Physiological Identification of the Targets of Cartwheel Cells in the Dorsal Cochlear Nucleus

NACE L. GOLDING AND DONATA OERTEL

Department of Neurophysiology, University of Wisconsin, Madison, Wisconsin 53706

Golding, Nace L. and Donata Oertel. Physiological identification of the targets of cartwheel cells in the dorsal cochlear nucleus. J. Neurophysiol. 78: 248–260, 1997. The integrative contribution of cartwheel cells of the dorsal cochlear nucleus (DCN) was assessed with intracellular recordings from anatomically identified cells. Recordings were made, in slices of the cochlear nuclei of mice, from 58 cartwheel cells, 22 fusiform cells, 3 giant cells, 5 tuberculoventral cells, and 1 cell that is either a superficial stellate or Golgi cell. Cartwheel cells can be distinguished electrophysiologically from other cells of the cochlear nuclei by their complex spikes, which comprised two to four rapid action potentials superimposed on a slower depolarization. The rapid action potentials were blocked by tetrodotoxin (n = 17) and were therefore mediated by voltage-sensitive sodium currents. The slow spikes were eliminated by the removal of calcium from the extracellular saline (n = 3) and thus were mediated by voltage-sensitive calcium currents. The spontaneous and evoked firing patterns of cartwheel cells were distinctive. Cartwheel cells usually fired single and complex spikes spontaneously at irregular intervals of between 100 ms and several seconds. Shocks to the DCN elicited firing that lasted tens to hundreds of milliseconds. With the use of these distinctive firing patterns, together with a pharmacological dissection of postsynaptic potentials (PSPs), possible targets of cartwheel cells were identified and the function of the connections was examined. Not only cartwheel and fusiform cells, but also giant cells, received patterns of synaptic input consistent with their having originated from cartwheel cells. These cell types responded to shocks of the DCN with variable trains of PSPs that lasted hundreds of milliseconds. PSPs within these trains appeared both singly and in bursts of two to four, and were blocked by 0.5 or 1 μM strychnine (n = 4 cartwheel, 4 fusiform, and 2 giant cells), indicating that cartwheel cells are likely to be glycinergic. In contrast with cartwheel cells, which are weakly excited by glycinergic input, glycinergic PSPs consistently inhibited fusiform and giant cells. Tuberculoventral cells and the putative superficial stellate cell received little or no spontaneous synaptic activity. Shocks to the DCN evoked synaptic activity that lasted ~5 ms. These cells therefore probably do not receive input from cartwheel cells. In addition, the brief firing of tuberculoventral cells and of the putative superficial stellate cell in response to shocks indicates that these cells are unlikely to contribute to the late, glycinergic synaptic potentials observed in cartwheel, fusiform, and giant cells.

INTRODUCTION

The cartwheel cells of the dorsal cochlear nucleus (DCN) are interneurons that are known to contact other cartwheel as well as fusiform cells, the main projecting neurons of the DCN (Berrebi and Muguinai 1991; Muguinai et al. 1987). Fusiform cells are in addition innervated tonotopically by auditory nerve fibers. The cartwheel cells thus form a network of interconnected cells that lies poised to influence the topographic array of fusiform cells as it transmits acoustic information to the contralateral inferior colliculus (Adams 1979; Oliver 1984; Ryugo et al. 1981).

The role played by cartwheel cells in vivo reflects the diverse sensory modalities encoded by the cochlear granule cells whose axons, the parallel fibers, provide glutamatergic excitation to cartwheel cells as well as other targets in the molecular layer of the DCN (Golding and Oertel 1996; Kane 1974; Manis 1989; Muguinai et al. 1980a,b; Wouterlood and Muguinai 1984; Wouterlood et al. 1984). The granule cells receive afferents from the cochlea, cochlear nucleus, superior olivary complex, inferior colliculus, auditory cortex, dorsal column nuclei, saccule, and vestibular nerve root (Berglund and Brown 1994; Brown and Ledwith 1990; Brown et al. 1988a,b; Burian and Gestoettner 1988; Caicedo and Herbert 1993; Feliciano et al. 1993; Golding et al. 1995; Itoh et al. 1987; Keveett and Parachio 1989; Spangler et al. 1987; Weedman and Ryugo 1996; Weedman et al. 1996; Weimbreg and Rustioni 1987; Wright and Ryugo 1996; Zhao et al. 1995). Any tonotopic arrangement of auditory inputs to granule cells ultimately is disrupted, because the trajectory of the parallel fibers is orthogonal to the isofrequency laminae of the DCN. The parallel fibers are thus in a position to spread excitation to cartwheel cells that lie over wide expanses of the tonotopic axis.

The responses of cartwheel cells to acoustic stimuli in vivo reflect the nontonotopic arrangement of parallel fiber inputs. These cells display little evidence of sharp tuning and are driven weakly by both tones and noise (Davis et al. 1996; Ding and Voigt 1996; Parham and Kim 1995). Cartwheel cells are also driven by electrical activation of somatosensory inputs from the dorsal column nuclei (Davis et al. 1996), and are likely to participate in the inhibition of principal cells (fusiform and/or giant cells) mediated by these same stimuli (Young et al. 1995).

Glycinergic and GABAergic inputs to cartwheel cells are depolarizing and can be excitatory (Golding and Oertel 1996). Because the reversal potential for glycinergic and GABAergic postsynaptic potentials (PSPs) was only slightly above threshold (about ~53 mV), however, these PSPs could also suppress rapid firing during strong membrane depolarizations. By contrast, glycinergic and GABAergic inputs to fusiform cells are conventionally inhibitory, with reversal potentials of about ~68 mV (Golding and Oertel 1996; Zhang and Oertel 1994).

The goal of the present experiments was to ascertain the integrative contribution of the cartwheel cells to the principal cells. We examine in detail the hypothesis that glycinergic input to both cartwheel and fusiform cells arises from the cartwheel cells (Golding and Oertel 1996). By correlating
the distinctive temporal firing patterns of cartwheel cells with corresponding patterns of synaptic activity in putative targets, we show that not only cartwheel and fusiform cells but also giant cells receive glycine-ergic PSPs that could have arisen from cartwheel cells. The present results, together with those of prior studies, indicate that cartwheel cells, using a single neurotransmitter, have different synaptic actions on principal cells and other cartwheel cells (Berrebi and Mugnaini 1991; Golding and Oertel 1996; Zhang and Oertel 1994).

**Methods**

**Slice preparation and intracellular recordings**

Parasagittal slices of the cochlear nuclei were cut from the brain stems of 18- to 26-day-old mice as described previously (Golding and Oertel 1996). Mice were decapitated and dissected under oxygenated (95% O₂-5% CO₂) normal saline at ~31°C. The saline contained (in mM) 130 NaCl, 3 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 20 NaHCO₃, 3 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 10 glucose, and 1.2 KH₂PO₄, pH 7.4. Slices were cut at ~300 µm thickness with an oscillating tissue slicer (Frederick Haer, New Brunswick, ME). The presence of the underlying restiform body on the medial face of the slice indicated that most of the circuitry remained intact in these slices. A slice was allowed to recover for >1 h in the recording chamber, where it was continuously superfused with saline at a rate of 9–12 ml/min and maintained at 34°C.

Intracellular recordings were made with sharp microelectrodes filled with 1% biocytin (Sigma) in 2 M potassium acetate (pH 7.3). Electrode impedances ranged from 120 to 200 MΩ. Voltages were recorded by a high-impedance amplifier (Dagan IX2–700, Minneapolis, MN) and filtered at 10 kHz. Membrane potentials were continuously monitored on a chart recorder. Data acquisition and analysis were performed with the use of a Digidata 1200 interface in conjunction with pClamp software (Axon Instruments, Foster City, CA).

Synaptic potentials were evoked with shocks (duration 100 µs, amplitude 0.1–100 V) through a bipolar electrode. In most experiments shocks were delivered to the surface of the DCN, dorsal to the site of recording, to activate directly the parallel fibers as well as the other neurons of the DCN. In a few experiments shocks were applied to the cut end of the auditory nerve (to activate the DCN circuitry polysynaptically through auditory nerve fibers) or to the surface of the ventral cochlear nucleus (VCN; to activate the DCN through the superficial granule cells).

All drugs were applied by exchanging test solutions for normal saline without interruption of the flow. Tetrodotoxin (TTX), strychnine, picrotoxin, di-2-aminophosphonovleric acid (APV), and 6,7-dinitroquinoxaline-2,3-dione (DNQX) were obtained from Sigma (St. Louis, MO) and were added to normal saline. The concentrations of these drug solutions (0.25–1.0 µM TTX; 0.5–1.0 µM strychnine; 10–100 µM picrotoxin; 100 µM APV; 40 µM DNQX) were sufficient to provide a complete blockade of their respective targets: sodium channels as well as glycine, y-aminobutyric acid-A (GABAₐ), N-methyl-D-aspartate, and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptors. In some experiments calcium was nominally eliminated by exchanging CaCl₂ for MgSO₄.

**Morphological identification of cells**

Biocytin was injected into recorded cells with 0.5- to 2-nA depolarizing current pulses 100 ms in duration delivered at a rate of 2.5 pulses per second for up to 5 min. Slices were fixed in 4% paraformaldehyde and stored refrigerated for 1–21 days. Slices were then embedded in a mixture of gelatin and albumin, sectioned at 60 µm, and processed for biocytin histochemistry with the use of the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) in conjunction with Co²⁺ and Ni²⁺ intensification (Adams 1981). Sections were mounted on coated slides and counterstained with cresyl violet. Reconstructions were made with a camera lucida.

**Results**

The conclusions of the present study are based on recordings of 38 cartwheel cells, 22 fusiform cells, 3 giant cells, 5 tuberculoventral cells, and 1 putative superficial stellate cell, all of which were anatomically identified. The resting potentials and input resistances of cells, as well as the duration of recordings, are summarized in Table 1.

**Complex spikes in cartwheel cells**

The ability to generate complex spikes distinguishes cartwheel cells from all other cell types in the cochlear nuclei thus far recorded. Complex spikes consist of a burst of fast, large action potentials superimposed on a slower, smaller depolarization (Manis et al. 1994; Zhang and Oertel 1993a), and were common both in the spontaneous activity and in responses to shocks. Figure 1A shows the characteristic simple and complex spikes fired by a cartwheel cell in response to depolarizing current pulses. The ionic basis of complex spikes was explored in experiments in which voltage-sensitive sodium and calcium currents were successively eliminated (Fig. 1B). The fast, large action potentials of complex spikes that were evoked with current pulses were sodium dependent, because they were reversibly blocked by TTX, an antagonist of voltage-gated sodium channels (n = 17). The slower, smaller action potential was calcium dependent, because it was reversibly eliminated by the removal of calcium from the bathing medium (n = 3).

Both complex and simple spikes were observed in the long and variable responses of cartwheel cells to shocks of synaptic inputs. Figure 2 illustrates the features of responses of cartwheel cells to shocks of the surface of the VCN. Synaptically evoked PSPs and action potentials lasted for hundreds of milliseconds, with responses to stronger shocks generally lasting longer than those to weaker shocks (Fig. 2). The recruitment of synaptic inputs with shock strength was evident in the shape of traces but not necessarily in the rate and timing of firing. As shock strength increased, the rate of rise of the excitatory PSP increased and the recruitment of synaptic inputs with shock strength was evident as irregularities in the membrane po-

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>n</th>
<th>Resting Potential, mV</th>
<th>Input Resistance, MΩ</th>
<th>Duration of Recordings, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartwheel cells</td>
<td>58</td>
<td>-63 ± 5</td>
<td>75 ± 22</td>
<td>1.41 ± 0.92</td>
</tr>
<tr>
<td>Superficial stellate (?) cell</td>
<td>1</td>
<td>-60</td>
<td>61</td>
<td>1.2</td>
</tr>
<tr>
<td>Fusiform cells</td>
<td>22</td>
<td>-58 ± 5</td>
<td>69 ± 21</td>
<td>1.62 ± 1.01</td>
</tr>
<tr>
<td>Giant cells</td>
<td>3</td>
<td>-56 ± 4</td>
<td>50 ± 10</td>
<td>1.71 ± 1.01</td>
</tr>
<tr>
<td>Tuberculoventral cells</td>
<td>5</td>
<td>-58 ± 3</td>
<td>74 ± 27</td>
<td>0.61 ± 0.47</td>
</tr>
</tbody>
</table>

Values with ± are means ± SD; n, number of cells. Values for the duration of recordings underestimate their stability because many recordings were terminated intentionally by pulling the electrode out of the cell.
FIG. 1. Simple and complex spikes in cartwheel cells. A: response of a cartwheel cell to a series of depolarizing current pulses comprising both simple and complex spikes. Cartwheel cells responded to depolarization with slow action potentials that triggered bursts of fast action potentials (complex spikes) as well as with rapid, all-or-none action potentials (simple spikes). Frequency of simple and complex spikes increased with magnitude of current pulse. B: ionic basis of complex and simple spikes. Cartwheel cell responded to a 0.4-nA depolarizing current pulse with a complex spike followed by a train of simple spikes. Large, fast action potentials were eliminated by tetrodotoxin (TTX), a blocker of voltage-gated sodium channels, indicating that they were sodium dependent. Smaller, slower action potential that remained was abolished when calcium was removed from bath.

Synaptic inputs to cartwheel, fusiform, and giant cells mirror the firing of cartwheel cells

Spontaneous synaptic activity in the targets of cartwheel cells should reflect the temporal pattern of the spontaneous firing of cartwheel cells. Cartwheel cells receive spontaneous depolarizing PSPs that may lead to spontaneous firing of simple and complex spikes at rates generally <10 Hz. Complex spikes presumably lead to bursts of synaptic potentials in the targets of cartwheel cells. Three cell types received spontaneous bursts of PSPs: cartwheel, fusiform, and giant cells. Figure 3 provides a comparison of the timing of spontaneous complex spikes in three cartwheel cells with the timing of spontaneous bursts of PSPs in six cartwheel, six fusiform, and three giant cells. The timing of the clusters of PSPs is remarkably similar despite the fact that they were recorded from different cell types in different preparations. The interval between the peaks of PSPs within a burst varied within a given cell, as did the interspike intervals of fast action potentials within the complex spikes of cartwheel cells. In cartwheel cells, the complex spikes can themselves be triggered by bursts of PSPs (Fig. 3, top left and top right traces), consistent with the earlier conclusion that input from cartwheel cells to other cartwheel cells is weakly excitatory at membrane potentials near rest (Golding and Oertel 1996). Some spontaneous bursts are not preceded by bursts of PSPs (Fig. 3, top middle trace).

Bursts of spontaneous inhibitory PSPs (IPSPs) are common and obvious in recordings from fusiform cells and have been noted in earlier recordings to be a consistent feature (Zhang and Oertel 1994). It seemed surprising, therefore, that despite the fact that obvious bursts of PSPs were observed in every giant cell, they were observed frequently in only one of the three examples. All three cells, however, received slow, irregular hyperpolarizations, many of which are probably bursts of PSPs smoothed by dendritic filtering. Figure 4 shows both single and bursts of spontaneous PSPs in two giant cells exhibiting a range of different rates of rise and decay. In both cells, it is difficult to distinguish whether some of the slower PSPs represent single events or bursts. The slow PSPs shown in Fig. 4A, fourth trace, and Fig. 4B, third trace, may, for example, reflect three individual PSPs,
the summation of which comprises the slowly rising edge. Slower PSPs were common in the cell shown in Fig. 4A, whereas more rapid PSPs predominated in the cell shown in Fig. 4B, suggesting that the proximity of spontaneous synaptic input to the presumed somatic recording site varies across giant cells.

The majority of spontaneous PSPs in cartwheel, fusiform, and giant cells was glycinergic. All three cell types received frequent spontaneous PSPs that occurred both singly and in bursts of tightly clustered PSPs that summed in time. One example of each cell type is illustrated in Fig. 5. In all cartwheel, fusiform, and giant cells tested, spontaneous PSPs were, at most, only partially driven by glutamatergic excitation, because many if not most PSPs remained in the presence of DNQX and APV (n = 6, 5, and 1, respectively). The residual spontaneous PSPs were glycinergic, because they were blocked by strychnine at 0.5 or 1 µM. In some cartwheel and fusiform cells, a few GABAergic PSPs were observed (3 of 6 and 3 of 5 cells, respectively) (Golding and Oertel 1996). When present, these PSPs occurred infrequently and singly. No spontaneous GABAergic PSPs were observed in the giant cells whose spontaneous activity was examined pharmacologically (n = 2).

In responses to shocks of the auditory nerve (Golding and Oertel 1996; Zhang and Oertel 1993a) or to the DCN or surface of the VCN (Golding and Oertel 1996) (Fig. 2), cartwheel cells generate trains of action potentials that are longer than those of most cells in the cochlear nuclei. Their targets should thus receive long trains of PSPs and those PSPs should all be eliminated by a common receptor antagonist. Figure 6 shows that this is indeed the case. Late PSPs in cartwheel, fusiform, and giant cells were eliminated by strychnine, indicating that they were mediated by glycine receptors. In the cartwheel cell shown in Fig. 6C, a shock to the DCN evoked a train of action potentials that lasted 200 ms. The addition of strychnine to the bath increased the magnitude of the initial depolarization but, significantly, also abbreviated the duration of firing. It is not surprising that the removal of inhibition causes an increase of excitation, as that observed early, but the loss of late excitation is inconsistent with a direct inhibitory action of glycinergic input on cartwheel cells. It is, however, consistent with the conclusion that glycinergic inputs are excitatory in cartwheel cells (Golding and Oertel 1996). The effects of strychnine were not reversed in every cell tested because >1 h was required to wash strychnine out of the slices. To resolve subthreshold synaptic responses in cartwheel cells, it was necessary to hyperpolarize them with current to reduce firing, as shown in Fig. 6C*. This record shows that strychnine eliminated late, depolarizing PSPs reversibly. In fusiform (Fig. 6F) and giant (Fig. 6G) cells, strychnine eliminated both early and late inhibition. In the fusiform cell, an additional GABAergic inhibitory influence was revealed by subsequent addition of 10 µM picrotoxin to a 1 µM strychnine solution (not shown).

Glycinergic inputs to cartwheel, fusiform, and giant cells could be activated monosynaptically with shocks to the DCN. The blockade of glutamatergic inputs with DNQX and APV revealed a residual PSP that was identified to be glycinergic by its subsequent elimination by strychnine. Examples of such experiments in a cartwheel, a fusiform, and a giant cell are shown in Fig. 7. A depolarizing PSP remained in the cartwheel cell when glutamatergic excitation was blocked with DNQX and APV (Fig. 7C). This PSP was glycinergic because it was subsequently blocked with strychnine, and could have arisen from the direct activation of local interneurons and/or axons of passage. The isolated glycinergic PSP in this cell could elicit action potentials and was therefore excitatory (not shown). Glycinergic PSPs were inhibitory in all fusiform and giant cells. The elimination of late synaptic activity by glutamatergic antagonists suggests that these PSPs were driven polysynaptically by glutamatergic inputs. In four of five cartwheel cells and three of six fusiform cells, a GABAergic component of the monosynaptic PSP was observed (Golding and Oertel 1996). However, late, presumably polysynaptic, GABAergic PSPs were never observed in response to shocks.

**Synaptic inputs to putative superficial stellate and tuberculoventral cells**

In contrast to cartwheel, fusiform, and giant cells, the putative superficial stellate and tuberculoventral cells...
showed neither spontaneous synaptic activity (Fig. 8A) nor late synaptic activity in responses to shocks of the DCN (Fig. 8B). In the putative superficial stellate cell, neither 1 μM strychnine nor 100 μM picrotoxin noticeably affected the cell’s synaptic responses (not shown). Although inhibition was not detected in the synaptic responses of the tuberculoventral cell in Fig. 8B, strychnine-sensitive IPSPs were observed in two other cells. However, these IPSPs occurred no later than 8 ms after the shock in both cases. These results indicate that the putative superficial stellate and tuberculoventral cells are unlikely to serve as targets of cartwheel cells.

**Cartwheel cells could be the source of late, glycinergic PSPs in cartwheel, fusiform, and giant cells**

Cartwheel, superficial stellate, and tuberculoventral cells are likely to be inhibitory interneurons and thus serve as a potential source of inhibition for other cells. A comparison of the timing of firing of these potential sources of PSPs (rasters) with potential targets (traces) is presented in Fig. 9. Each trace and each line in the raster were recorded from a separate cell. To be comparable, each response was chosen to be the longest of those recorded in that cell. The traces from the three cartwheel cells were recorded while the cells were hyperpolarized with current to reveal sub-threshold PSPs. The raster plot below shows firing after a single shock in cartwheel cells, tuberculoventral cells, and the putative superficial stellate cell. The filled diamonds within each row represent the timing of action potentials within the longest response recorded in one cell. Although cartwheel cells, without exception, fired trains of spikes lasting tens to hundreds of milliseconds, the tuberculoventral and putative stellate cells fired a single action potential that occurred no later than 5 ms after the stimulus. Although the population of tuberculoventral cells and putative superficial stellate cells sampled is considerably smaller than that of cartwheel cells, these findings are consistent with a previous study in which the temporal responses of these cell types to shocks of the auditory nerve were analyzed (Zhang and Oertel 1994). Cartwheel cells, but not tuberculoventral or superficial stellate cells, fire late enough after a shock to the DCN to produce the late PSPs in cartwheel, fusiform, or giant cells.

**Morphology of DCN neurons**

Examples are shown in Figs. 10–12 of cartwheel, putative superficial stellate, fusiform, giant, and tuberculoventral cells that were recorded, labeled with biocytin, and reconstructed with a camera lucida. The dendrites are the potential targets and the terminal arbors the potential sources of some of the connections that were examined electrophysiologically above.
Examples of neurons with cell bodies in the molecular layer are shown in Fig. 10. Both cells have dendrites in the molecular layer and terminals in the molecular and fusiform cell layers. The cell depicted in Fig. 10A shows the features that were characteristic of each of the 58 labeled cartwheel cells. The thick dendrites with the characteristic branching pattern for which the cells are named extend to the ependyma and have a dense covering of spines. Identification of the cell shown in Fig. 10B is less certain; the cell body and the dendrites lie in the molecular layer, like the one other described superficial stellate cell (Zhang and Oertel 1993a, their Fig. 9). On the other hand, the dendrites shown in Fig. 10B are less straight and the axon terminates not only in the molecular but also in the fusiform cell layer. The differences between these two cells raise the possibility that the cell shown in Fig. 10B is a Golgi cell (Mugnaini and Floris 1994; Mugnaini et al. 1994). The fact that the identification of Golgi and superficial stellate cells is based mainly on electron microscopic evidence and that the sample of singly labeled superficial stellate and Golgi cells is small makes the positive identification of this cell impossible.

**FIG. 4.** Spontaneous IPSPs in giant cells show evidence of dendritic filtering. A: spontaneous IPSPs in a giant cell show heterogeneity in their shapes and durations. Whereas bursts of individual IPSPs are clearly resolved within some faster IPSPs (e.g., bottom trace), they are more difficult to distinguish from single synaptic events in the more slowly rising IPSPs (e.g., 3rd and 4th traces). Resting potential: −56 mV. Dotted line: beginning of each spontaneous PSP. B: spontaneous IPSPs in a different giant cell also show heterogeneity in their temporal patterns and in their rates of rise and decay. Morphology of this cell is presented in Fig. 11B. Resting potential: −53 mV.

**FIG. 5.** Spontaneous synaptic activity in cartwheel, fusiform, and giant cells is largely glycinergic. Each of these cells received spontaneous PSPs that occurred singly as well as in bursts of summing PSPs (a few are indicated by *). Much of this spontaneous activity remained in the presence of the glutamatergic antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX) and D,L-2-amino-5-phosphonovaleric acid (APV). The remainder of spontaneous activity was eliminated by strychnine (STR), indicating that it was glycinergic. Recorded cells were in different slices. Concentration of antagonists: DNQX, 40 μM; APV, 100 μM; strychnine, 0.5 or 1 μM.
FIG. 6. Late PSPs in responses to shocks of dorsal cochlear nucleus (DCN) in cartwheel, fusiform, and giant cells are at least in part glycinergic. In a cartwheel cell (C), shocks gave rise to a train of simple spikes lasting 200 ms. In the presence of strychnine, the same stimulus evoked a complex spike followed by a subthreshold depolarization. In a different cartwheel cell (C*), a DCN shock given while the cell was hyperpolarized with −0.2 nA current evoked a series of late depolarizing PSPs. These late PSPs were reversibly eliminated by strychnine. In the fusiform (F) and giant (G) cells, late IPSPs were also reversibly eliminated by strychnine. Synaptic activity was evoked with constant voltage shocks (∇) to DCN in cartwheel and fusiform cells; synaptic activity was evoked from auditory nerve (∇) in the giant cell. Cells were recorded from different slices. Concentration of strychnine: 0.5 or 1 μM.

does not arborize in the DCN and was cut medially as it traversed the deep layer, presumably in its path to the dorsal acoustic stria. The cell bodies of giant cells lie in the deep layer (Fig. 11B). The dendrites of giant cells are not restricted in an isofrequency lamina in the deep layer but extend widely. Some dendrites extend through the fusiform cell layer and into the molecular layer. Because the axons of cartwheel cells are largely restricted to the molecular and fusiform cell layers in mice, their input must be located largely on distal dendrites of giant cells. The axon of this giant cell is unusual in that it is the only one (of 8 total) (Zhang and Oertel 1993b; present study) that had local collaterals. These collaterals terminated in the fusiform cell and deep layers of the DCN as well as in the lamina of granule cells that separates the DCN from the VCN. The main axon was cut as it entered the dorsal acoustic stria. Although some principal cells have axon collaterals in the DCN, the small number of such cases indicates that fusiform and giant cells are not a major source of input to neurons in the DCN.

Tuberculoventral cells lie intermingled with giant cells in the deep layer (Fig. 12). Their dendrites, like those of fusiform cells, are aligned within isofrequency laminae. The axon of the cell illustrated in Fig. 12, like those of other tuberculoventral cells in mice (Oertel and Wu 1989; Zhang and Oertel 1993c), terminates in the deep layer of the DCN as well as in the VCN. The terminal arbors of tuberculoventral cells lie mingled among dendrites of fusiform and giant cells but are separated from the dendrites of cartwheel and superficial stellate cells.

DISCUSSION

We conclude that the major integrative contribution of the superficial layers of the DCN to principal cells is inhibitory and primarily mediated by cartwheel cells. The cartwheel cells’ distinctive patterns of simple and complex spikes are reflected in the patterns of glycinergic PSPs in cartwheel, fusiform, and giant cells. Glycinergic PSPs are inhibitory in fusiform and giant cells, but are depolarizing in cartwheel cells. Depolarizing, glycinergic PSPs can excite cartwheel cells to threshold while inhibiting other targets with the same neurotransmitter (Golding and Oertel 1996). The present
results explore further the consequences of the connections of cartwheel cells on the output of the DCN.

**Complex spikes in cartwheel cells**

Cartwheel cells are the only cells in the cochlear nuclei known to fire complex spikes. Complex spikes occur spontaneously as well as in synaptic responses to shocks of the auditory nerve, VCN, and DCN, and in responses to depolarizing current injected through the recording electrode (Golding and Oertel 1996; Manis et al. 1994; Zhang and Oertel 1993a). The present results show that complex spikes in cartwheel cells comprise fast, sodium-dependent action potentials superimposed on slower, calcium-dependent depolarizations. Complex spikes in cartwheel cells resemble those of the cerebellar Purkinje cells (Llinás and Sugimori 1980), consistent with other studies showing extensive ontogenetic, morphological, and immunocytochemical similarities shared by these two cell types (Altman and Bayer 1985; Berrebi and Mignaini 1991; Berrebi et al. 1990; Mignaini and Morgan 1987; Pierce 1967; Ryugo et al. 1995; Wouterlood and Mignaini 1984). In Purkinje cells, calcium spikes are generated in the dendrites, and undergo passive electrotonic decay to the soma, where the recorded waveform appears small and slowly rising (Lev-Ram et al. 1992; Llinás and Sugimori 1980; Tank et al. 1988). The similar shape of calcium spikes in cartwheel cells suggests that the underlying voltage-gated channels may also be dendritically located.

**Glycinergic synaptic interactions between cartwheel cells**

Connections among cartwheel cells have been demonstrated anatomically (Berrebi and Mignaini 1991; Mignaini et al. 1987) and physiologically (Golding and Oertel 1996; present results). The synaptic activity in cartwheel cells that mirrors the firing patterns of cartwheel cells is blocked by strychnine. This result, together with immunocytochemical evidence demonstrating that cartwheel cells show strong glycinerlike immunoreactivity, support the conclusion that cartwheel cells are glycineric (Gates et al. 1996; Oertel and Wicksberg 1993; Osen et al. 1990; Wenthold et al. 1987; Wicksberg et al. 1994). Golding and Oertel (1996) demonstrated that the reversal potential for glycineric PSPs (estimated to be $-53 \text{ mV}$) was near the firing threshold of cartwheel cells. In cartwheel cells, glycineric synaptic input from other cartwheel cells can either promote or suppress firing, depending on how the synaptic inputs interact with the intrinsic conductances. These findings indicate that cartwheel cells form an interconnected network with both positive and negative feedback. The network is driven by excit-
cells, mirror the characteristic timing of firing of cartwheel cells both spontaneously and in responses to shocks. In an earlier report based on recordings from five giant cells it was concluded that giant cells lacked spontaneous inhibition (Zhang and Oertel 1993b). Indeed, clear examples of bursts of IPSPs were not common in two of the three giant cells in the present sample. More common were slow hyperpolarizations that probably represent dendritically filtered versions of such bursts. Because cartwheel cell terminals lie in the molecular and fusiform cell layers, their inputs to giant cells must commonly be to distal dendrites. It is therefore not surprising that PSPs arising from those inputs appear filtered. The conclusion that giant cells are targets of cartwheel cells is consistent with anatomic results that show that cartwheel cells contact not only fusiform cells but also large

FIG. 8. Synaptic activity in superficial stellate and tuberculoventral cells. A: spontaneous PSPs were not observed in putative superficial stellate cell (S) or tuberculoventral cell (T). B: shocks to DCN evoked action potentials within 5 ms in both putative superficial stellate and tuberculoventral cell.

atatory input from parallel fibers (Kane 1974; Wouterlood and Mugnaini 1984) that is probably glutamatergic (Golding and Oertel 1996; Hunter et al. 1993).

Feedforward inhibition of fusiform and giant cells by cartwheel cells

The present results support the conclusion that cartwheel cells inhibit fusiform cells (Berrebi and Mugnaini 1991; Golding and Oertel 1996; Mugnaini et al. 1987; Zhang and Oertel 1994). The electrophysiological observation that the temporal patterns of glycinergic PSPs mirror the firing patterns of cartwheel cells is consistent with earlier results (Zhang and Oertel 1994) and supports the finding from anatomic studies that cartwheel cells contact fusiform cells (Berrebi and Mugnaini 1991). The molecular layer has opposing influences on fusiform cells. Parallel fibers excite fusiform cells, whereas cartwheel cells inhibit them. The present study confirms that the inhibitory influence through cartwheel cells is strong, long lasting, and predominant under the experimental conditions of the slice.

A new finding is that giant cells are also targets of cartwheel cells. This conclusion is based on the observation that the temporal patterns of IPSPs in giant cells, as in fusiform

FIG. 9. Comparison of synaptic responses evoked by shocks to DCN in potential targets of cartwheel cells with timing of firing of potential sources of glycinergic input. Traces: responses of 3 cartwheel (C), 3 fusiform (F), and 2 giant (G) cells show that late PSPs occur nearly 300 ms after shocks to DCN. Cartwheel cells were hyperpolarized with current (between −0.2 and −0.4 nA) to reveal late depolarizing PSPs in the absence of firing. Longest duration of synaptic responses recorded in each cell is represented. Raster plots: firing patterns of 18 cartwheel cells, 3 tuberculoventral cells, and 1 putative superficial stellate cell. Each row of filled diamonds represents timing of action potentials in longest of the responses recorded in 1 cell.
neurons in the deep layer (Berrebi and Mugnaini 1991). A connection of cartwheel cells with giant cells is also supported by results obtained in vivo. In cats, the responses of principal cells (type IV units) to activation of parallel fibers are dominated by inhibition lasting from 10 to 30 ms (Young et al. 1995). Such units were recorded in both the fusiform and deep layers of the DCN, and thus may have included giant cells as well as fusiform cells. The timing of this inhibition is correlated temporally with the firing of units presumed to be cartwheel cells (Davis et al. 1996).

**Superficial stellate and Golgi cells**

The superficial stellate and Golgi cells reside in the molecular layer and could potentially provide feedforward inhibition onto fusiform and giant cells (Osen et al. 1990). Superficial stellate and Golgi cells are small to medium-sized multipolar cells with smooth or slightly beaded dendrites that have largely been described on the basis of electron microscopy (Mugnaini et al. 1980a; Osen et al. 1990; Wouterlood et al. 1984). At the light microscopic level, distinctions between these interneurons are not entirely clear, making the identification of the cell shown in Fig. 10B uncertain. In slices of the cochlear nuclei one cell has been identified as a superficial stellate cell (Zhang and Oertel 1993a). The cell shown in Fig. 10B resembles the stellate cell in its smooth dendrites but, in contrast with the earlier example, terminates both in the molecular layer and in the fusiform cell layer; the physiological responses of the two cells were similar. With a sample of only two cells, it is not clear
whether the cells are similar or different. Whether it is a superficial stellate or a Golgi cell, is it likely to be inhibitory. Both cell types were labeled with antibodies against GABA conjugates and against glutamic acid decarboxylase; Golgi cells were in addition labeled by antibodies against glycine conjugates (Adams and Mugnaini 1987; Mugnaini 1985; Osen et al. 1990). The putative superficial stellate cell is unlikely to be the source of late, glycinerergic PSPs because the cell fired a single, early spike in response to shocks of the auditory nerve or DCN within 5 ms of the shock. This cell could, however, be the source of GABAergic inhibition in cartwheel, fusiform, and giant cells.

Other inhibitory interneurons

Tuberculoventral cells are glycinerergic interneurons that likely contact fusiform and giant cells (Oertel and Wickesberg 1993; Oertel and Wu 1989; Zhang and Oertel 1993c, 1994). Tuberculoventral cells are confined to the deep layer of the DCN. Their dendrites are aligned parallel to, and receive excitatory input from, a restricted group of primary auditory nerve fibers (Brown and Ledwith 1990; Ryugo and May 1993; Wickesberg and Oertel 1988; Zhang and Oertel 1993c). Their axonal arbor have one group of terminals within roughly the same isofrequency lamina as the dendrites, and another group of terminals in an isofrequency band of the VCN. Because neither their auditory nerve inputs nor any processes lie dorsal to the cell body, it is not surprising that tuberculoventral cells were activated only weakly even at the highest shock strengths. The brief firing of tuberculoventral cells is inconsistent with their giving rise to late, glycinerergic activity in cartwheel, fusiform, and giant cells.

The DCN also receives a putative inhibitory influence from the axon collaterals of D-stellate cells of the VCN (Oertel et al. 1990; Smith and Rhode 1989). The firing of D-stellate cells does not account for the spontaneous and evoked bursts of PSPs recorded in cartwheel, fusiform, and giant cells, because they neither fire action potentials in bursts nor fire spontaneously in vitro (Oertel et al. 1990; M. J. Ferragamo, N. L. Golding, and D. Oertel, unpublished results).

Functional significance of the circuitry in the DCN

In considering these results in the context of the DCN as a whole it is useful to think of neurons not just singly but in arrays (Fig. 13). The principal cells combine information from two anatomically and functionally distinct neuronal circuits, one conveying multimodal sensory information through the molecular layer and one conveying...
tonotopic acoustic information through the deep layer. In the molecular layer, the network of cartwheel cells is excited by an array of glutamatergic parallel fibers (Manis 1989). The interconnections among cartwheel cells shape the spatial as well as the temporal pattern of activation of the network of cartwheel cells by parallel fibers. Activity in cartwheel cells ultimately imposes inhibition on the arrays of principal cells in the deeper layers. The array of fusiform cells preserves the tonotopic arrangement of the auditory nerve fiber inputs but not of the parallel fibers that cross the tonotopic array orthogonally. Less is known about giant cells, which also combine the same two classes of information but in different proportions and spatial patterns.

The superposition of excitation and inhibition from the neuronal arrays and networks in DCN principal cells underlies their sensitivity to spectral peaks and notches in sound stimuli (Evans and Nelson 1973; Nelken and Young 1994; Sproul and Young 1991; Young and Brownell 1976). These spectral features are produced by the passive filtering characteristics of the external ear and serve as potential cues for sound localization (Musacian et al. 1990; Rice et al. 1992). Young and colleagues (Kanold and Young 1996; Young et al. 1995) have proposed that somatosensory input to the molecular layer of the DCN could contribute information concerning the position of the pinnae and head that would be needed for the central auditory system to interpret the spectral cues encoded by DCN principal cells. Findings in vivo (Davis et al. 1996) indicate that the cartwheel cells convert the excitation from somatosensory inputs (and their granule cell intermediaries) into robust inhibition of principal cells. The diversity of other sensory and nonsensory inputs to granule cells would suggest, however, that such inhibition could subserve a more ubiquitous role in the DCN.

We thank our colleagues in the Department of Neurophysiology, especially J.A. Ekloferry, J. Meister, and I. Siggelkow in the histology lab and P. Heinritz, L. Lokken, A. Rassbach, and J. Hineline in the office. We also thank M. Ferragamo, D. Geisler, B. Rhode, P. Smith, and L. Trussell for comments on the manuscript. This work was supported by National Institute of Deafness and Other Communications Disorders Grant ROI DC-00176. N. Golding was supported by a predoctoral fellowship from the National Science Foundation. Present address of N.L. Golding: Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Address for reprint requests: D. Oertel, Dept. of Neurophysiology, University of Wisconsin, 1300 University Ave., Madison, WI 53706.

Received 2 July 1996; accepted in final form 17 March 1997.

REFERENCES


MAAS, P. B. SCOTT, J. C., and SPROU, G. A. Physiology of the dorsal cochlear nucleus molecular layer. In: The Mammalian Cochlear Nuclei;