is similar to the tonotopic arrangement of the AV of the cat (Bourk et al. 1981). According to our data, the frequency representation in the AV does not strictly coincide with the cytoarchitectonic subdivisions, AVa and AVp respectively. For HRP-injections into the frequency range from 46 to 91 kHz (which occupy about 80% of the volume of the AV), the slabs of labeled auditory nerve fibers clearly crossed the border between AVa and AVp. Only very low (<46 kHz) or very high frequencies (>91 kHz) were restricted

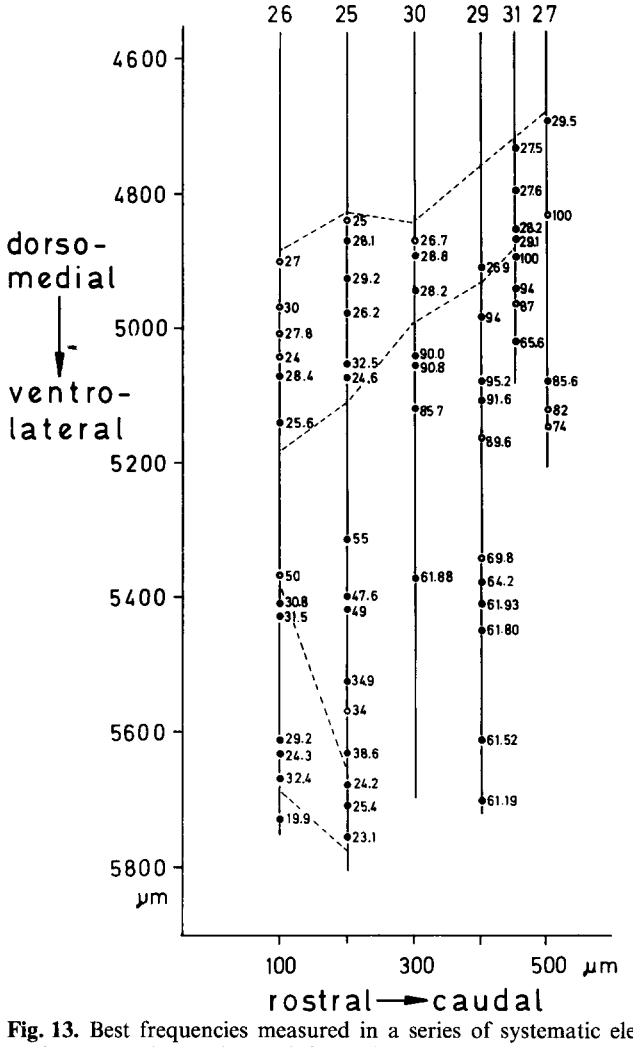

Fig. 13. Best frequencies measured in a series of systematic electrode penetrations advanced from dorsomedial to lateroventral through the medial MA and adjacent AV (open circles: single units, filled circles: multiunits). Areas with best frequencies below 31 kHz are outlined with broken lines. Note the abrupt jump from low to high BFs, taking place at the dorsomedial border of the recording area

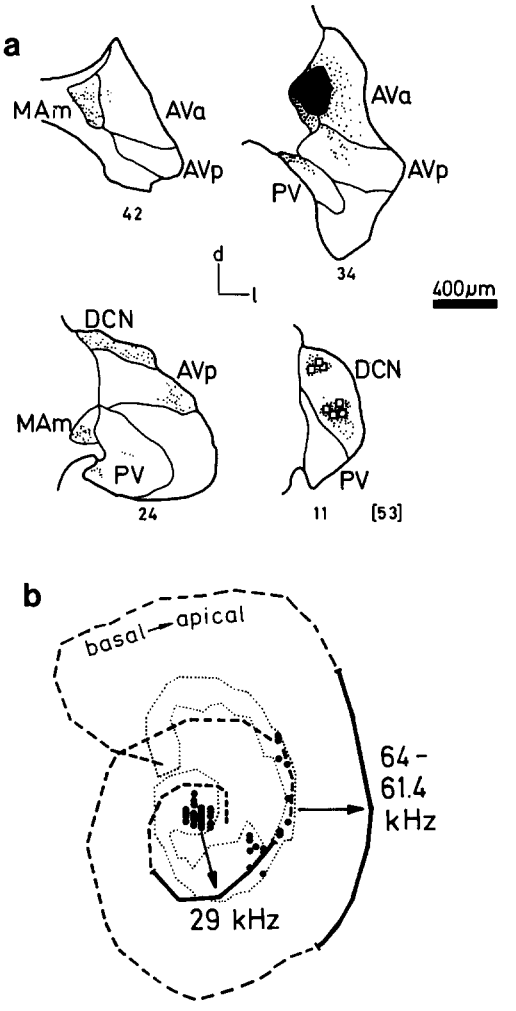

Fig. 14. a HRP-transport pattern derived from an injection which covers part of the medial MA and the adjacent AV. At the CN-injection site a single unit activity with a BF of 29 kHz was recorded, in addition to a background multiunit activity in the range of 61.4 to 64 kHz; Symbols as in Figs. 6–10. **b** Corresponding label in the cochlea shown in a horizontal projection. The course of the basilar membrane is given by a thick broken line and basilar membrane regions where labeled afferent fibers terminate are shown as thick solid lines. In the spiral ganglion (outlined with dotted lines) two groups of labeled cell bodies are found (filled circles) which project to two clearly different regions of the basilar membrane that are separated by about 6 mm. The basal termination area is located in the second half turn where the CF component of the 2nd harmonic around 60 kHz is represented (Kössl and Vater 1985b), the apical label is located in the fourth half turn which is responsive to the first harmonic

to either AVa or AVp. A similar organizational feature is also reported for the AV of the cat (Bourk et al. 1981).

Analysis of retrograde transport of HRP from focal injections into the IC of *Pteronotus* (Ross et al. 1988) shows a rostro-caudal organization from low to high frequencies in the AV, which is similar to our data. However, differing from our results, their data suggest a clear correlation of cytoarchitectonic subregions of the AV with the frequency representation: Neurons in the AVa were labeled from injections into the low frequency range and the neurons in the AVp from injections into the 60 kHz range, respectively. These data are not necessarily contradictive, if one takes into account that focal HRP-injections into the IC will only label a subset of the total population of input neurons. This raises the question whether in *Pteronotus* there are segregations of central projections from the different cell types comprising an isofrequency lamina in the AV to the corresponding isofrequency lamina in the IC or a differential strength of their respective projections as discussed for the CN of the cat (Adams 1979).

In the PV and DCN of *Pteronotus*, the isofrequency planes are oriented almost horizontally and thus perpendicular to those in the AV. Low frequencies are located ventrally and higher frequencies progressively more dorsally, a pattern which again is similar to data from the cat and the horseshoe bat (Rose et al. 1959; Feng and

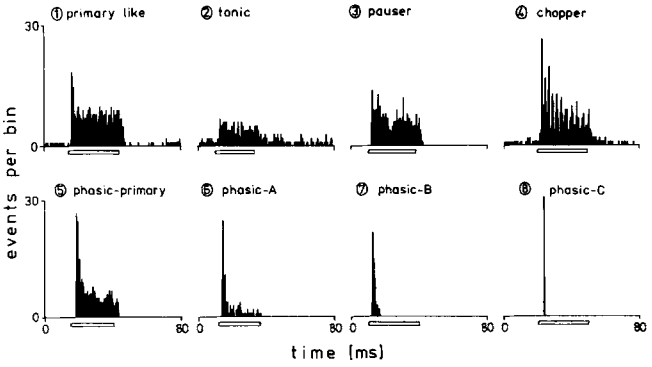

Fig. 15. Neuronal response patterns (post-stimulus-time, PST-histograms) recorded in the anteroventral CN and the MAm. See text for further details. Stimulus duration is given by bars and ranges from 20 to 30 ms; 40 stimulus presentations; bin width of 200 μs

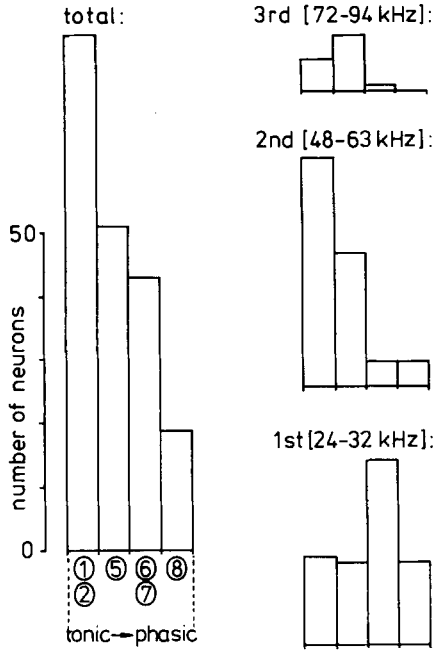

Fig. 16. Proportion of different response types found within the entire population (left) and for subpopulations of neurons responding within the frequency ranges of the 1st, 2nd and 3rd harmonics (right). For neurons with BFs in the 1st harmonic range, the proportion of phasic responders is clearly higher than it is for neurons with BFs in the range of the 2nd and 3rd harmonic

Vater 1985). In the PV of *Pteronotus*, the medial division (PVm) processes low frequencies (< 54 kHz), whereas in the lateral division (PVl) the whole range of higher frequencies, is orderly represented. A basically similar dorsoventral gradient of the frequency representation in the PV is reported from HRP-injections into the IC (Ross et al. 1988).

The regular dorsoventral organization of high to low frequencies in the DCN of *Pteronotus* as reported here, is basically similar to the organization of the DCN in the cat (Rose et al. 1959) and the horseshoe bat (Feng and Vater 1985), however less specialized than in the latter species. Such a clear cut organization was not evident in analysis of the retrograde HRP-transport from the IC (Ross et al. 1988), which may indicate a more complex central projection pattern of this CN-division than expected from a simple tonotopic scheme.

A common feature of the CN of two CF-FM bats, *Rhinolophus* (Feng and Vater 1985) and *Pteronotus* is the overrepresentation of the frequency range of the dominant second harmonic of the echolocation signal in all 3 subdivisions. However, in *Pteronotus*, the high frequencies of the third harmonic and the FM-range of the fourth harmonic are well represented in the CN, in contrast to *Rhinolophus*, where neurons with such BFs are not found (Feng and Vater 1985). This difference is correlated with differences in the orientation calls. In the echolocation calls of *Rhinolophus*, the third harmonic appears only occasionally (Neuweiler et al. 1987) whereas in *Pteronotus* the third and fourth harmonics are well pronounced, the third harmonic is only 10 dB less intense and the fourth harmonic 20 dB less intense than the dominant second harmonic (Suga 1984). A further pronounced species difference is the representation of the first harmonic frequency range, which as discussed below, is extremely specialized in *Pteronotus*.

Frequency representation in the marginal cell group

A deviation from the general mammalian scheme of tonotopy in the cochlear nucleus was found for the marginal cell group of *Pteronotus*. The rostral 2/3 of this area respond to low frequencies between 24 and 32 kHz as shown by the HRP-injections in Fig. 12 and the physiological data of Fig. 13. There is a sharp discontinuity between neurons tuned to FM/CF_1 frequencies which are located in the MAm and neurons in the adjacent AV which respond to higher frequencies. The patterns of labeled terminals after an injection of HRP into the AV (Figs. 6, 7, 8) abruptly terminate at the border between AV and MAm indicating that the discontinuity in frequency representation indeed is related to this border.

Since the MAm is located at the medial edge of the CN, close to the fiber bundles of the trapezoid body and the intermediate acoustic stria, our recordings could be obtained from fibers of passage instead of cell bodies. Several lines of evidence argue against this possibility:

1. The shape of action potentials in the marginal cell group was similar to recordings in the AV. The main difference to AV-recordings was the size of the spikes. In general the spikes recorded in low frequency neurons within the MAm were larger than for instance in neurons of the AV tuned to 60 kHz. We attribute this to the fact that the cell bodies in the marginal cell group are distinctly larger than in the rest of the ventral cochlear nucleus.

2. Iontophoretic injections of various neurotransmitters (GABA, glycine, glutamate, acetylcholine, noradrenaline) onto MAm neurons (Kössl and Vater 1989) produced profound changes in spike rate and temporal

auditory response patterns. This implies that we indeed recorded from cell bodies.

3. HRP-injections in the MAm resulted in labeled spiral ganglion cells that projected to apical parts of the basilar membrane where low frequencies are represented (Fig. 14, Table 1). This provides evidence that the MAm cells are supplied by auditory nerve fibers like the rest of the CN and most directly shows the frequency range they are tuned to.

We have no detailed physiological data on the exact frequency representation in the caudal third of the MAm and the MAl. Neurons responding to 111 kHz were recorded at the center of a HRP-injection site located at the border between caudal MAm and PV (Fig. 12). Injections in AV regions responsive to 85 and 91 kHz resulted in labeled fibers in the MAl. This indirectly suggests that higher frequencies are represented in these areas. Additional evidence for this notion could come from the study of Ross et al. (1988), who found retrogradely labeled cell bodies in the marginal area adjacent to the most caudal AV after injections in IC-sites responsive to frequencies above 60 kHz. Contrasting with these data, pronounced retrograde label in the entire MAm and MAl was found by Zook and Casseday (1985) after injecting HRP in the lateral part of the LSO where according to Ross et al. (1988) low frequencies are represented. This raises the question if HRP-transport from higher integration centers can be exclusively interpreted in terms of tonotopic arrangement according to BF. Further data on the specific projections of the MAm cells are needed.

The MAm differs from the rest of the CN not only in its frequency representation but also in its innervation by descending fiber systems. Catecholaminergic fibers and fibers staining positive with acetylcholine-esterase establish particularly dense and distinct terminal plexi within the MAm (Kössl et al. 1988). This indicates that the neuronal processing of the MAm cells may be specifically modulated by descending fiber systems.

Phasic characteristics of single unit tuned to low frequencies

Most of the neurons in the anteroventral CN of *Pteronotus* showed tonic or primary-like response patterns which closely reflect the auditory nerve activity, just as they do in the cat (Kiang et al. 1965; Evans and Nelson 1973). Phasic responses are predominantly seen in low frequency neurons of the AV with BFs between 24 and 32 kHz. This response feature is most probably not related to cochlear specializations since some of the low frequency neurons display normal primary-like responses which indicates that the auditory nerve input to low frequency regions of the CN is primary-like and not phasic. Instead, several factors intrinsic to the CN could contribute to the phasic response characteristics:

1. Onset responses are common in large neurons of the CN, such as octopus or giant cells (Rhode et al. 1983a, b). In *Pteronotus* the MAm cells which preferentially represent the range between 24 and 32 kHz are among the largest neurons in the cochlear nucleus. Due to the large membrane surface, it is possible that only the first well synchronized input spikes can induce depolarizations large enough to elicit an output spike.

2. Phasic responses could result from feedback inhibition by recurrent axon collaterals innervating inhibitory interneurons. Phasic neurons in the PV of the rat have been shown to send axon collaterals back into the CN (Friauf and Ostwald 1988). No cell bodies in the AV and MAm of *Pteronotus* stain positively with antibodies against GAD (glutamic acid decarboxylase), a marker for the inhibitory transmitter GABA (gamma-amino-butyric-acid) (Vater et al., unpublished observation). Thus, any inhibitory interneurons would have to use a different transmitter, e.g. glycine, which has been shown to exert inhibitory actions in the CN of other mammals (Caspary et al. 1979). Feedforward inhibition by inhibitory collaterals of the auditory nerve as discussed for octopus cells (Kane 1973; Walsh and McGee 1987) could also result in phasic onset response patterns if the inhibitory input had a longer latency than the excitatory input.

3. Low frequency areas in the CN of *Pteronotus*, in particularly the MAm, are densely innervated by catecholaminergic fibers (Kössl et al. 1988). Noradrenaline administered iontophoretically enhances phasic responses and depresses tonic responses in the CN of *Pteronotus* (Kössl and Vater 1989). This suggests that the catecholaminergic innervation might play a role in producing the phasic response type.

Phasic low frequency responses could be of special biological significance for target range measurement during echolocation. Echolocating bats derive the distance of their targets from the time lag between emitted call and returning echo (Simmons 1973). In the auditory cortex of *Pteronotus* there is a distinct population of neurons which are able to code target range information (O'Neill and Suga 1979, 1982). These neurons respond to second or third harmonic components of the echo only if it is preceded by the first harmonic FM of the call and both components have to be separated by a specific temporal delay to yield a maximum neuronal response. Different temporal delays correspond to different target ranges. A large proportion of the MAm cells in the CN of *Pteronotus* is tuned to the frequency range of the first harmonic and the predominant response type is phasic. Consequently, the output of these MAm cells provides a sharp and precise trigger pulse which should optimize the time delay measurements at higher levels of the auditory system. Thus, the MAm cells may represent the most peripheral stage of a neural network designed for precise temporal measurements in the context of the processing of target range information.

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