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RESEARCH ARTICLE

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The projection from auditory cortex to cochlear nucleus in guinea pigs: an in vivo anatomical and in vitro electrophysiological study

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Abstract Previous anatomical experiments have demonstrated the existence of a direct, bilateral projection from the auditory cortex (AC) to the cochlear nucleus (CN). However, the precise relationship between the origin of the projection in the AC and the distribution of axon terminals in the CN is not known. Moreover, the influence of this projection on CN principal cells has not been studied before. The aim of the present study was two-fold. First, to extend the anatomical data by tracing anterogradely the distribution of cortical axons in the CN by means of restricted injections of biotinylated dextran amine (BDA) in physiologically characterized sites in the AC. Second, in an in vitro isolated whole brain preparation (IWB), to assess the effect of electrical stimulation of the AC on CN principal cells from which intracellular recordings were derived. BDA injections in the tonotopically organized primary auditory cortex and dorsocaudal auditory field at high and low best frequency (BF) sites resulted in a consistent axonal labeling in the ipsilateral CN of all injected animals. In addition, fewer labeled terminals were observed in the contralateral CN, but only in the animals subjected to injections in low BF region. The axon terminal fields consisting of boutons *en passant* or *terminaux* were found in the superficial granule cell layer and, to a smaller extent, in the three CN subdivisions. No axonal labeling was seen in the CN as result of BDA injection in the secondary auditory area (dorsocaudal belt). In the IWB, the effects of ipsilateral AC stimulation were tested in a population of 52 intracellulary recorded and stained CN principal neurons, distributed in the three CN subdivisions. Stimulation of the AC evoked slow late excitatory postsynaptic potentials (EPSPs) in only two cells located in the dorsal CN. The EPSPs were induced in a giant and a pyramidal cell at latencies of 20 ms and 33 ms, respectively, suggesting involvement of polysynaptic circuits. These findings are consistent with anatomical data showing sparse projections from the AC to the CN and indicate a limited modulatory action of the AC on CN principal cells.

Keywords Biotinylated dextran amine (BDA) · Intracellular recording · Intracellular staining · Corticobulbar axons

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Introduction

The cochlear nucleus (CN) occupies a strategic position in the auditory system. Being the first relay in the auditory pathways, it receives stereotyped inputs from the auditory nerve (AN), modifies this information, and then sends output signals to multiple nuclei of the brainstem and midbrain. In addition, it receives reciprocal, descending auditory inputs which constitute a substantial proportion of connections in the auditory system (Huffman and Henson 1990; Romand and Avan 1997; Rouiller 1997). The sources of the descending auditory projections to the CN have been shown to arise from the contralateral CN (Cant and Gaston 1982; Wenthold 1987; Shore et al. 1992; Schofield and Cant 1996), the superior olivary complex (Adams 1983; Spangler et al. 1987; Brown et al. 1988; Schofield and Cant 1999), the dorsal nucleus of the lateral lemniscus (DLL) (Caicedo and Herbert 1993), and the inferior colliculus (IC) (Van Noort 1969; Carey and Webster 1971; Adams and Warr 1976; Kane and Finn 1977; Andersen et al. 1980; Conlee and Kane 1982; Hashikawa 1983; Faye-Lund 1986, 1988; Coleman and Clerici 1987; Herbert et al. 1991; Malmierca et al. 1996;

Schofield 2001). The auditory cortex (AC) is the most remote auditory region giving rise to descending auditory projections to the CN. For a long time, it was believed that the AC might influence the CN only via indirect pathways involving the midbrain. However, recent anatomical experiments (Feliciano et al. 1995; Weedman and Ryugo 1996a, 1996b; Saldaña et al. 1996; Schofield et al. 2001; Doucet et al. 2002) have demonstrated the existence of direct, bilateral projection from the AC to the CN. It has been shown that projections from AC to CN in the rat and the guinea pig arise from layer V neurons of the AC and terminate mainly in the granule cell domain of the CN (Weedman and Ryugo 1996a, 1996b; Schofield et al. 2001). However, the precise relationship between the origin of the projection in the AC and the distribution of axon terminals in the CN remains largely unknown. Although the existence of descending auditory inputs to CN suggests a possible mechanism for cortical feedback control of afferent information at peripheral levels, the functional nature of the influence exerted by direct descending projections from the AC to CN remains unknown.

Therefore, the aim of the present study was two-fold. The first aim was to extend the available anatomical data, by establishing the distribution of the corticobulbar axons in the CN. The second objective was to assess the effects of activating the descending projections from the AC on CN neurons. Two types of experiments were conducted in the guinea pig to address these questions. First, the distribution of corticobulbar axons in the CN was established neuroanatomically, based on restricted injections of the anterograde tracer biotinylated dextran amine (BDA) in physiologically characterized sites in the AC. Second, an in vitro isolated whole brain (IWB) preparation was used to perform intracellular recording and staining of single neurons in the CN in order to test the presence and the physiological properties of synaptic responses to electrical stimulation of the AC. Since, in the IWB preparation, it was impossible to record intracellularly from small neurons, the effects of AC stimulation could be assessed only in CN principal cells.

Materials and methods

In vivo experiments

BDA injections into the AC were performed in five adult guinea pigs (body weight 530–850 g). Under general anesthesia (diazepam 8 mg/kg, followed 15 min later by pentobarbital 20 mg/kg; see Evans 1979), a craniotomy (about 5×8 mm) was made in the temporal bone. Small punctures were made in the dura under microscopy control to insert low impedance glass pipettes (<3 MΩ) that allowed multiunit recordings (3–5 neurons) at a depth of 500–1000 μm. The frequency tuning at each recording site was tested between 20 and 80 dB SPL using a previously described sound delivery system (Manunta and Edeline 1997, 1999). Briefly, ascending sequences of pure tones (frequency 0.1–35 kHz, 100 ms duration, 1 s intertone interval) were delivered to the contralateral ear. Online displays of the peri-stimulus time histograms allowed determination of the frequency tuning at each intensity. A regular progression of the best frequency (BF) from low to high when moving from rostral to caudal was interpreted as a location in the primary auditory cortex (AI), whereas a regular progression of the BF from high to low was assumed to be typical of the dorsocaudal field (DC) (Redies et al. 1989; Wallace et al. 2000). Locations in secondary auditory fields (DCB dorsocaudal belt, VCB ventrocaudal belt; Wallace et al. 2000) were characterized by (1) long-latency responses, (2) very broad tuning, and (3) a lack of tonotopy. At a previously characterized locus, a pipette (tip 25–50 μm) filled with BDA (10%; Molecular Probes, Eugene, OR, USA) was lowered 1500 μm below the pial surface and the tracer was iontophoretically ejected for 45 min (8 μA positive current, 7 s on/off). After a survival time of 7–10 days, the animals were deeply anesthetized (pentobarbital, 120 mg/kg) and perfused with a 4% paraformaldehyde (PFA) solution in 0.1 M phosphate buffer (PB, pH 7.4). After 24 h postfixation, the brain was placed in a solution of 30% sucrose in 0.1 M PB until it sank. Serial coronal sections (40 μm) were prepared with a freezing microtome and collected in 0.05 M Tris (pH 7.6). BDA labeling was vizualized using previously described protocols (see for details Bajo et al. 1999). Individual sections were examined under brightfield illumination (×4, ×10, ×20, and ×40), and reconstruction of the corticobulbar axons in the CN was made using a drawing tube.

The parcellation of the CN and the corresponding nomenclature used in the present study were derived from the original description of the CN in the guinea pig by Hackney et al. (1990) in Nissl material. Two main divisions of the CN are clearly distinguishable. Caudally, the dorsal cochlear nucleus (DCN) has been subdivided in four layers. Rostrally, the ventral cochlear nucleus (VCN) contains two distinct regions, the anteroventral (AVCN) and posteroventral (PVCN) subdivisions separated by the cochlear nerve root. AVCN comprises mainly the spherical cell area (sca), whereas PVCN includes mainly the globular and octopus cell areas (gca and oca, respectively). In addition, several territories formed by small neurons surround the CN. They consist of the superficial granular layer (sgrl) covering the lateral and dorsal surface of VCN, the granular lamina (lam) separating the DCN and PVCN, and the small cell cap region (cap) located medially between the dorsal edge of PVCN and the sgrl. For simplification, lam and sgrl were grouped in the present report and referred to as the granule cell domain (GCD).

In vitro experiments

The physiological experiments were performed on guinea pigs of both genders, weighing 200–600 g. The methods used to isolate the brain and maintain it in vitro were similar to those described previously (Babalian et al. 1997, 1999, 2002a). Briefly, the animals were deeply anesthetized with pentobarbital (150–300 mg/kg i.p.) and perfused through the ascending aorta with a cold (10–13°C) Ringers solution for 5–6 min. Then the animal was decapitated and the skull bones covering the brain were removed. The exposed brain was transected rostrally at the level of olfactory bulbs and caudally at the cervical segments C1–C2. The isolation of the brain from the skull was completed by gradual cutting of cranial nerves and arteries on the ventral surface of the brain. The brain was transferred to the incubation/recording chamber filled with saline at 13°C, and secured in the ventral side up position. The brain was cannulated through one of the vertebral arteries with a metallic tube (external diameter 0.4 mm) that was connected to the perfusion system. Following elimination of all major leaks from the brains arterial system by ligating the arteries severed during the dissection (second vertebral artery, carotids, hypophyseal and labyrinthine arteries), the temperature of the chamber and perfusate was gradually increased to 29°C. The perfusion rate was progressively increased during rewarming to reach 4.5–5 ml/min. The perfusion solution had the following composition (in mM): NaCl 126; $NaHCO_3$ 26; $MgSO_4$ 1.3; KH_2PO_4 1.2; KCl 3; $CaCl_2$ 2.4; glucose 15; dextran 1.5% (MW 70000, Macrodex 70; Braun Medical AG, Sempach, Switzerland). The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2.

The auditory nerves and the ipsilateral auditory cortex were stimulated using bipolar metallic (stainless steel or tungsten) electrodes with interpolar distance of 0.5 and 3–4 mm for AN and AC stimulation, respectively. The electrodes were inserted in the stump of the AN and 1–1.5 mm below the surface of the AC, at a location where a field potential was present in response to AN stimulation. Intracellular recordings from CN neurons were made by micropipettes filled with a solution of 1–2% neurobiotin in 2 M potassium acetate (resistance 120–200 MΩ). At the end of the recording session, neurons were stained by passing positive currents of 0.5–1.5 nA for 5–30 min in a 200 ms on/off duty cycle. The recorded signals were displayed and stored using a computer-based data acquisition system (PowerLab; ADInstruments, Castle Hill, Australia). After each successful experiment, the brain was fixed with a solution of 4% PFA in 0.1 M PB delivered through the perfusion system for 30–45 min. The brain was then postfixed overnight and placed in 30% sucrose solution until further processing. Injected cells were visualized on coronal brain sections (100 μm) using standard ABC histochemistry with some modifications (Wan et al. 1992).

Results

In vivo experiments

BDA injection sites are shown in Fig. 1. Two guinea pigs received an injection into the area AI at sites where the BF was 21 kHz and 2.1 kHz, respectively. In two other guinea pigs, BDA injections were placed in the area DC, where the BFs were 29 kHz and 0.9 kHz, respectively. Finally, one guinea pig received an injection into the area DCB at a site where the BF was 29 kHz (Table 1). In all animals injected in AI or DC (n=4), BDA-labeled terminal fields, including axons, terminal endings and boutons *en passant*, were present in the CN either bilaterally or ipsilaterally. In contrast, no labeled axons were found in the CN either ipsilaterally or contralaterally as a result of injection into the area DCB (animal GP 38, Table 1).

Concerning the trajectory of the BDA-labeled axons, these were seen to travel from AC in the most dorsal part of cerebral peduncle and reach the lateral lemniscus in its rostral corner. From the lateral lemniscus, AC fibers traveled in the dorsal acoustic stria and entered the DCN. In the DCN, they ran in layers III and IV, from dorsal to ventral and from medial to lateral. More rostrally, the majority of axons were seen in the GCD and small cell cap surrounding the CN, whereas few of them reached the PVCN. Very few BDA-labeled axons traveled in the trapezoid body.

BDA-labeled axon terminals were observed in restricted zones of the ipsilateral CN in four animals. In two of these guinea pigs, some axon terminals were also found in the contralateral CN, but they were much less numerous than in the CN ipsilateral to the cortical injection. Interestingly, BDA-labeled axon terminals in the contralateral CN were seen only in the two animals subjected to injection in low BF region (Table 1). An example of the distribution of BDA-labeled terminal fields in the ipsilateral and contralateral CN is shown in Fig. 2 for two animals. Figure 2A illustrates that, following an injection at BF 21 kHz in the area AI, BDA-labeled axons were present only in the

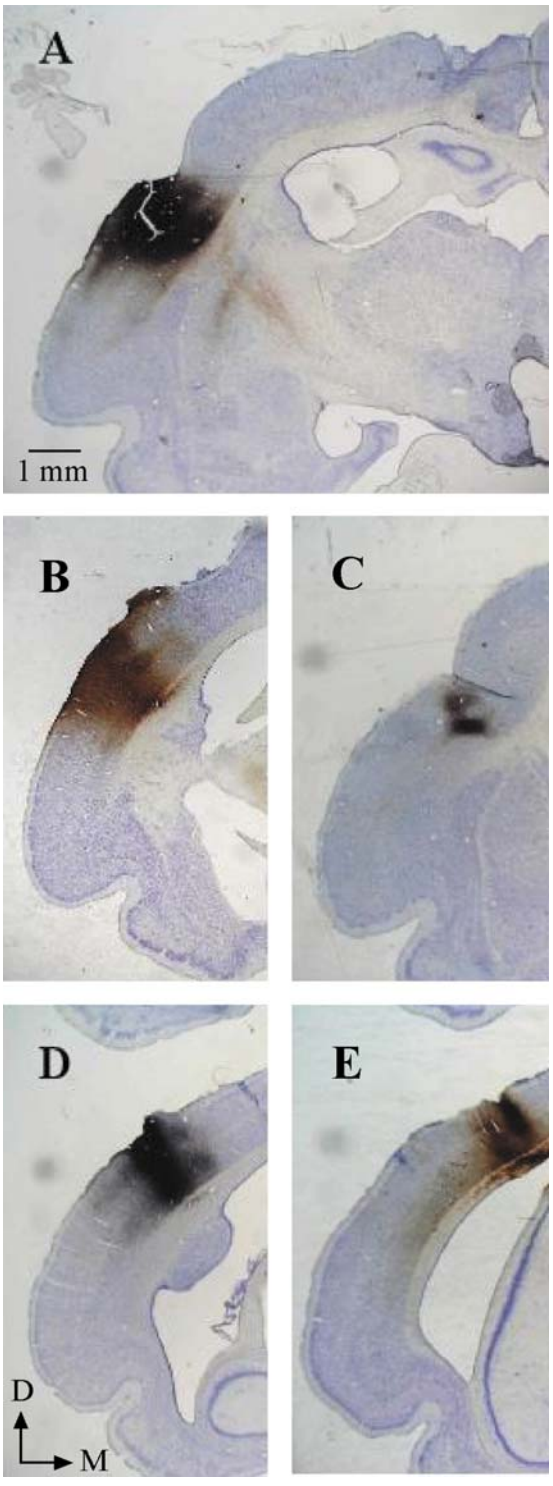

Fig. 1A–E Photomicrographs showing the injection sites of biotinylated dextran amine. **A** Guinea pig (GP) 39 at a best frequency (BF) site of 2.1 kHz in the area AI (primary auditory cortex). **B** GP 36 at a BF site of 21 kHz in the area AI. **C** GP 40 at a BF site of 0.9 kHz in the area DC (dorsocaudal auditory field). **D** GP 34 at a BF site of 29 kHz in the area DC; **E** GP 38 at a BF site of 29 kHz in the area DCB (dorsocaudal belt) (*D* dorsal, *M* medial)

Table 1 Properties of biotinylated dextran amine injection sites and presence of labeled axons in the cochlear nucleus (CN). *AI* Primary auditory cortex, *DC* dorsocaudal auditory field, *DCB* dorsocaudal belt

Guinea pig	Location of injection	Best frequency (kHz)	Presence of labeled axon terminals	
			Ipsilateral CN	Contralateral CN
GP 39	AI	2.1	Yes	Yes
GP 40	DC	0.9	Yes	Yes
GP 36	AI	21	Yes	No
GP 34	DC	29	Yes	No
GP 38	DCB	29	No	No

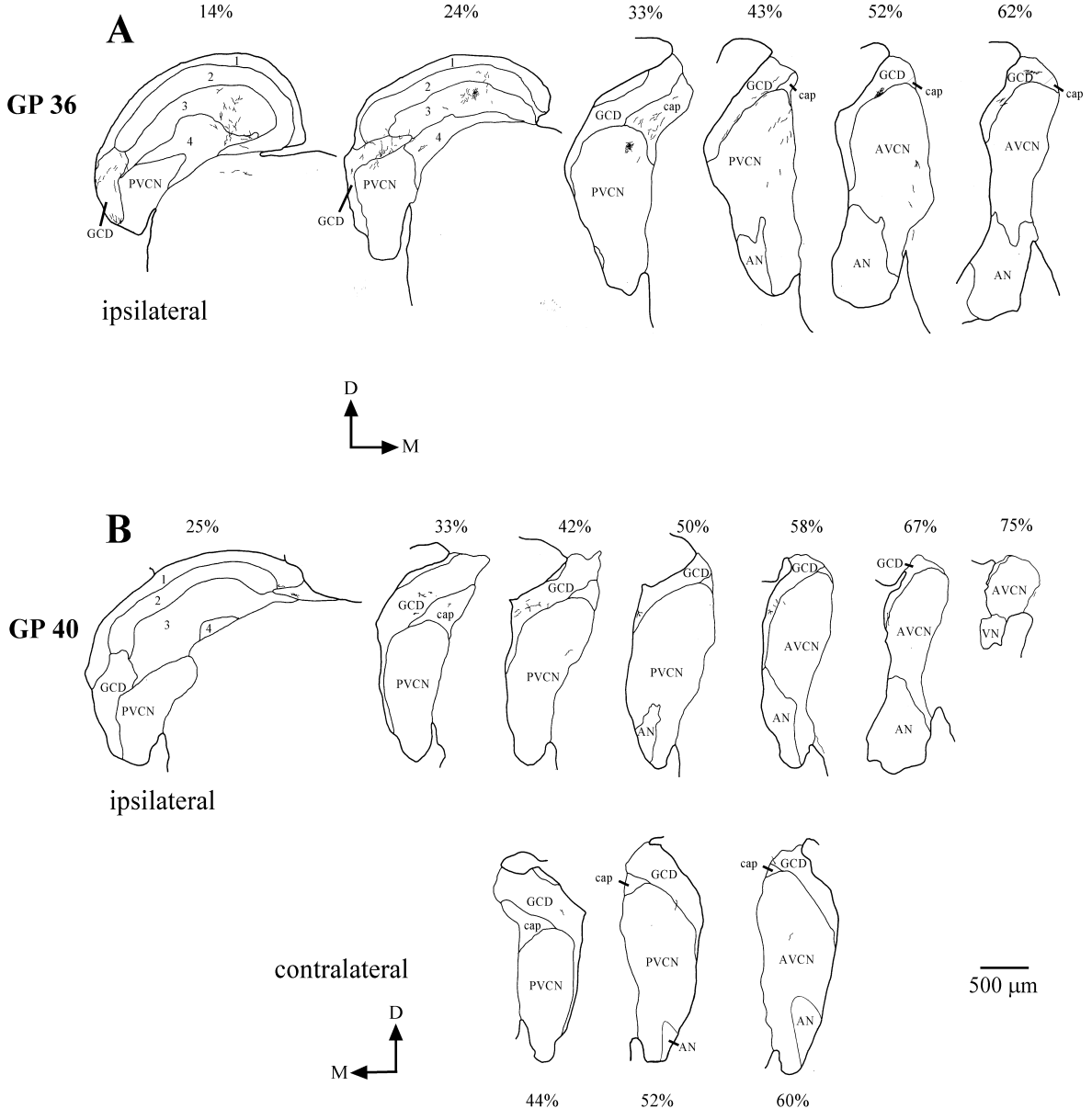

Fig. 2A,B Distribution of biotinylated dextran amine (BDA)-labeled axons in the ipsilateral cochlear nucleus (CN) of guinea pig (GP) 36 and bilaterally in the CN of GP 40, as seen on coronal sections. **A** GP 36, BDA was injected in primary auditory cortex (AI) at a best frequency (BF) site of 21 kHz. **B** GP 40, BDA was injected in dorsocaudal auditory field (DC) at a BF site of 0.9 kHz. The sections of the CN were arranged from caudal (*left*) to rostral (*right*). *Percentages* indicate the caudorostral level of each section, where 0% is the caudal limit of the CN and 100% its rostral limit. CN parcellation: *GCD* granule cell domain, *PVCN* posteroventral cochlear nucleus, *AVCN* anteroventral cochlear nucleus, *AN* auditory nerve, *cap* small cell cap region; *1*, *2*, *3*, *4* are the four laminae of the dorsal cochlear nucleus

ipsilateral CN. Figure 2B shows the location of labeled terminal fields in the CN bilaterally, as a result of BDA injection in the area DC at BF 0.9 kHz. As a general finding, labeled terminal fields were present predominantly in the GCD of the CN and in the small cell cap. Fewer labeled axons were seen in the CN subdivisions, with the density decreasing from DCN to PVCN and to AVCN. After BDA injection in high BF sites in the AC, axonal labeling tended to be located more caudally in the CN (Fig. 2A) than after injection in low BF sites (Fig. 2B *upper row*). Confirming previous observations (Schofield et al. 2001), the axon terminals consisted of spherical boutons *en passant* or *terminaux*, as illustrated in Fig. 3 for axon terminal fields in DCN and GCD. The diameter of the boutons was less than 1 μm.

In vitro experiments

Taking into account that the AC projections to CN are predominantly ipsilateral (see tracing data above), the physiological effects in CN cells were tested by electrical stimulation of the ipsilateral AC. We tested the effects of AC stimulation on 52 CN principal cells in 28 IWB preparations. Tested neurons were located in the three subdivisions of the CN: AVCN, PVCN and DCN. In the AVCN, the activity of eleven neurons was recorded. Seven of them were recovered on histological sections and identified as stellate (n=6) or bushy (n=1) cells. The other four neurons were not recovered, being only partially stained or unable to be stained after physiological characterization. Among 24 neurons recorded in the PVCN, seven were characterized as stellate cells, and two as octopus cells, whereas 15 were not identified. Finally, the population of 17 cells recorded in DCN comprised three giant, three pyramidal, two vertical, two stellate and seven unidentified neurons. The location in the CN of the cell body of all identified neurons is illustrated in Fig. 4 as a result of the transposition of the IWB sections onto a CN of reference (Hackney et al. 1990; see Methods section).

Single-pulse electrical stimulation of the AC induced responses in only 2 of the 52 tested cells (4%). No response to AC stimulation was observed in the great

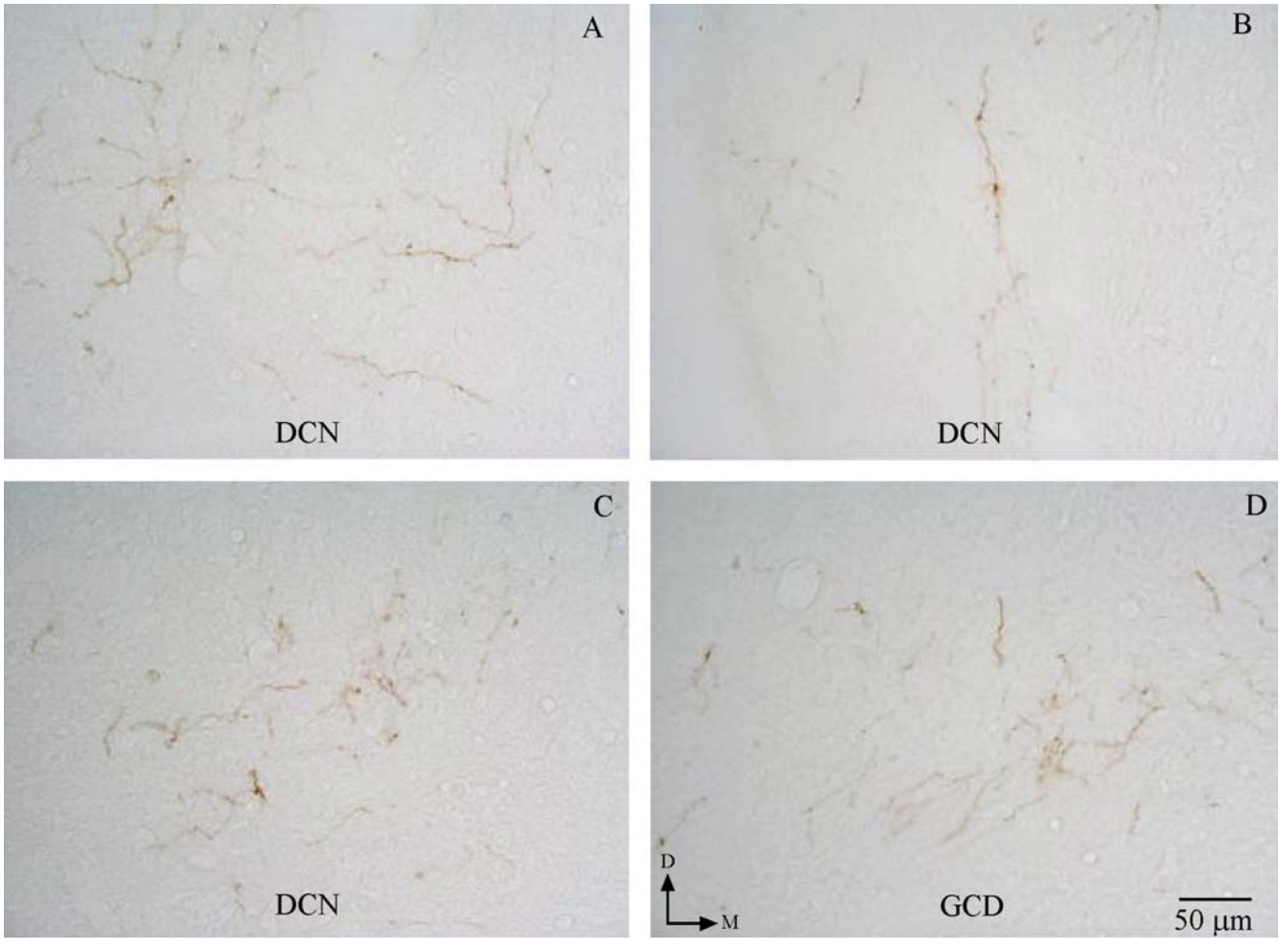

Fig. 3A–D Photomicrographs illustrating examples of axons anterogradely labeled with biotinylated dextran amine in the cochlear nuclus (CN) of guinea pig 36. **A,B** Labeled axon terminal fields in the dorsal cochlear nucleus (DCN) of the section at 14% of caudorostral level shown in Fig. 2A. **C,D** Labeled axon terminal fields in the DCN and granule cell domain (GCD) of the section at 24% of caudorostral level shown in Fig. 2A

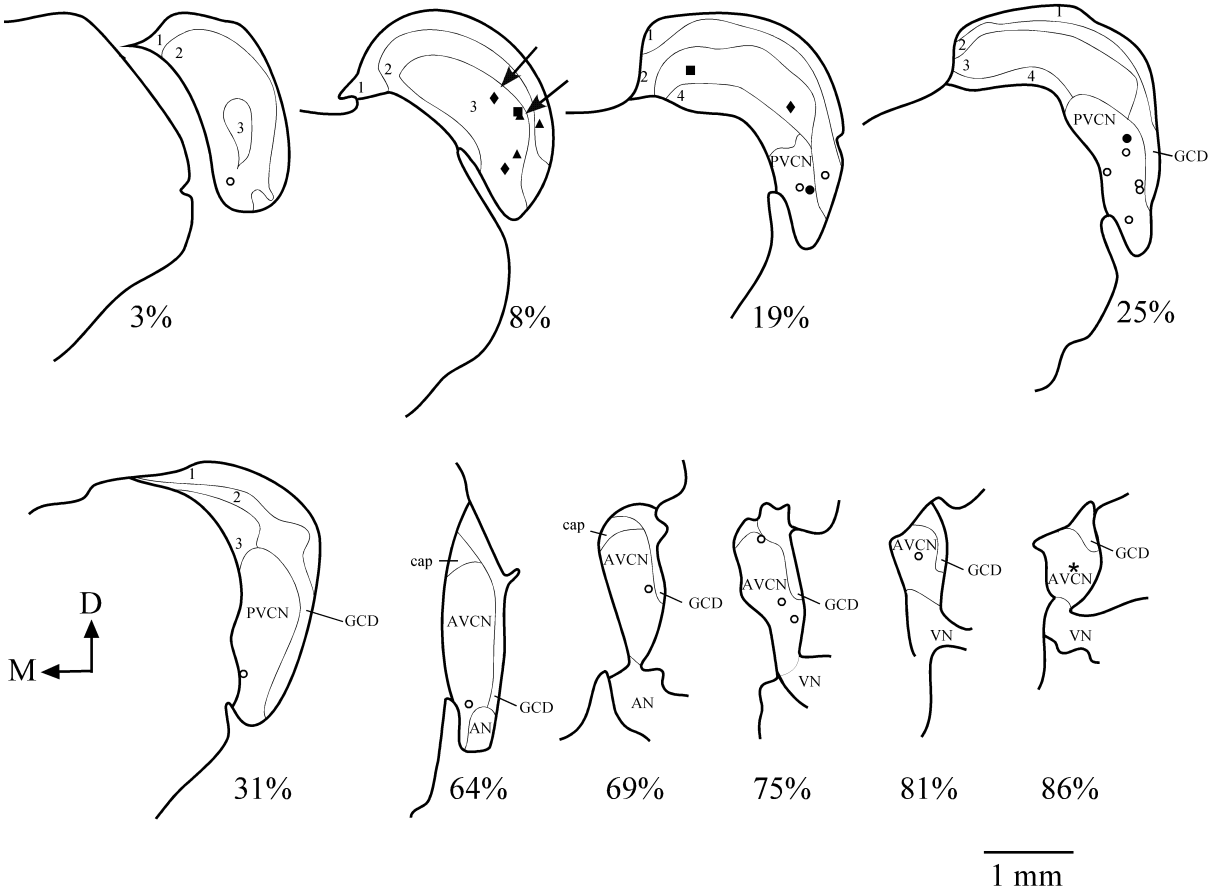

Fig. 4 Distribution of all identified neurons in the cochlear nucleus (CN) derived from the in vitro experiments. The brain of one guinea pig was sectioned in the coronal plane and Nissl-stained in order to establish a CN of reference (atlas). Individual sections derived from the isolated whole brain preparations were superimposed to the corresponding rostrocaudal section of the atlas in order to position the intracellularly stained neurons. *Percentages* indicate caudorostral levels as in Fig. 2. *Arrows* indicate the two cells that responded to the stimulation of the auditory cortex; *asterisk* a bushy cell, *open circles* stellate cells, *solid diamonds* giant cells, *solid triangles* pyramidal cells, *solid squares* vertical cells, *solid circles* octopus cells. CN parcellation: *GCD* granule cell domain, *PVCN* posteroventral cochlear nucleus, *AVCN* anteroventral cochlear nucleus, *AN* auditory nerve, *VN* vestibular nerve, *cap* small cell cap region; *1*, *2*, *3*, *4*, are the four laminae of the dorsal cochlear nucleus

majority of neurons even though many of them responded to stimulation of other auditory and non-auditory inputs (e.g., contralateral AN, dorsal column nuclei, and trigeminal nerve). Electrical stimulation of AC with trains of 3–6 pulses (100–250 Hz), tested in about 50% of cells, did not result in any effect different from that observed with single-pulse stimulation. The two CN cells responsive to AC stimulation were located in the DCN (*arrows* in Fig. 4), and had morphological properties typical of a giant and a pyramidal cells, respectively. The morphology of the giant cell is illustrated in Fig. 5A, together with its location in the CN (Fig. 5B). The axon of the cell left the CN in the direction of the dorsal acoustic stria (Fig. 5B, *arrows*), without emitting recurrent collaterals in the CN itself.

The two cells had similar physiological properties, which are illustrated in Fig. 5C for the giant cell. Both cells responded to stimulation of the ipsilateral AN with a sequence of excitatory (EPSPs) and inhibitory postsynaptic potentials (IPSPs), and exhibited long latency EPSPs to stimulation of AC. Stimulation of the AC evoked an EPSP of 2.9 mV at a latency of 20 ms in the giant cell (Fig. 5C). For the pyramidal cell, the EPSP had an amplitude of 7 mV and a latency of 33 ms (not shown). These long latencies suggest that responses of CN neurons to AC stimulation were induced polysynaptically.

In order to ensure stimulation of adequate sites in the AC, field potentials induced by stimulation of auditory nerve were systematically recorded through the stimulating electrode in the AC. Figure 5D illustrates an example of responses in the AC to stimulation of the ipsilateral or contralateral AN in the same animal. Stimulation of auditory nerves evoked a monophasic field potential in the AC. The latency of the field potentials was in the range of 10–15 ms and their amplitudes varied from 0.1 to 0.5 mV across the experiments. These data confirm that the location of the electrical stimulations was in the AC.

Discussion

The original contribution of the present study is to extend previous tracing data on the projection of the AC to the

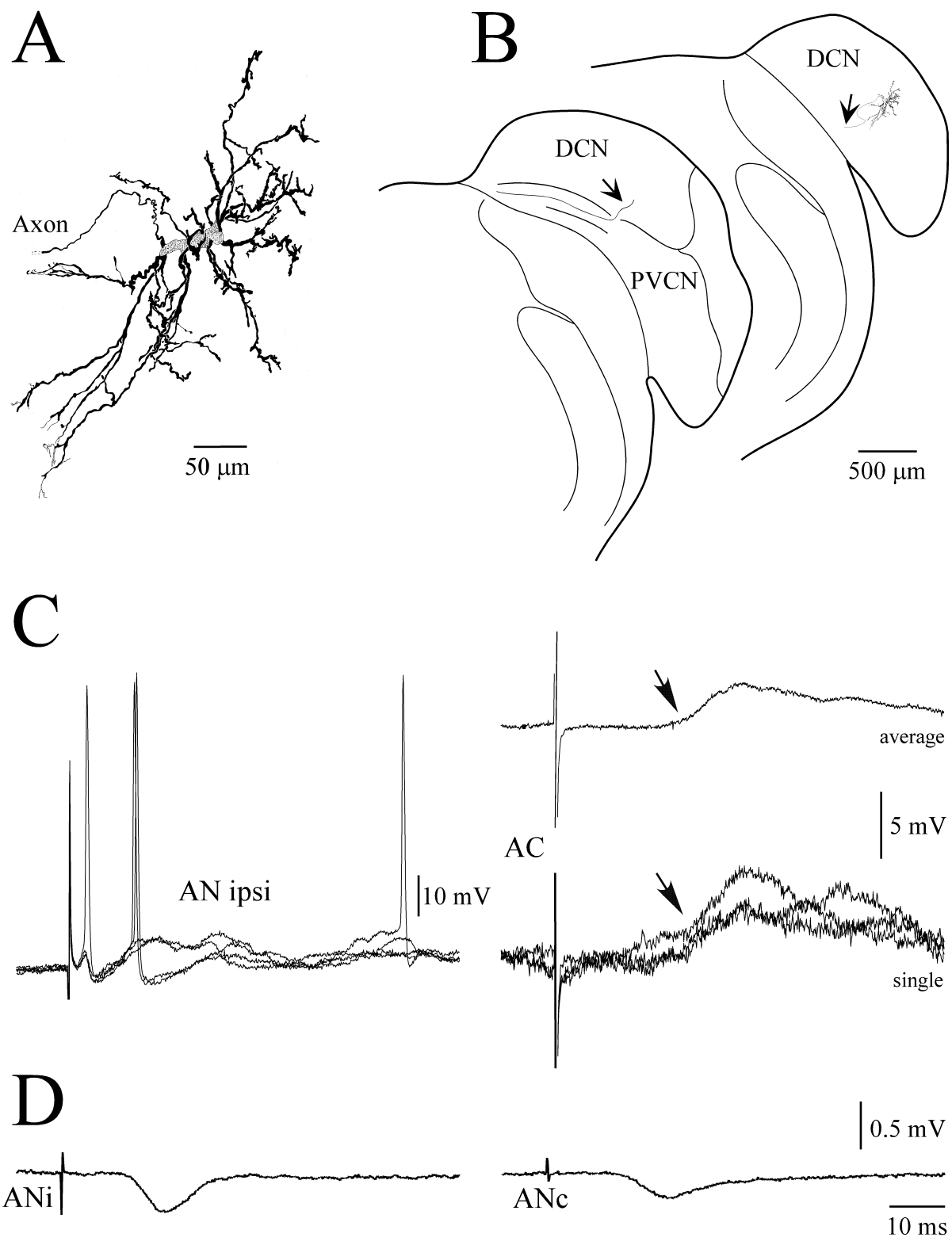

Fig. 5A–D Physiological and morphological characteristics of a giant cell in the dorsal cochlear nucleus (DCN). **A** High magnification reconstruction of the neuron whose location is shown on **B** a transverse section of the cochlear nucleus. *Arrows* indicate the cell axon (*PVCN* posteroventral cochlear nucleus). **C** Intracellular recordings from the cell at a membrane potential of −60 mV. Responses induced by stimulation of the ipsilateral auditory nerve (*AN ipsi*, *left* panel, individual traces) or auditory cortex (*AC*, *right* panel, averaged and individual records). *Arrows* indicate the response onset. **D** Field potentials in AC in response to contralateral (*ANc*) or ipsilateral auditory nerve (*ANi*) stimulations recorded in the same experiment. Averages of 10–20 individual responses. Same time scale is used in **C** and **D**

CN, by introducing physiological characterization of the AC injection sites, and an investigation of corticobulbar effects in the IWB preparation. The anterograde transport of BDA was used to localize the axon terminal fields formed by the direct projection from the AC to the CN. The corticobulbar axons terminate mainly in the GCD of the ipsilateral CN and, to a smaller extent, in the DCN and the PVCN. This result is consistent with previous retrograde and anterograde tracing studies in the rat and guinea pig showing direct projections from the AC to the CN (Weedman and Ryugo 1996a, 1996b; Schofield et al. 2001). We found some axon terminals in the contralateral CN but they were much less numerous than in the ipsilateral CN. This observation is also in accordance with a recent anterograde tracing study in the guinea pig demonstrating bilateral projections from the AC to the CN, with predominance of the ipsilateral ones (Schofield et al. 2001). A new finding of the present study is that contralateral AC projections were not observed in the two animals subjected to BDA injection in the high BF regions of AI or DC. This result is not due to a particularly small injection site (see Fig. 1B,D) and/or poor labeling (see Fig. 3). Nevertheless, the observed difference between projections originating from high versus low BF regions is based on a small number of animals (n=4) and needs further confirmation.

As mentioned above, in our physiological experiments using the IWB preparation, the effects produced by AC stimulation could be tested only in CN principal cells. The AC-induced responses observed in two DCN cells had very long latencies suggesting their polysynaptic origin. Thus it can be concluded that AC projections to the three CN subdivisions and GCD most likely do not establish direct contacts with somata or dendrites of CN principal cells. Moreover, even the polysynaptic effects were observed in a very low percentage of recorded CN principal neurons (4%). To some extent, this is consistent with the relatively sparse corticobulbar projection observed in AVCN, PVCN and DCN as a result of BDA injection in the AC. In contrast, a much stronger projection was seen in the GCD and small cell cap. The GCD consists of a continuous sheet of small cells that surrounds the magnocellular VCN and penetrates into the DCN as layer II (Mugnaini et al. 1980). These small cells project to some CN principal cells and other CN cells, which in turn can contact CN principal cells. Therefore, one may wonder why so few polysynaptic responses induced by AC stimulation were observed in the present study. One possible explanation could be that the stimulation of AC and/or position of stimulating electrodes in the AC were not optimal. This argument can be ruled out for at least two reasons. First, the position of the stimulating electrodes in the AC was confirmed by recording, through the same electrodes, of field potentials in response to AN stimulation. Second, a large interpolar distance of the stimulating electrodes and high stimulation currents suggest that a large area of AC and, consequently, a large number of projecting AC cells was activated in our experiments. The observation of rare AC-induced effects in the CN principal cells is probably not due to the impaired GCD and other polysynaptic circuits in the IWB preparation. Indeed, in the same set of experiments, a large proportion of CN principal cells responded to stimulation of other sources of inputs to the CN (e.g., dorsal column nuclei, and trigeminal nerve; see Babalian et al. 2001) known also to operate mainly through the GCD (Itoh et al. 1987; Wright and Ryugo 1996; Shore et al. 2000; Haenggeli et al. 2002; Ryugo et al. 2003). This comparison under the same experimental conditions thus supports the interpretation that the AC projection exerts indeed a limited influence on CN principal cells as compared to the other two mentioned sources of non-auditory inputs. The reasons for that are not known. One may, however, speculate that the density of projections and/or the reliability of synaptic transmission in the GCD and other polysynaptic circuits are lower for the projections originating from the AC. For example, AC stimulation might produce predominantly subthreshold modulation of the membrane potential in small interneurons, which would not result in any visible effects on CN principal cells. Possible failure of AC inputs to generate action potential in small relay cells would thus account for the rare observation of responses at the level of CN principal cells. This explanation somewhat contradicts our experiments using train stimulation of AC intended to produce temporal summation of synaptic responses and discharges in relay neurons between AC and CN principal cells. Yet it is possible that parameters of train stimulation were not optimal in our study. Thus, influences of AC on granule cells and other CN interneurons remain to be elucidated.

The polysynaptic nature of the observed excitatory responses in two DCN cells suggests an alternative possibility that these effects were due to activation of AC projections to the IC and/or superior olivary complex, which in turn project to the CN. The existence of these pathways is well documented (Herbert et al. 1991; Feliciano and Potashner 1995; Saldaña et al. 1996; Malmierca et al. 1996; Games and Winer 1998; Schofield and Cant 1999; Budinger et al. 2000; Schofield 2001; Coomes and Schofield 2001). However, this possibility seems to be unlikely for the following reasons. Our previous results in the IWB preparation indicate that electrical stimulation of IC and various auditory and non-auditory brainstem inputs to the CN produce predominantly inhibitory effects in the vast majority of CN cells (Babalian et al. 2002b; Jacomme et al. 2002). In contrast, the two responses observed in the present study were excitatory. Moreover, stimulation of the IC and brainstem sources of inputs to CN induces synaptic responses in CN cells, usually at relatively short latencies (below 10 ms). Even if we take into account propagation and synaptic delay time related to the additional activation of AC projections to IC and/or brainstem, the resulting values will be hardly compatible with response latencies in the present study (20 and 33 ms). Therefore, we think that the observed long-latency responses are mediated through the AC projections to CN and slow signal processing compatible with the granule cell circuits or other

interneuronal circuits in the CN. The fact that AC stimulation effects were observed only in DCN cells may indicate that the AC preferentially influence, though weakly, output activity of the DCN. Such a conclusion would be consistent with our observation of higher density of AC projections in the DCN relative to other CN subdivisions.

In conclusion, the anatomical data show sparse projections from the AC to the three CN subdivisions and more dense projections to the GCD. Physiological results in the IWB preparation indicate the existence of a limited modulatory action of the AC on CN principal cells. Thus, in the guinea pig, the auditory cortex might modulate the CN activity essentially at the level of granule cells and CN interneurons.

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