Golgi Cells in the Superficial Granule Cell Domain Overlying the Ventral Cochlear Nucleus: Morphology and Electrophysiology in Slices

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ABSTRACT

Golgi cells are poised to integrate multimodal influences by participating in circuits involving granule cells in the cochlear nuclei. To understand their physiological role, intracellular recordings were made from anatomically identified Golgi cells in slices of the cochlear nuclei from mice. Cell bodies, dendrites, and terminals for all seven labeled cells were restricted to the narrow plane of the superficial granule cell domain over the ventral cochlear nucleus. The axonal arborization was the most striking feature of all Golgi cells; a dense plexus of terminals covered an area 200–400 μm in diameter in the vicinity of the cell body and dendrites. Axonal beading often surrounded granule cell bodies, indicating that granule cells are probable targets. Cells had input resistances up to 130 MΩ and fired regular, overshooting action potentials. Golgi cells probably receive auditory nerve input, because shocks to the cut end of the auditory nerve excited Golgi cells with excitatory postsynaptic potentials (EPSPs). The latency of EPSPs shortened to a minimum and the amplitude of EPSPs grew in several steps as the strength of shocks was increased. The minimum latency of EPSPs in Golgi cells was on average 1.3 milliseconds, 0.6 milliseconds longer than the minimum latencies of EPSPs in nearby octopus and T stellate cells. The long latency raises the possibility that Golgi cells receive input from slowly conducting, unmyelinated auditory nerve fibers. Golgi cells are also excited by interneurons with N-methyl-D-aspartate receptors, probably granule cells, because repetitive shocks and single shocks in the absence of extracellular Mg2+ evoked late EPSPs that were reversibly blocked by DL-2-amino-5-phosphono-valeric acid. J. Comp. Neurol. 400:519–528, 1998. © 1998 Wiley-Liss, Inc.

Indexing terms: dorsal cochlear nucleus; GABAergic neuron; type II auditory nerve fiber

Granule cells lie in clusters within and around the cochlear nuclei, comprising more than half of all neurons in the cochlear nuclei. In addition to the numerous granule cells, two types of interneurons are associated with granule cells both in the cerebellum and in the cochlear nuclei, Golgi and unipolar brush cells (Mugnaini et al., 1980a,b; 1997). Together these three cell types comprise the granule cell domains. The observations that these regions integrate auditory, somatosensory, and vestibular inputs indicate that their role goes beyond relaying acoustic information (Itoh et al., 1987; Weinberg and Rustioni, 1987; Brown et al., 1988a,b; Burian and Goestettner, 1988; Kezirian and Perachio, 1989; Calcedo and Herbert, 1993; Feliciano et al., 1995; Golding et al., 1995; Weedman and Ryugo, 1996). It has long been recognized that the output of the granule cell domains influences the principal cells of the dorsal cochlear nucleus (DCN; Parham and Kim, 1995; Young et al., 1995; Davis and Young, 1997; Golding and Oertel, 1997), but the possibility of synaptic interactions between cells in the granule cell domains and the cells in
the magnocellular regions of the ventral cochlear nucleus (VCN) has received little consideration. The present results, along with those of Ferragamo et al. (1998), suggest that such interactions exist and are bidirectional.

Golgi cells are sparse and relatively small and are located superficially in the cochlear nuclei. As a consequence, little is known about cochlear nuclear Golgi cells. Immunocytochemical labeling for γ-aminobutyric acid (GABA), glutamic acid decarboxylase (GAD), and glycine in the cochlear nucleus indicates that Golgi cells are probably inhibitory (Mugnaini, 1985; Adams and Mugnaini, 1987; Kolston et al., 1992). They are suspected of terminating on granule and unipolar brush cells (Mugnaini et al., 1980a, 1994; Dunn et al., 1996; Mugnaini et al., 1997; Schuerver et al., 1997).

MATERIALS AND METHODS

Tissue preparation

The protocols of this study were conducted with the approval of the University of Wisconsin Animal Care Committee.

Slices that contained a large proportion of the neuronal circuitry of the cochlear nuclei were made from 19–23-day-old, juvenile CBA mice as described previously (Wickesberg et al., 1994; Ferragamo et al., 1998). After the brain was removed from the skull and the block of tissue affixed, the cochlear nuclei were removed from the brainstem with a single parasagittal cut with a tissue slicer (Frederick Haer, New Brunswick, ME). Dissections were performed at about 31°C. Slices, between 250 and 400 μm at the thickest point, were immersed in oxygenated (95% CO₂/5% O₂) saline in a tissue chamber with a volume of 0.3 ml and continuously perfused at a rate of 10–12 ml/min (Oertel, 1985). Saline (130 mM NaCl, 3 mM KCl, 1.2 mM K₂HPO₄, 2.4 mM CaCl₂·H₂O, 1.3 mM MgSO₄, 3 mM HEPES, 20 mM NaHCO₃, and 10 mM glucose; pH 7.4) was maintained at 34°C with a thermostimulator (University of Wisconsin–Madison Medical Electronic Shop), with feedback supplied by a temperature probe in the chamber (Physitemp, Clifton, NJ). Slices were incubated in the chamber for between 60 and 90 minutes before recording was begun.

Pharmacological agents were dissolved in normal saline or saline in which MgSO₄ was replaced with CaCl₂ (0 Mg²⁺) and introduced into the chamber without a break in the flow.strychnine, bicuculline methiodide, DL-2-amino-5-phosphonovaleric acid (APV), 6,7-dinitroquininaline-2,3-dione (DNQX) were obtained from Sigma (St. Louis, MO).

Electrophysiological recording

The techniques for recording have been described previously (Ferragamo et al., 1998). Recording electrodes had impedances of 120–250 MΩ and were filled with 1% biocytin (Sigma) in 2 M K⁺-acetate, pH 7.0. Intracellular potentials were amplified and low-pass filtered at 10 kHz (ICX2–700; Dagan, Inc., Minneapolis, MN). Membrane potential was monitored videoiusisically and recorded on chart paper (Gould, Inc., Valley View, OH), and individual traces were recorded digitally. Data acquisition, current injection, and shock triggering were performed by using a Digitdata 1200 A computer interface under control of pCLAMP software (Axon Instruments, Inc., Foster City, CA) on an IBM-compatible computer (Micron, Inc., Nampa, ID). Responses to both current injection and synaptic stimulation were sampled at 25 kHz. Shocks were delivered to the severed eighth nerve through a pair of insulated tungsten electrodes (Bak Electronics, Rockville, MD), each with a 50-μm exposed tip. Stimulation voltage (0.1–100 V; 100-μs duration) was produced by an isolated DC source (S-100; Winston Electronics Co., Millbrae, CA) under control of a digitally triggered timer (A-65; Winston Electronics Co.).

Histology

Recorded cells were labeled anatomically by iontophoretic injection of biocytin with depolarizing current steps (0.5–2.0 nA; 150–200 milliseconds) at a rate of 2 Hz for roughly 2 minutes. At the end of the experiment, slices were fixed in 4% paraformaldehyde, and 0.1 M phosphate buffer (pH 7.4) and stored at 4°C for between 24 hours and 2 weeks. Tissue was embedded in a mixture of gelatin and albumin cross-linked with glutaraldehyde and sectioned at 60 μm on a Vibratome. Sections were incubated with avidin conjugated to horseradish peroxidase (Vector ABC kit, Vector Laboratories, Burlingame, CA) and processed for horseradish peroxidase with CoCl₂ and NiCl₂ intensification. Sections were mounted on coated slides and were counterstained with cresyl violet.

Cells were reconstructed from two or three sections with a camera lucida. Reconstructions and film negatives of photomicrographs were scanned into application software (Photoshop; Adobe, Inc., San Jose, CA) and labeled (Illustrator; Adobe, Inc.) without alteration.

RESULTS

Morphology

Seven Golgi cells were labeled with biocytin. Each cell lay entirely within the superficial granule cell domain overlying the posterior and anterior divisions of the VCN and in the granule cell lamina between the VCN and DCN (Table 1, Figs. 1, 2). Recordings were made from four of the seven labeled Golgi cells. Their small size and sparse representation within the granule cell regions overlying the VCN probably account for the low rate of impalement. The anatomical and electrophysiological properties were consistent among the cells in our small sample and are summarized in Table 1.

Smooth, tapering dendrites, between 50 and 100 μm long, emanated in all directions from a cell body of about 15 μm in diameter (Figs. 1, 2A). A dense, axonal plexus, limited to the plane of the granule cell domain, extended about 250 μm from the soma in all directions (Fig. 1). The axonal processes of Golgi cells were densely beaded

<table>
<thead>
<tr>
<th>Cell</th>
<th>Location</th>
<th>Membrane potential (mV)</th>
<th>Input resistance (MO)</th>
<th>EPSP latency (milliseconds)</th>
<th>Late EPSPs</th>
<th>Glycinergic IPSPs</th>
<th>Spontaneous PSPs</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>s-VCN</td>
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<td>67</td>
<td>1.22</td>
<td>+</td>
<td>EPSPs</td>
<td></td>
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<tr>
<td>2</td>
<td>GCL</td>
<td>−60</td>
<td>128</td>
<td>1.31</td>
<td>+</td>
<td>EPSPs</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>GCL</td>
<td>−66</td>
<td>93</td>
<td>1.28</td>
<td>+</td>
<td>EPSPs, IPSPs</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>s-VCN</td>
<td>−68</td>
<td>131</td>
<td>1.25</td>
<td>+</td>
<td>EPSPs, IPSPs</td>
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1 s- superficial; PVCN, posterior ventral cochlear nucleus; AVCN, anterior ventral cochlear nucleus; GCL, granule cell lamina; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; PSP, postsynaptic potential.
Fig. 1. Camera lucida reconstructions of three Golgi cells. The dense axonal plexus is characteristic of Golgi cells; it lies confined in the plane of the granule cell domain. The smooth, thick appendages radiating from the soma are dendrites. The position of the reconstructed cells on the surface of the ventral cochlear nucleus (VCN) is shown superimposed on reconstructions of the slices at one tenth the magnification. Cell numbers correspond to numbers in Table 1. GCL, granule cell lamina; VIII N., auditory nerve. Scale bar = 50 μm.

throughout their course, each cell having between 1,000 and 2,000 beads that were presumably synaptic terminals (Fig. 2). The shape suggests that each Golgi cell influences a patch of hundreds of granule cells that extend over a large portion of the VCN.

Responses to current pulses
Resting potentials of Golgi cells were -62 mV on the average. Figure 3Ai shows the responses of one cell to pulses of injected current as typical of the behavior observed in all four cells. Injection of depolarizing pulses of current elicited a tonic discharge of simple spikes whose frequency rose with current strength. A slight sag was evident in responses to hyperpolarizing current, indicating that Golgi cells show inward rectification. The relationship of the peak hyperpolarization as a function of injected current was linear and had a slope of 131 MΩ. The mean input resistance of the four cells was 105 MΩ.

Synaptic excitation and inhibition
Synaptic inputs in Golgi cells were evident spontaneously and in responses to shocks to the cut end of the
auditory nerve. Spontaneous synaptic inputs consisted of frequent unitary excitatory postsynaptic potentials (EPSPs) in three cells and in one of those cells EPSPs were accompanied by occasional unitary inhibitory postsynaptic potentials (IPSPs; Table 1, Fig. 3Aii).

Shocks to the auditory nerve evoked strong excitation. EPSPs had durations of about 40 milliseconds (Fig. 3Aii). In responses to strong shocks, a depolarization arose during the undershoot of the evoked action potential, occasionally producing a second discharge with a latency between 4 and 10 milliseconds. Monosynaptic responses had latencies of less than approximately 3 milliseconds. The observation that this depolarization was present in some traces (Fig. 3Aiii; 7.5 and 20 V) and absent in others (Fig. 3Aiii; 3.5, 4.5, and 6 V) indicates that in some trials an additional source of excitation had been recruited. The lateness of the second depolarization indicates that this EPSP is likely to be polysynaptic, resulting from the activation of excitatory interneurons. Behavior similar to that described in Figure 3Aiii was observed in two other cells.

Although the balance of synaptic inputs favored early excitation in three of the cells, early inhibition was prominent in the fourth cell (Fig. 3B). The earliest inhibitory responses occurred later than the earliest excitatory responses, preventing them from reaching threshold (see Fig. 6). Increasing shock strength enhanced late EPSPs (Fig. 3B; 55 and 65 V).
The minimal latencies of EPSPs in Golgi cells (1.27 ± 0.05 milliseconds) were longer than those of the large cells in the VCN and DCN. In recordings made under similar conditions, latencies from the beginning of the shock to the beginning of the rise of EPSPs in the large cells of the VCN were between 0.5 and 0.9 milliseconds (Oertel, 1983; Wu and Oertel, 1987; Oertel et al., 1990; Golding et al., 1995). Because the neurons from which earlier measurements were made were scattered throughout the nucleus, it was conceivable that the differences in latency reflected differences in the distance between stimulating electrodes and target neurons. Therefore, a comparison was made of minimal delays in T stellate cells and octopus cells in this same series of experiments from electrode penetrations in the same dorso-ventral part of the posterior ventral cochlear nucleus (PVCN) in which the Golgi cells were found (Fig. 4). The mean minimal latencies in this sample of T stellate and octopus cells were on average 0.7 milliseconds in comparison with those in Golgi cells that followed on average 1.3 milliseconds after the shock. It is significant that the minimal latencies do not overlap. These results indicate that Golgi cells receive input either through a different, more slowly conducting group of fibers or that their excitatory input is polysynaptic.

To mimic the trains of action potentials that are evoked in the auditory nerve in responses to sound, trains of shocks were presented to cells in slices. The results of one such measurement are illustrated in Figure 5A (top trace). Whereas each shock in a train at 100 Hz evoked a distinct EPSP only the first EPSP was reliably suprathreshold. Subsequent EPSPs were often subthreshold, but they could sum over two or more shocks within the train to produce action potentials (inset). After trains of shocks, the Golgi cell continued to be excited by late EPSPs for

Fig. 3. Electrophysiological responses of Golgi cells 7 (A) and 5 (B). Responses to injected current were similar to the two cells: responses to shocks of the auditory nerve were dominated by excitation in one cell and included prominent inhibition in the other. A: Golgi cell fired overshooting action potentials when depolarized with 0.1 nA current pulses. It hyperpolarized with a time constant of 5.1 milliseconds when hyperpolarized with −0.1 nA; after reaching a peak hyperpolarization, the voltage sagged slightly back toward rest. The current-voltage relationship is linear. The current-voltage relationship at the peak hyperpolarization, measured at the time denoted by the filled circle, is linear and has a slope of 131 MΩ (r = 0.99). ii: Spontaneous synaptic responses include both excitatory postsynaptic potentials (EPSPs; black diamonds) and inhibitory postsynaptic potentials (IPSPs; arrow). iii: Responses to shocks to the auditory nerve (arrow). The EPSP in response to a weak shock (3.5 V) occurs late. A stronger shock (4.5 V) evokes three EPSPs whose arrival is asynchronous, giving the rise of the synaptic response a stepped appearance. Shocks of 6 V or more cause rapidly rising, suprathreshold excitation. The strongest shocks (7.5 and 20 V) also evoke late excitation that rises during the undershoot of the action potential (black dot). Subthreshold responses are shown on a larger scale than suprathreshold responses. Action potentials are digitally truncated. B: A series of synaptic responses in another cell. The response with the lowest threshold (2 V) was an IPSP. Increasing the shock strength resulted in stronger excitation that became suprathreshold. With increasing shock strength, late EPSPs become increasingly prominent. Suprathreshold response is digitally truncated.
hundreds of milliseconds. The behavior of this cell in response to trains of shocks was observed in each of the other two cells in which it was tested.

The sensitivity of synaptic excitation to blockers of glutamate receptors was used to dissect synaptic responses pharmacologically. Although late EPSPs could be infrequent in response to a single shock (Fig. 5B), repetitive stimuli consistently evoked late EPSPs for hundreds of milliseconds beyond the stimulus and could therefore arise only from excitatory interneurons (Fig. 5A, top trace). This enhancement of late EPSPs by repetitive stimulation suggested that N-methyl-D-aspartate (NMDA) receptors might play a role in the excitatory interneuron (Fig. 5C). We tested whether the removal of extracellular Mg$^{2+}$, which is known to block these receptors in a voltage-dependent fashion (Nowak et al., 1984), promoted the appearance of EPSPs. Figure 5C shows that removal of Mg$^{2+}$ reversibly enhances the appearance of late EPSPs even in responses to single shocks. Late EPSPs were also abolished by the NMDA-receptor antagonist APV. These results indicate that Golgi cells are contacted by excitatory interneurons that are driven at least in part through NMDA receptors. Both the initial EPSP (Fig. 5C) and late EPSPs (Fig. 5A, middle trace) were reversibly blocked by the AMPA-receptor antagonist DNQX. A similar experiment was performed in another cell and yielded similar results.

The one Golgi cell in which inhibition was prominent received glycinergic input. Over a wide range of stimulus strengths, shocks evoked excitation that was only rarely suprathreshold when the slice was bathed in normal saline. Those synaptic responses that were suprathreshold appeared to have a notch on the rising phase. In the presence of strychnine, the notch disappeared, and excitation consistently became suprathreshold (Fig. 6).

**DISCUSSION**

Granule cell domains, comprising intermingled Golgi, granule, and unipolar brush cells, are prominent in the cochlear nuclei of most mammals, but their sparse representation and peripheral location has made them elusive not only in electrophysiological studies but also in anatomical studies. By labeling single Golgi cells, we reveal the remarkable axonal arbors of Golgi cells that permeate a patch of the superficial granule cell domain 0.5 mm in diameter with a uniformly dense network of beaded terminals. The present experiments show that synaptic responses of Golgi cells to shocks at the root of the auditory nerve have three components, early EPSPs, late EPSPs, and inhibition. On the basis of what is known anatomically about neuronal connections in granule cell domains, it is possible to identify the sources of those inputs with reasonable confidence. We will argue below that the early EPSPs may have arisen through type II auditory nerve fibers, that late EPSPs arose through granule cells, and that glycinergic inhibition probably arose from D stellate cells of the magnocellular region of the VCN.

**Morphology of Golgi cells**

The Golgi cells in cochlear nuclei closely resemble the Golgi cells in the cerebellum. Golgi cells are present in regions with granule cells both in the cerebellum and in the cochlear nuclei (Palay and Chan-Palay, 1974; Mugnaini et al., 1980a; Alvarez-Otero and Anadón, 1992; Midggaard, 1992; Mugnaini and Floris, 1994; Mugnaini et al., 1994, 1997). As in the cerebellum, the dendrites of Golgi cells lay in the vicinity of granule cell dendrites; dendrites of the cochlear nuclear Golgi cells in this study lie within the plane of the layer of superficial granule cells on the lateral surface of the VCN. A distinctive attribute of Golgi cells is their extensive, local axonal arbor. Although investigators have commented on the extensive arborization of the axon, it is generally not possible to reconstruct axons of individual Golgi-impregnated Golgi cells in their entirety; the present study confirms this impression and reveals the full extent of the axonal arborization.

Golgi cells associated with the ventral cochlear nucleus have not been previously examined systematically at the light- or electron-microscopic levels, but their close resem-
blance to cerebellar Golgi cells has been reported to extend to the electron-microscopic level in the dorsal cochlear nucleus (Mugnaini et al., 1980a, 1997). Ultrastructural descriptions of Golgi cells both in the cerebellum and in the cochlear nuclei precede the finding of a previously unknown class of cell, the unipolar brush cell, with which Golgi cells were in some cases confused (Mugnaini and Floris, 1994; Mugnaini et al., 1994). The distinctions between Golgi and unipolar brush cells are summarized by Mugnaini et al. (1997).

**Golgi cells are inhibitory interneurons**

Golgi cells are inhibitory and probably GABAergic. Golgi cells overlying the VCN are labeled with antibodies to GABA (Kolston et al., 1992) and GAD, an enzyme involved in the synthesis of GABA (Mugnaini, 1985), and glycine (Kolston et al., 1992). Colabeling with GABAergic and glycnergic markers raises the question of whether Golgi cells are GABAergic or glycnergic or both. Several indirect lines of evidence suggest that Golgi cells are GABAergic, but the possibility that they also release glycine cannot be excluded. Ferragamo et al. (1998) observed GABAergic components in responses to shocks in T stellate cells of the ventral cochlear nucleus. In those slice preparations, Golgi cells were a likely source of GABA because they were the only cells that are labeled by GABAergic markers and whose dendrites and terminals remained connected and which therefore could be driven synaptically (Mugnaini, 1985; Adams and Mugnaini, 1987; Kolston et al., 1992). Studies of granule and Golgi cells of the cerebellum have also led to the conclusion that Golgi cells are GABAergic (Baude et al., 1992; Dieudonne, 1995; Kaneda et al., 1995).

**Input to the granule cell domains**

In the cochlear nuclei, as in the cerebellum, granule cell domains receive input from widespread regions of the brain (Hámori and Szentágothai, 1966; Dunn et al., 1996). Somatosensory input arises from the dorsal column nuclei and from spinal trigeminal nucleus (Itoh et al., 1987; Weinberg and Rustioni, 1987; Young et al., 1995; Davis et al., 1996; Davis and Young, 1997); vestibular input arises through vestibular primary afferents (Burian and Gusttten, 1988; Kevetter and Perachio, 1989). The granule cell domains receive auditory input from many levels. Unmyelinated, primary auditory nerve afferents terminate in or near the granule cell domains (Brown and Ledwith, 1990; Benson et al., 1996; Berglund et al., 1996). Two types of cells from the magnocellular VCN terminate in granule cell domains, octopus cells (Golding et al., 1995), and D stellate cells (Oertel et al., 1990). There are descending projections from the olivocochlear efferents (Brown et al., 1988b; Benson et al., 1996), from the inferior colliculus (Caicedo and Herbert, 1993), and from the auditory cortex (Feliciano et al., 1995; Weedman and

![Figure 5](image-url)
Ryugo, 1996). Inputs to the granule cell domains are inhomogeneous, raising the possibility that granule cell domains may be functionally subdivided.

Often, but not always, these inputs to granule cell domains are in the form of large, mossy fiber terminals that form the core of synaptic glomeruli. Golgi cells, as well as granule and unipolar brush cells, have been observed to be contacted by mossy fiber terminals (Mugnaini et al., 1997; Schuerger et al., 1997). The fact that mossy terminals are ultrastructurally heterogeneous indicates that the terminals may be functionally heterogeneous even when they innervate the granule cell domain through similar glomeruli (Dunn et al., 1996). Mossy fiber terminals mediate the input of the cochlear nuclei from octopus cells (Golding et al., 1995), from the auditory cortex (Feliciano et al., 1995; Weedman and Ryugo, 1996) and from the cuneate nucleus (Wright and Ryugo, 1996). Unipolar brush cells within granule cell domains not only receive input through mossy fiber terminals but also terminate locally with mossy terminals (Mugnaini and Floris, 1994; Mugnaini et al., 1994, 1997; Rossi et al., 1995). The dendrites of granule cells surround the mossy fibers and are contacted not only by mossy fibers but also by smaller boutons that contain pleomorphic vesicles and are thought to arise from Golgi cells. This entire cluster of processes is wrapped in glial processes (Kane, 1974; Palay and Chan-Palay, 1974; Mugnaini et al., 1980a; Dunn et al., 1996; Weedman et al., 1996). A second type of glomerulus associated with unipolar brush cells has been described more recently in the vestibulocerebellum and in the cochlear nuclei (Mugnaini et al., 1994, 1997; Rossi et al., 1995; Weedman et al., 1996). In these, mossy fiber terminals are intertwined with processes of unipolar brush cells, forming extensive appositions.

**Inputs to Golgi cells**

The present experiments leave no doubt that Golgi cells are activated by shocks to the root of the auditory nerve. Such shocks evoked early EPSPs in Golgi cells after a latency of about 1.3 milliseconds. These responses had a latency that was longer than monosynaptic responses in large cells nearby whose input is known to arise through the myelinated, type I, auditory nerve afferent fibers (0.5–0.9 milliseconds). The difference in latency of responses in Golgi cells could arise either because input from the auditory nerve is through a disynaptic pathway or because it is carried by slowly conducting fibers.

To assess the possibility that input is through a disynaptic pathway, excitatory interneurons must be identified that terminate in the granule cell domain. Two groups of cells from the magnocellular region of the VCN are known to send collaterals to the granule cell domains, D stellate (Oertel et al., 1990), and octopus cells (Golding et al., 1995), one of which, the D stellate cells, is inhibitory. Octopus cells are excitatory and end in granule cell domains with large, mossy terminals (Golding et al., 1995). In the sample of more than 30 individually labeled octopus cells, terminals have been observed in the immediate vicinity of the octopus cell area and in the granule cell lamina but not in the superficial granule cell domain over the anterior ventral cochlear nucleus. Thus while this pathway could account for excitatory responses to shocks of Golgi cells near the octopus cell area and in the granule cell lamina, it is unlikely to account for responses in the three Golgi cells over the anterior ventral cochlear nucleus (Table 1). Furthermore, the wide dynamic range of responses to sound reported by Ghoshal and Kim (1995) in the granule cell domain are unlikely to arise from octopus
cells whose responses have an exceptionally narrow dynamic range.

Unmyelinated, type II auditory nerve fibers could mediate responses to shocks in slices and to sound in vivo. It has also been shown that type II auditory nerve fibers end in the superficial granule cell domain. Brown and Ledwith (1990) have traced unmyelinated auditory nerve fibers into the superficial granule cell domain of mice. The type II auditory nerve fibers terminate on proximal dendrites of unidentified multipolar cells in the granule cell domains (Benson et al., 1996). Because these terminals are in the same region where Golgi cells were recorded in the present study, it seems likely that at least some responses to shocks of the auditory nerve were transmitted along this pathway. Conduction through unmyelinated fibers would also account for the latencies measured in the present study. Of the 1.3-millisecond latency, about 0.5 milliseconds is expected to be synaptic, leaving a 0.8-millisecond conduction delay. Conduction over approximately 0.5 mm, from the cut end of the auditory nerve where the stimulating electrodes were placed to the location of the Golgi cell bodies in the present experiments, in 0.8 milliseconds implies that the conduction velocity would have to be approximately 0.6 m/second, a value that is within the range of conduction velocities of unmyelinated fibers in the peripheral nerve, 0.5 to 2.5 m/second (Paintal, 1967).

Two results show that interneurons also excite Golgi cells. First, because isolated axons consistently fire only a single action potential in slices, the EPSPs that arise tens to hundreds of milliseconds after a shock reflect the presence of excitatory interneurons. Second, this conclusion was confirmed by the finding that NMDA receptors affect the frequency of late EPSPs. These actions could not have been mediated through axons or synaptic terminals, which lack such receptors (Wickesberg and Oertel, 1990).

Granule and unipolar brush cells are excitatory and are thus possible sources of the late excitation. Both have been shown to contact Golgi cells (Mugnaini et al., 1994, 1997) and both possess NMDA receptors (Farrant et al., 1994; Rossi et al., 1995). In the cerebellum, unipolar brush cells fire long, regular trains of action potentials. The finding that late EPSPs were irregular argues against unipolar brush cells being the source of that excitation.

The presence of glycinergic inhibition in one Golgi cell is intriguing because it could reflect a functional connection between large cells that lie just beneath the superficial granule cell domain and neurons within the granule cell domain. Magnocellular cochlear nuclear stellate cells, the D stellate cells, have been shown to terminate in superficial granule cell domain in mice and are a possible source of glycinergic inhibition (Oertel et al., 1990). The response of these cells to shocks of the nerve root in similar slices has been demonstrated (Oertel et al., 1990; Ferragamo et al., 1998). The observation that the influence of glycinergic inhibition was subtle is consistent with the finding that the projection from D stellate cells is a minor one.

Functional role of Golgi cells

The paucity of information about the function of the granule cell domain, and of Golgi cells in particular, necessarily makes the consideration of the functional role of Golgi cells somewhat speculative. The possibilities are intriguing, however, and can be tested in future experiments.

The shape of Golgi cells suggests that they coordinate the activity of groups of granule cells. Golgi cells have extensive axonal arbors that encompass a group of granule cells with uniformly dense appositions in the cochlear nuclei (present results) as well as in the cerebellum (Alvarez-Otero and Anadón, 1992). In sharing a common input, the group of granule cells that is innervated by one Golgi cell functions to some degree as a unit. These observations raise the question of whether terminal arbors of neighboring Golgi cells overlap. It is possible that Golgi cells bind granule cells into functional groups.

Golgi cells may also serve to control the gain in underlying T stellate cells. As discussed above, Golgi cells are a likely source of GABAergic inhibition in T stellate cells (Ferragamo et al., 1998). If Golgi cells are the source of direct GABAergic inhibition in T stellate cells, the question arises of where that synaptic interaction might occur. It has been shown recently that the distal dendrites of stellate cells reach toward the superficial granule cells and receive contacts from axonal terminals containing pleomorphic vesicles in the overlying granule cell lamina (Josephy and Morest, 1998). Synaptic interactions at the tips of dendrites could account for the slow subtle GABAergic IPSPs seen in somatic recordings. It seems likely that some of the recordings by Ghoshal and Kim (1997) of neurons with wide dynamic ranges were from Golgi cells because action currents in Golgi cells are larger and would be more easily recorded extracellulary than those of granule cells. If GABAergic inhibition in T stellate cells has a wide dynamic range, its action would be expected to modulate the membrane potential and the input conductance of its targets of stellate cells over a wide range of intensity. A similar action of GABA has been proposed by Evans and Zhao (1993) in the DCN. In this context, it has been noted that the dynamic range of amplitude modulation in the corresponding cells in cats (choppers) is high (Rhode, 1994).

Golgi cells also influence the DCN. In influencing the spatial and temporal firing patterns of granule cells, Golgi cells influence targets of granule cells in the molecular layer of the DCN. Stimulation of the medullary somatosensory nuclei evoked short-latency inhibition from inhibitory interneurons other than cartwheel cells (Davis et al., 1996; Davis and Young, 1997) for which Golgi cells are a possible source.

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LITERATURE CITED


