Acoustic and Current-Pulse Responses of Identified Neurons in the Dorsal Cochlear Nucleus of Unanesthetized, Decerebrate Gerbils

JIANG DING, THANE E. BENSON, AND HERBERT F. VOIGT

Departments of Biomedical Engineering and Otolaryngology, Hearing Research Center, Boston University, Boston, Massachusetts 02215-2407

Ding, Jiang, Thane E. Benson, and Herbert F. Voigt. Acoustic and current-pulse responses of identified neurons in the dorsal cochlear nucleus of unanesthetized, decerebrate gerbils. J. Neurophysiol. 82: 3434–3457, 1999. In an effort to establish relationships between cell physiology and morphology in the dorsal cochlear nucleus (DCN), intracellular single-unit recording and marking experiments were conducted on decerebrate gerbils using horseradish peroxidase (HRP)- or neurobiotin-filled micropipettes. Intracellular responses to acoustic (tone and broadband noise bursts) and electric current-pulse stimuli were recorded and associated with cell morphology. Units were classified according to the response map scheme (type I to type V). Results from 19 identified neurons, including 13 fusiform cells, 2 giant cells, and 4 cartwheel cells, reveal correlations between cell morphology of these neurons and their acoustic responses. Most fusiform cells (8/13) are associated with type III unit response properties. A subset of fusiform cells was type I/III units (2), type III-i units (2), and a type IV-T unit. The giant cells were associated with type IV-i unit response properties. Cartwheel cells all had weak acoustic responses that were difficult to classify. Some measures of membrane properties also were correlated with cell morphology but to a lesser degree. Giant cells and all but one fusiform cell fired only simple action potentials (APs), whereas all cartwheel cells discharged complex APs. Giant and fusiform cells all had monotonic rate versus current level curves, whereas cartwheel cells had nonmonotonic curves. This implies that inhibitory acoustic responses, resulting in nonmonotonic rate versus sound level curves, are due to local inhibitory interactions rather than strictly to membrane properties. A complex-spiking fusiform cell with type III unit properties suggests that cartwheel cells are not the only complex-spiking cells in DCN. The diverse response properties of the DCN’s fusiform cells suggest that they are very sensitive to the specific complement of excitatory and inhibitory inputs they receive.

INTRODUCTION

The cochlear nucleus (CN) provides the first stage for processing acoustic information in the central auditory pathway. It consists of three tonotopically organized subnuclei: the anteroventral, posteroventral, and the dorsal cochlear nuclei (Lorente de Nó 1981; Osen 1969; Ramon y Cajal 1909; Rose et al. 1960). Of these, the laminated dorsal CN (DCN) is the most complex. The DCN contains a diverse set of morphologically distinct neuron types, including fusiform/pyramidal cells, giant cells, cartwheel cells, granule cells, Golgi cells, unipolar brush cells, stellate cells, chestnut cells, and vertical/corn/tuberculoventral cells (Brawer et al. 1974; Floris et al. 1994; Kane 1974; Lorente de Nó 1981; Mugnaini et al. 1980a,b; Osen 1969; Osen and Mugnaini 1981; Weedman et al. 1996; Wickettberg and Oertel 1988; Wouterlood and Mugnaini 1984; Wouterlood et al. 1984; Zhang and Oertel 1993a–c). These neurons participate in complex neural circuitry thought capable of performing sophisticated signal processing tasks (Davis et al. 1996; Nelkin and Young 1994; Young et al. 1992). Extensive extracellular studies have revealed a variety of response patterns from DCN units responding to acoustic stimulation. These responses have been classified according to response maps (Davis et al. 1996; Evans and Nelson 1973; Young and Brownell 1976; Young and Voigt 1982) and/or temporal discharge patterns (Gdowski and Voigt 1997; Pfeiffer 1966; Rhode and Smith 1986a,b; Shofer and Young 1985; Young et al. 1988). Little is known, however, about which neurons give rise to these responses.

To understand DCN neural circuitry and function, it is important to associate acoustic response properties with morphologically defined neuron types. Several in vivo intracellular studies on cat have been successful in this regard for a few cell types (Rhode et al. 1983a,b; Smith and Rhode 1985, 1989). For example, Rhode et al. (1983a,b) showed that fusiform/pyramidal cells possessed pauser-buildup and chopper peristimulus time histogram response patterns depending on stimulus parameters. These studies were performed in barbiturate-anesthetized preparations, and units were classified according to their temporal discharge patterns. Response map properties for identified DCN neurons still remain largely unknown.

The effects of barbiturates on the response properties of DCN neurons is a confounding factor in these past studies. Since 1973, the profound effects of barbiturates on the response maps of presumed fusiform/pyramidal cells have been well known (Evans and Nelson 1973). Young and Brownell (1976) showed how injections of pentobarbital sodium (Nembutal) reduced much of the inhibition for moderate level best frequency (BF) tones transforming type IV units into type III units. In gerbil, the effects of Brevital, an ultrashort-acting barbiturate, and Nembutal reduce the spontaneous activity of all spontaneously active DCN cells in decerebrate preparations, and transform units with one response map into another (Fan and Voigt 1997). Gdowski and Voigt (1997) found that nearly 90% of all units recorded in the barbiturate-anesthetized gerbil DCN have no spontaneous activity and that some measures used to classify DCN units in unanesthetized, decerebrate animals (e.g., relative noise response and normalized tone slope) are not useful for this purpose for anesthetized preparations. Thus it is hardly surprising that the response maps of
DCN units recorded in the presence of barbiturates are not well known. This study is an attempt to achieve a better understanding of the neural circuitry and function of the DCN by investigating acoustic response properties of morphologically defined DCN neurons and establishing relationships between unit physiology and cell morphology. Intracellular single-unit recording and horseradish peroxidase (HRP)- and neurobiotin-marking techniques in vivo were used to achieve this goal. An anesthetized, decerebrate preparation was selected to eliminate the influence of barbiturates on neural response properties (Evans and Nelson 1973; Fan and Voigt 1997; Young and Brownell 1986). Portions of this work have been reported at scientific meetings (Ding et al. 1994a,b; Voigt et al. 1998).

Portions of this work were submitted by J. Ding in partial fulfillment of the requirements for a doctoral degree in Bio-medical Engineering at Boston University.

METHODS

Animal choice

Mongolian gerbils (Meriones unguiculatus Tumblebrook Farms) were chosen as subjects because they have well-developed auditory systems capable of low-frequency hearing (Ryan 1976), have neurons and a basic organization similar to cats (Benson and Voigt 1995; Schwartz et al. 1987), have relatively large bullae providing easy access to the DCN (Frisina et al. 1982; Lay 1972; Plassmann et al. 1987), and allow a stable preparation for in vivo intracellular studies (Ding and Voigt 1997). In addition, an extensive database of DCN neurons and establishing relationships between unit physiology and a basic organization similar to cats (Benson and Voigt 1995; Schwartz et al. 1987), have relatively large bullae providing easy access to the DCN (Frisina et al. 1982; Lay 1972; Plassmann et al. 1987), and allow a stable preparation for in vivo intracellular studies (Ding and Voigt 1997). In addition, an extensive database of DCN unit response properties from both extracellular (Davis et al. 1996) and intracellular (Ding and Voigt 1997) studies in the decerebrate preparation and extracellular studies in the barbiturate anesthetized gerbil (Gdowski and Voigt 1997) is available.

Surgical procedures

All experiments were performed in a sound-attenuating chamber (IAC, model 1202A) using institutionally approved protocols. Detailed surgical procedures can be found in Davis et al. (1996). Female gerbils (~3 mo old) first were administered atropine (0.04 mg/kg im) to reduce respiratory secretions. The animals then were anesthetized by an intraperitoneal injection of Brevalid sodium (methohexital sodium, 65 mg/kg), an ultrashort-acting barbiturate. Supplemental doses of Brevalid sodium (32 mg/kg) were administered during surgery as needed. Body temperature was maintained at ~38°C using a Harvard Apparatus heating blanket and controller. After an incision in the throat, a small slit was made on the ventral surface of the trachea, and the common carotid arteries were ligated with silk. After the pinnae were removed, the animal was placed in a stereotaxic device (KOPF, model 1730). A hole was made in the skull, and all brain tissue rostral to the superior colliculus was aspirated. The empty portion of the skull was packed gently with gelfoam to promote blood clotting and to provide mechanical support for the remaining brain tissue. The DCN was accessed using Frisina et al.’s transbulla approach (Frisina et al. 1982). The animal was allowed to stabilize for ~30 min after the anesthetic was discontinued.

Acoustic system and calibration procedures

Acoustic stimuli were delivered to the left ear by an earphone (Beyer Dynamic DT48A, 200 Ω) coupled to a hollow earbar. A probe-tube microphone (0.5-in Bruel and Kjaer, model 4134) was placed near the tympanic membrane to measure sound pressure levels during acoustic calibration procedures. Pure tone stimuli were generated by a programmable (Wavetek, model 5100) or manual (Wavetek, model 188) oscillator with low harmonic distortion (less than ~60 dB). Broadband noise stimuli were generated digitally on a personal computer (Gateway 2000, 486DX) and output through a D/A converter (Tucker/Davis) at a sampling frequency of 100 kHz (Davis et al. 1996). Acoustic stimuli were gated on and off with 5-ms rise/fall times using an electronic cosine switch (Wilsonics, model B5). The signals were attenuated by a programmable attenuator (Wilsonics model PATT) to achieve the desired stimulation levels. Stimulus presentation and data acquisition were under the control of a DEC PDP 11/73 computer. The acoustic system was calibrated in each experiment by delivering a click (5 V, 20 μs) to the earphone and measuring the resulting sound pressure near the tympanic membrane. The system’s frequency response was computed by performing a fast Fourier transform (FFT) of the click response and dividing through by the FFT of the click. A 10-band equalizer (BSR, model EQ-3000) was used to achieve a maximally flat frequency response by modifying the frequency spectrum of the click before delivery to the ear. To compensate for the nonflat frequency response, the digital noise stimuli were created with a spectrum the magnitude of which was the inverse of the magnitude of the system’s frequency response (~25 kHz). Davis et al. (1996) provide more details and a representative frequency response curve.

Electrodes and recording system

Glass microelectrodes were pulled from capillary tubes (WPI) with 1-mm ID using a Flaming-Brown micropipette puller (Sutter Instrument, model P80/PC), and filled with 0.5 M potassium chloride, 0.05 M Tris buffer, and either 4% HRP or 2% neurobiotin using standard techniques. The tip diameters were usually <0.5 μm, and the initial impedances were >100 MΩ, measured at 1 kHz using a microelectrode impedance meter (Winston Electronics, model BL-1000). The electrodes were beveled in a saline-silicon carbide (Buehler) slurry to reduce the impedance to between 40 and 80 MΩ before use.

Electrodes were advanced into the DCN through the paraflocculus by a stepper-motor microdrive (Kopf Instruments, model 660). A silver/silver chloride (Ag/AgCl) wire was inserted into the electrode and connected to the headstage of an Axoclamp 2A (x1) amplifier (Axon Instruments). Another such wire was placed in the neck musculature as a reference electrode. The electrical activity recorded by the Axoclamp was further amplified by a DC amplifier (Tektronix, model AM 502), digitized, and stored on a PDP 11/73 computer system.

Experiment protocol

MEASUREMENT OF BRAIN STEM AUDITORY-EVOKED RESPONSES. Click-induced brain stem auditory-evoked responses (BAERs) were measured in each animal as described in Ding and Voigt (1997) to gauge the integrity of the auditory brain stem pathways. The experiment would continue only if BAER threshold was within normal range (Burkard and Voigt 1989).

STIMULUS AND DATA COLLECTION PROCEDURES. As the electrode advanced through paraflocculus, broadband noise bursts were used to evoke background driving. Once background driving was observed, signaling DCN penetration, the noise stimuli were replaced with tone bursts that clearly elicited background responses. After penetrating a neuron, its BF and threshold (θ) were estimated audiovisually. The transmembrane voltage then was recorded for various stimuli. First, three trials of 50- or 100-ms tone bursts (10-ms delay, 250-ms interstimulus interval) were presented at three frequencies (BF, BF −0.7 octave, and BF +0.7 octave) and 17 sound pressure levels each

*Before December 1994, 50-ms (acoustic) stimuli were used. In December 1994 the experimental system was updated and longer data records could be acquired. The stimulus duration and interstimulus interval were increased to 100 ms and 500 ms, respectively.*
(0–80 dB SPL, 5-dB steps). This was followed by three trials of 50- or 100-ms broadband noise bursts (10-ms delay, 250-ms interstimulus interval) at each of 17 levels (0–80 dB SPL, 5-dB steps). To collect rate versus level curves, longer-duration tone and noise bursts (200-ms duration, 1,000-ms interstimulus interval) were used, and the unit’s spike times were recorded. Stimulus level typically started at 0 dB SPL and increased in 2-dB steps.

To assess a cell’s rate-current and I-V relationships, 50-ms current pulses (10-ms delay, 250-ms interstimulus interval) were delivered through the recording electrode (16 trials, from −1.0 to 1.0 nA or from −2.0 to 2.0 nA in equal steps). To study electrotonic properties, multiple (16–100) trials of short-duration hyperpolarizing current pulses (50 ms, −1.0 nA or 0.5 ms, −5.0 nA) were injected into the cell.

After a cell was marked (see following text), data collection would resume if the neuron was still acoustically responsive. Extensive intracellular response maps were measured if possible. In such cases, tone frequency was changed in 0.1-octave steps starting at BF and then alternating above and below BF for ≤50 frequencies; seven sound pressure levels were used beginning at threshold and then increasing in 10 dB steps. Only seven levels were used because of concerns regarding limited intracellular contact.

CELL MARKING. After an initial set of data was collected, the cell was iontophoretically injected with HRP or neurobiotin using 4.0-nA, 4-Hz depolarizing current pulses (150-ms on, 100-ms off) for ≥2 min. The membrane potential was closely monitored and recorded to determine whether the electrode remained inside the cell throughout this procedure. A near constant membrane potential was interpreted as strong evidence that the cell marked was the same cell from which the physiological data were recorded. To avoid out-of-cell current leakage, the injection was stopped immediately after observing a sudden change in the measured voltage.

Data processing

CRITERIA FOR ACCEPTABLE INTRACELLULAR RECORDINGS. A recording was considered to be intracellular and acceptable if the cell’s resting potential was less than or equal to −50 mV and if the cell’s maximum action potential (AP) was ≥40 mV in amplitude. One exception was made, however, for a giant cell, since these cells are only infrequently encountered. In this case, the resting potential was less than −45 mV and its maximum AP amplitude was ≈30 mV.

DRIVEN AND SPONTANEOUS RATE ESTIMATES. Discharge rates were obtained by counting the number of APs over a specific time window. For estimating driven rates, the analysis window was either the same as the stimulus duration (for electric stimulation) or the last 80% of the stimulus duration (for acoustic stimulation). The time window used for estimating spontaneous activity rate (SpAc) was the same as that for the driven rate but positioned at the end of each trial. For example, when the tonal stimulus duration was 100 ms, the driven rate was estimated during a 80-ms period, starting 20 ms after the stimulus onset, whereas the SpAc was computed during the last 80 ms of the 250-ms trial. The only exception was when the duration of the acoustic stimulus was 50 ms, then the SpAc was estimated at 0 dB SPL during a 100-ms interval.

Rate versus sound pressure level (RSL) curves and rate versus current level (RCL) curves were constructed from the experimental data. To reduce rate variations due to the short time intervals used to estimate acoustically driven rates, the RSL curves were smoothed using a three-point filter that has the following equation:

\[ R_s(x) = \frac{R(x-1) + 2R(x) + R(x+1)}{4} \]

where \( R(x) \) and \( R_s(x) \) are the \( x \)th rate in the data array before and after filtering, respectively.

ANALYSIS OF ACOUSTIC RESPONSES: UNIT CLASSIFICATION. In classifying a unit physiologically, deviations from SpAc were used to determine excitation and inhibition (Davis et al. 1995, 1996). A driven rate that was larger (smaller) than SpAc by >2 SD of the SpAc was taken to be excitation (inhibition). In situations where SpAc was estimated at only one level (0 dB SPL), a criterion of 20% above/below SpAc was used to determine excitation/inhibition. When SpAc was zero, a driven rate >2.5 spikes/s was considered excitation.

Units were classified on the basis of the response map scheme as adapted for the gerbil by Davis et al. (1996). According to this scheme, acoustically driven units in the DCN can be grouped into type I, type I/III, type II, type III, type III-i, type IV, type IV-i, and type IV-T units. These are defined as follows: type I units have SpAc and give excitatory responses to both tone and noise stimuli. Type I/III units have SpAc rates <2.5 spikes/s, excitatory responses to BF tone and noise, and no detectable side-band inhibition (i.e., inhibition at frequencies above or below BF). Type II units also have little or no SpAc (<2.5 spikes/s), excitatory responses to BF tones, and little or no responses to noise. Type III units have SpAc rates exceeding 2.5 spikes/s, excitatory responses to BF tones and noise, and side-band inhibition; type III-i units are similar to type III units except that they are inhibited by noise at low levels and excited at higher levels (Davis et al. 1996). Type IV units are excited by near-threshold BF tones but inhibited at higher levels. They also have inhibitory side-bands and excitatory responses to noise. Type IV-i units have type IV features except that they are inhibited by noise at higher levels (Davis et al. 1996). Type IV-T units have excitatory responses at all levels for BF tones but have highly nonmonotonic BF rate versus level curves (Shofner and Young 1985). These are characterized by having BF tone-driven rates that drop below one-half of the sum of the maximum driven rate and the SpAc rate within 35 dB SPL of the peak. Their response maps actually resemble those of type III units.

ANALYSIS OF RESPONSES TO CURRENT-PULSE STIMULATION. In addition to obtaining RCL curves, responses to 50-ms current pulses also were investigated for poststimulus hyperpolarization, anode break excitation, and membrane voltage sags. Estimates of current-voltage relationship (I-V curves) were made for hyperpolarizing current pulses by measuring the steady-state voltage responses near the end of the current pulse. In cases where the amplifier bridge was not balanced (normal case), estimates of the voltage drop across the recording electrode were made by fitting the sum of three decaying exponentials to the entire voltage response. The coefficient of the fastest decaying exponential was taken to represent the electrode contribution, and this component was subtracted from the voltage response to yield the cell’s responses to the current pulses. Estimates of the cell’s input resistance were made from the slopes of these I-V curves.

TISSUE PROCESSING AND CELL RECONSTRUCTION. At least 2 h after labeling a cell, the animal was killed by administering a dose of pentobarbital sodium (60.0 mg/kg), exsanguinated by perfusion with normal saline solution and 1% sodium nitrite, and fixed with buffered mixed aldehydes. After overnight fixation at 4°C, the brain tissue was removed from the skull, embedded in gelatin/albumin, and sectioned serially with a Vibratome (series 1000) into 50-μm-thick slices. For neurobiotin, the tissues were processed overnight in Vector ABC reagent solution. The tissue slices then were processed with diaminobenzidine histochemistry (Graham and Karnovsky 1966), intensified with cobalt chloride (Adams 1981). The tissue slices were mounted on glass slides and counterstained with cresyl violet or further processed for flat-mount epoxy embedment.

Images of labeled cells were obtained using a CCD video camera (Sony, Model SSC-M256), a charge-coupled device camera (Olympus, Model BHS), and image-processing software (Image-Pro Plus, Media Cybernetics). To reveal neuronal processes spanning multiple tissues sections, camera lucida drawing techniques were used to reconstruct several representative labeled cells.
TABLE 2. Distribution of response types of intracellularly recorded DCN units

<table>
<thead>
<tr>
<th>Unit Type</th>
<th>I/III</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>IV-T</th>
<th>Weak</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple-spiking cells</td>
<td>12</td>
<td>12</td>
<td>37</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>68 (86)</td>
</tr>
<tr>
<td>Complex-spiking cells</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>11 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>12 (15)</td>
<td>12 (15)</td>
<td>39 (49)</td>
<td>5 (6)</td>
<td>2 (3)</td>
<td>9 (11)</td>
<td>79 (100)</td>
</tr>
</tbody>
</table>

Percentages are in parentheses. DCN, dorsal cochlear nucleus.

The axons of several labeled cells were found in, or leading to, the dorsal acoustic stria (DAS) and could be traced back to the soma. Away from the initial segment, axons appeared to be of fairly uniform diameter (compared with the dendrites) and would sometimes reveal periodic darkenings of the process presumably corresponding to the location of Nodes of Ranvier. When axons traversed tissue sections, sometimes only the parts of the axons entering and leaving each section would be labeled, presumably because the interruption of the myelin sheath at these points allowed the DAB reaction to occur.

CONFIDENCE OF ASSOCIATION BETWEEN PHYSIOLOGY AND MORPHOLOGY. 'Confidence of association' is a qualitative measure of the certainty that the recovered cell was the same cell from which data were recorded. The confidence of association would be high if one or both of the following conditions were satisfied: the resting potential was stable throughout the injection of HRP or neurobiotin and response properties recorded before and after iontophoresis were similar. The confidence of association would be low if the data failed to meet either criteria or if no data were available to assess these criteria. In this report, only cells with high confidence of association are discussed. Six other cells that failed to meet either of these criteria were discarded.

RESULTS

Unit incidence

In 114 experiments, 133 spiking cells were successfully impaled. Of these, 120 (90%) cells fired only simple APs (called simple-spiking cells), and 13 cells (10%) fired both simple and complex APs (called complex-spiking cells). A complex AP consists of a burst of simple spikes superimposed on a slow and transient depolarization (see Fig. 10). Intracellularly recorded nonspiking cells frequently were encountered. These nonspiking cells had much lower resting potentials (less than −80 mV) than spiking cells, and did not respond to either acoustic or electric stimulation. They were not studied further.

All simple-spiking cells were acoustically responsive, and 68 of these were classified according to the response map scheme. Table 1 shows the distribution of unit response types for these cells. Notice that type III (including type III-i) units are the most common response type encountered and type IV (including type IV-i) and IV-T units are the least common. Also included in Table 1 are 11 complex-spiking cells. Only two of these could be classified and both were type III units. The other complex-spiking cells were unclassifiable due to poor acoustic responses (high-thresholds and low driven rates). Another 52 simple-spiking cells and 2 complex-spiking cells could not be classified because of a lack of data (cells were either lost or injured during the recording).

TABLE 2. Physiological features of identified fusiform cells

<table>
<thead>
<tr>
<th>ID</th>
<th>Type</th>
<th>BF, kHz</th>
<th>RP, mV</th>
<th>AP, mV</th>
<th>SpAc, Hz</th>
<th>Iₕ, nA</th>
<th>SPL</th>
<th>Rₛ, Hz</th>
<th>θₛ, dB SPL</th>
<th>Rₛ, Hz</th>
<th>θₛ, dB SPL</th>
<th>Rₛ, Hz</th>
<th>θₛ, nA</th>
<th>Rₛ, Hz</th>
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</thead>
<tbody>
<tr>
<td>j61793-10-1</td>
<td>IV-T</td>
<td>5.66</td>
<td>−63</td>
<td>78</td>
<td>69</td>
<td>5</td>
<td>171</td>
<td>15</td>
<td>207</td>
<td>0.07</td>
<td>280</td>
<td></td>
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<tr>
<td>j90229-3-1</td>
<td>III</td>
<td>1.48</td>
<td>−62</td>
<td>78</td>
<td>5</td>
<td>15</td>
<td>173</td>
<td>35</td>
<td>207</td>
<td>0.07</td>
<td>320</td>
<td></td>
<td></td>
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<tr>
<td>j80993-3-1</td>
<td>III</td>
<td>1.7</td>
<td>−61</td>
<td>65</td>
<td>92</td>
<td>25</td>
<td>154</td>
<td>35</td>
<td>182</td>
<td>0.07</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>j92942-1</td>
<td>III+</td>
<td>2.68</td>
<td>−61</td>
<td>73</td>
<td>20</td>
<td>20</td>
<td>298</td>
<td>40</td>
<td>259</td>
<td>0.07</td>
<td>320</td>
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<td></td>
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<tr>
<td>j22795-5-1</td>
<td>III</td>
<td>2.68</td>
<td>−70</td>
<td>73</td>
<td>40</td>
<td>40</td>
<td>142</td>
<td>40</td>
<td>146</td>
<td>0.07</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>j31495-4-1</td>
<td>III</td>
<td>2.07</td>
<td>−62</td>
<td>72</td>
<td>47</td>
<td>25</td>
<td>263</td>
<td>45</td>
<td>183</td>
<td>0.07</td>
<td>240</td>
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<tr>
<td>j40595-4-1</td>
<td>III</td>
<td>14.9</td>
<td>−65</td>
<td>55</td>
<td>44</td>
<td>25</td>
<td>181</td>
<td>35</td>
<td>215</td>
<td>0.07</td>
<td>280</td>
<td></td>
<td></td>
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<tr>
<td>j60695-6-1</td>
<td>III</td>
<td>2.9</td>
<td>−58</td>
<td>62</td>
<td>32</td>
<td>25</td>
<td>160</td>
<td>50</td>
<td>102</td>
<td>0.07</td>
<td>240</td>
<td></td>
<td></td>
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<tr>
<td>j90695-8-1</td>
<td>III</td>
<td>11.21</td>
<td>−65</td>
<td>73</td>
<td>26</td>
<td>25</td>
<td>226</td>
<td>40</td>
<td>171</td>
<td>0.07</td>
<td>220</td>
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<tr>
<td>j92595-12-1</td>
<td>III+</td>
<td>2.81</td>
<td>−71</td>
<td>67</td>
<td>40</td>
<td>15</td>
<td>281</td>
<td>45</td>
<td>175</td>
<td>0.07</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>j81959-10-1</td>
<td>III</td>
<td>7.61</td>
<td>−64</td>
<td>75</td>
<td>10</td>
<td>5</td>
<td>160</td>
<td>20</td>
<td>129</td>
<td>0.07</td>
<td>180</td>
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<tr>
<td>j32695-13-1</td>
<td>I/III</td>
<td>7.15</td>
<td>−63</td>
<td>65</td>
<td>1</td>
<td>10</td>
<td>108</td>
<td>25</td>
<td>104</td>
<td>0.07</td>
<td>140</td>
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<tr>
<td>j81959-9-1</td>
<td>I/III</td>
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<td>−65</td>
<td>70</td>
<td>0</td>
<td>5</td>
<td>268</td>
<td>20</td>
<td>238</td>
<td>0.07</td>
<td>380</td>
<td></td>
<td></td>
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</tbody>
</table>

Shaded cells are discussed in detail in the text. BF, best frequency; RP, resting potential; AP, maximum action potential (AP) size; SpAc, spontaneous activity average at BF; Iₕ, threshold to BF tone bursts; Rₛ, maximum BF-driven discharge rate; θₛ, threshold to depolarizing current pulse injection; Rₛ, driven rate at 1.0-nA depolarizing current pulse input.
of injury-discharging APs. An earlier paper describes in detail the responses properties of all intracellularly recorded units that were not marked or recovered (Ding and Voigt 1997).

Acoustic response properties of identified DCN neurons

The 19 identified DCN cells, for which the confidence in the association of physiology to morphology is high, include 13 fusiform/pyramidal cells, 2 giant cells, and 4 cartwheel cells. Listed in Tables 2–4 are the response types and several physiological properties for each morphologically defined cell class. Most of the fusiform cells (10/13) have type III unit response properties, although two of these were type III-i units. A small number were associated with either type I/III (2/13) or type IV-T (1/13) unit response properties (Table 2). Both giant cells had type IV-i unit responses (Table 3). One of the fusiform cells with type III unit response properties (J22795-5-1) was a complex-spiking cell. All other fusiform/pyramidal cells and both giant cells were simple-spiking cells. All cartwheel cells were complex-spiking cells and had weak acoustic responses that were difficult to classify using the response map scheme (Table 4).

Detailed results from seven fusiform cells with different response types (2 type III units, 2 type III-i units, 2 type I/III units, and 1 type IV-T unit), two giant cells with type IV-i response properties, and two cartwheel cells with weak acoustic responses are presented. These cells were selected because of the completeness of the data record and the quality of the labeling. To emphasize the link between morphology and physiology, aspects of both are shown in each figure. Also included is a plot of the membrane potential during the marking process and/or selected responses before and after cell marking.

Fusiform/pyramidal cells with type III and III-i unit response properties

Figure 1A is a camera lucida reconstruction of a fusiform/pyramidal cell (Jd0993-3-i-1) with type III response properties. Its cell body (* in inset) is located in the DCN’s fusiform cell layer. This neuron has a well-developed apical dendritic tree that projects into the DCN’s molecular layer, reaching its surface. The apical dendrites have dendritic spines (not shown in the drawings). Basal dendrites are less elaborate or complex than the apical dendrites and project into deep DCN. Spines are not apparent on the basal dendrites. The axon does not have local branches and could be traced into the DAS.

The cell’s resting potential was $-61\, \text{mV}$, its largest APs were $61\, \text{mV}$, and its initial average spontaneous activity (SpAc) was 92 spikes/s. BF was 1.7 kHz and BF-threshold ($\theta_b$) was $\sim 25\, \text{dB SPL}$. Figure 1B shows examples of intracellular responses to 50-ms tonal and broadband noise bursts at various levels. The cell was excited by BF tones at all levels. The cell’s firing pattern and membrane behavior, however, were level dependent. At levels between 25 and 35 dB SPL, the cell showed a pause discharge pattern, and the membrane was hyperpolarized during the pause (e.g., 1.7 kHz, 35 dB SPL). At higher levels, the firing pattern became more regular. Although the membrane was not notably depolarized during the stimulus, there was a $\sim 10\, \text{mV}$ after-stimulus hyperpolarization that lasted for $>20\, \text{ms}$ (e.g., 1.7 kHz, 70 dB SPL). Side-band inhibition was seen at frequencies both above and below BF. When strongly inhibited, the cell fired an initial spike or two, followed by a silent period, during which the membrane was hyperpolarized (e.g., 2.76 kHz, 50 dB SPL). Responses to broadband noise were excitatory and the membrane behavior was similar to that at BF except that there were no pauser-like patterns (e.g., noise, 45 dB SPL). After-stimulus hyperpolarization also was present.

Shown in Fig. 1C are the cell’s RSL curves. These were derived from intracellular responses to tones of three frequencies [BF and BF $\pm 0.7$ octave] and broadband noise, with levels ranging from 0 to 80 dB SPL in 5-dB steps. The curves were smoothed by a three-point filter. The BF-driven rate increased monotonically with increasing level until saturating at $\sim 55\, \text{dB SPL}$ with a maximum discharge rate of $\sim 160$ spikes/s. Side-band inhibition was clearly seen between 30 and 60 dB SPL at the lower frequency and at levels $>30\, \text{dB SPL}$, at the higher frequency. Responses to noise were nonmonotonic with a maximum discharge rate $\sim 180$ spikes/s.

The cell was labeled by injecting HRP for $\sim 2$ min. Figure

### TABLE 3. Physiological features of identified giant cells

<table>
<thead>
<tr>
<th>ID</th>
<th>Type</th>
<th>BF, kHz</th>
<th>RP, mV</th>
<th>AP, mV</th>
<th>SpAc, Hz</th>
<th>$\theta_b$, dB SPL</th>
<th>$R_b$, Hz</th>
<th>$\theta_h$, dB SPL</th>
<th>$R_h$, Hz</th>
<th>$\theta_s$, nA</th>
<th>$R_s$, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jd0993-3-i</td>
<td>IV-i</td>
<td>8.79</td>
<td>-60</td>
<td>58</td>
<td>99</td>
<td>10</td>
<td>122</td>
<td>5</td>
<td>157</td>
<td>0.07</td>
<td>220</td>
</tr>
<tr>
<td>J22394-4-i</td>
<td>IV-i*</td>
<td>7.47</td>
<td>-47</td>
<td>34</td>
<td>74</td>
<td>10</td>
<td>111</td>
<td>15</td>
<td>213</td>
<td>0.07</td>
<td>180</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-54 ± 10</td>
<td>46 ± 17</td>
<td>86 ± 17</td>
<td>10 ± 0</td>
<td>116 ± 8</td>
<td>10 ± 7</td>
<td>185 ± 39</td>
<td>0.07 ± 0.00</td>
<td>200 ± 28</td>
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<td></td>
</tr>
</tbody>
</table>

See notes in Table 2 for a description of columns. Shading denotes cells detailed in text. * This is not a typical type IV-i unit. See the text for a detailed description.

### TABLE 4. Physiological features of identified cartwheel cells

<table>
<thead>
<tr>
<th>ID</th>
<th>Type</th>
<th>BF, kHz</th>
<th>RP, mV</th>
<th>AP*, mV</th>
<th>SpAc, Hz</th>
<th>$\theta_b$, dB SPL</th>
<th>$R_b$, Hz</th>
<th>$\theta_h$, dB SPL</th>
<th>$R_h$, Hz</th>
<th>$\theta_s$, nA</th>
<th>$R_s$, Hz</th>
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</thead>
<tbody>
<tr>
<td>jo1493-8-i</td>
<td>Weak</td>
<td>2.00</td>
<td>-72</td>
<td>58</td>
<td>0</td>
<td>40</td>
<td>67</td>
<td>&gt;80</td>
<td>8</td>
<td>0.07</td>
<td>80</td>
</tr>
<tr>
<td>j31694-6-2</td>
<td>Weak</td>
<td>6.70</td>
<td>-61</td>
<td>65</td>
<td>16</td>
<td>30</td>
<td>65</td>
<td>35</td>
<td>75</td>
<td>0.07</td>
<td>20</td>
</tr>
<tr>
<td>j72695-13-1</td>
<td>Weak</td>
<td>21.17</td>
<td>-62</td>
<td>61</td>
<td>9</td>
<td>65</td>
<td>25</td>
<td>65</td>
<td>8</td>
<td>0.07</td>
<td>40</td>
</tr>
<tr>
<td>Jo1395-5-i</td>
<td>Weak</td>
<td>5.48</td>
<td>-68</td>
<td>74</td>
<td>12</td>
<td>30</td>
<td>61</td>
<td>&gt;70</td>
<td>26</td>
<td>0.07</td>
<td>60</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-66 ± 5</td>
<td>64 ± 7</td>
<td>9 ± 7</td>
<td>41 ± 17</td>
<td>54 ± 20</td>
<td>&gt;63 ± 19</td>
<td>29 ± 32</td>
<td>0.07 ± 0.00</td>
<td>50 ± 26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See notes in Table 2 for a description of columns. Shaded cells are detailed in text. * Refers only to simple APs.
1D shows a stable resting potential measured between current pulses during the injection, indicating the electrode remained within the cell. After the cell was marked, its response properties did not change drastically. For example, Fig. 1E shows the cell’s RSL curves derived from responses to 200-ms noise bursts before and after HRP injection. The two curves are similar in that they have similar thresholds (≈15 dB SPL), reach maximum at similar sound pressure levels (≈45 dB SPL), and became slightly nonmonotonic afterward with similar slopes. Spontaneous rates were also comparable before and after the injection. In fact, this cell was held long enough after the HRP injection that extensive intracellular responses were collected. Figure 2A shows the responses from which a three-dimensional response map was derived (Fig. 2B). The V-shaped excitatory area at BF and side-band inhibition above and below BF clearly indicate a type III unit response map. The similarity of response properties before and after HRP injection provides high confidence in the association between morphology and physiology.

Figure 3A shows a camera lucida reconstruction of a fusiform/pyramidal cell (J30294-2-1) with type III-i unit response properties. The cell body (* in the inset), located in the DCN’s fusiform cell layer, has a darkly stained nucleus, but the processes were lightly stained, possibly due to intranuclear labeling. The main apical dendrites project into the molecular layer, whereas it appears that a second dendritic process was thin and unbranched, possibly indicating that the cell was undergoing some degeneration. The basal dendrites project into the deep DCN. The axon was traced out of the DCN through the DAS.

Shown in Fig. 3B are some intracellular responses of this cell to 50-ms tones and noise bursts. The cell had large, over- and undershooting APs (≈73 mV in size), and the resting potential was −61 mV. BF-tone (2.68 kHz) responses were excitatory and monotonic. Compared with the previous cell, the firing patterns were more regular (e.g., 2.68 kHz, 25 and 60 dB SPL). Side-band inhibition, though not strong, was seen at both higher and lower frequencies. Unlike the previous cell, there were no sustained hyperpolarizations seen at off-BF frequencies (e.g., 4.35 kHz, 30 dB SPL). Noise responses were excitatory and similar to BF-tone responses except that the after-stimulus hyperpolarization was stronger for noise (e.g., Noise, 70 dB SPL). Such hyperpolarization would totally suppress SpAc.

At suprathreshold levels (>20 dB SPL), the discharge rate
for BF tones quickly rose to ~300 spikes/s before starting to saturate (Fig. 3C). After collecting BF-tone responses, the SpAc increased significantly. These changes in SpAc were reflected in shifted RSL curves for off-BF stimuli. The responses to off-BF stimuli and noise were inhibitory for a range of levels and excitatory at higher levels. Although such responses at side-band frequencies are characteristic of type III units, the inhibitory responses for low levels of noise are specific for the type III-i unit subtype. This feature is more clearly seen in the RSL curve derived from responses to

**FIG. 2.** A: intracellular responses from the fusiform/pyramidal cell Jd0993-3-1 in Fig. 1 as a function of sound pressure level (from spontaneous activity to 65 dB SPL in 10 dB steps) and frequency (40 logarithmically spaced frequencies, 0.49–7.28 kHz; responses to every 3rd frequency shown in plot). Three responses at each displayed frequency and sound level shown to convey response variability. B: typical type III unit 3-dimensional response map derived from A. Data smoothed by a 9-point spatial filter.
200-ms broadband noise bursts, shown in Fig. 3E, where the stimulus levels were changed in 2-dB steps and SpAc was more accurately estimated at each sound pressure level. The cell was inhibited by broadband noise at low levels (25–40 dB SPL) but excited at higher levels; therefore it was classified as a type III-i unit.

The RSL curve, derived from responses to 200-ms broadband noise after HRP injection, was similar to the curve obtained immediately before the labeling (Fig. 3E), although shifted to the left. The preservation of such unique noise responses, together with the stable membrane potential during the labeling process (Fig. 3D), strongly indicate that the cell marked was the one recorded.

Shown in Fig. 4A is a digitized image of another fusiform/pyramidal cell (*J92595-12-1*) with type III-i unit response properties. The cell is located in the fusiform cell layer in the caudal DCN. In this plane of section, the soma is more rounded, something that is not typical. Thick branches of apical and basal dendrites extend into the molecular and deep layers, respectively. Some spines are seen at the distal parts of the apical dendrites. The axon arises from a branch of basal dendrite and does not have collaterals. Part of the axon is seen in the DAS, although it cannot be traced farther.

Shown in Fig. 4B are examples of intracellular responses to 100-ms tones and noise bursts at various frequencies and levels. The cell had a resting potential of ~71 mV, and its largest APs measured 67 mV. Responses to BF tones (2.81 kHz) were excitatory, and the discharges were regular (e.g., 20 and 60 dB SPL). A mild membrane depolarization was seen at levels ~20 dB SPL, followed by a stronger after-stimulus hyperpolarization. This long-lasting (>200 ms) hyperpolarization would suppress significantly the interstimulus activity. When inhibited at off-BF frequencies, however, there was no sustained hyperpolarization (e.g., 4.55 kHz, 65 dB SPL). The noise responses, although excitatory, were not as strong as the BF responses, and the firing patterns were less regular (e.g., noise, 60 dB SPL).

The cell responded monotonically to BF tone bursts (Fig. 4C). Starting with an initial average SpAc rate of 40 spikes/s and a BF threshold of 15 dB SPL, the discharge rate increased with level to ~280 spikes/s at 60 dB SPL, saturating with increased levels. Like other type III units, weak side-band inhibition was seen within a narrow region (15–30 dB) 0.7 octaves below BF but much stronger and wider region 0.7 octaves above BF. Unlike the primarily excitatory responses at the lower frequency, the responses at the higher frequency...
were never excitatory. The RSL curve for short-duration (100 ms) noise bursts was linear at suprathreshold levels, and the noise-driven rate did not saturate at ≤80 dB SPL. When longer duration (200 ms) noise bursts were applied in smaller steps (2 dB), the responses were inhibitory between 5 and 20 dB SPL (Fig. 4E). Such responses indicate that the cell had type III-i unit response properties.

The cell was labeled with neurobiotin. Although the membrane potential decreased from −50 to about −90 mV during the iontophoresis (Fig. 4D), there was no indication that the electrode left the cell. Thus, we conclude that the recording and labeling were confined to the same cell.

The cells of Figs. 3 and 4 are both type III-i units and are included because they show slightly different morphology and responses to BF tones and noise stimuli. These are rare units and have not been reported in other species to date. Showing two such cells does not seem to be excessive.

Shown in Fig. 5A is a camera lucida reconstruction of a complex-spiking fusiform/pyramidal cell (J22795-5-1) associated with type III unit response properties. The oval-shaped cell body was located in the fusiform cell layer. Thick trunks of apical and basal dendrites extended toward the surface of the DCN and into the deep layer, respectively. Spines (not shown) were seen on the dendrites reaching the surface. No axon was found in the vicinity of the cell body. Sections of an axon, however, were seen in the DAS.

This was the only fusiform/pyramidal cell that fired complex APs. These complex spikes were seen in addition to simple APs. Shown in Fig. 5B are some intracellular responses to 100-ms tone and broadband noise bursts. Complex APs from this cell consisted of doublet and triplet bursts of simple spikes of decreasing amplitude superimposed on a small (≤5 mV) and transient depolarization (e.g., 2.68 kHz, 25 dB SPL). Notice that many of the other single spikes also rode on small, transient depolarizations, and these spikes had undershoots. When excited by BF tones at high levels (e.g., 2.68 kHz, 65 dB SPL), the cell responded mainly with simple spikes. There was sustained depolarization during the stimulus, followed by a much stronger after-stimulus hyperpolarization as much as 30 mV below the resting potential and as long as 200 ms, during which SpAc was suppressed. When inhibited by off-BF frequencies, the membrane was heavily hyperpolarized (~20 mV; e.g., 4.35 kHz, 80 dB SPL). Responses to broadband noise were excitatory and similar to responses at BF (e.g., noise, 65 dB SPL).
The RSL curves (Fig. 5C) indicate that BF threshold was ~40 dB SPL, the highest of all the fusiform/pyramidal cells, and the maximum BF-driven rate was ~140 spikes/s, which was among the lowest for this cell type. The RSL curve for noise is similar to that for BF tones, both became slightly nonmonotonic at the highest levels. Like all fusiform/pyramidal cells with type III unit response properties, side-band inhibition for this cell was more obvious for frequencies above BF (e.g., RSL curve at BF +0.7 octave).

The membrane potential was quite stable as neurobiotin was injected into the cell (Fig. 5D), decreasing slightly with time. SpAc, however, decreased after the injection, as can be seen in the RSL curves derived from responses to 200-ms noise bursts (0–80 dB SPL in 2-dB steps) presented immediately before and after neurobiotin injection (Fig. 5E). The RSL curve after neurobiotin injection was shifted downward probably because of the decrease in SpAc. The fluctuations in the two curves were due to the bursting APs. The confidence of association between cell morphology and physiology is considered to be high because the membrane potential was flat during cell marking and because the responses before and after labeling were similar.

**Fusiform/pyramidal cells with type I/III unit response properties**

Figure 6A is a camera lucida of a fusiform/pyramidal cell (J22795-5-1) with type I/III unit response properties. The cell body was located in the fusiform cell layer. Heavy branches of spinous apical dendrites were seen extending into the molecular layer, some reached the ependyma. The basal dendrites had fewer branches and extended into the deep layer. A single axon emerged from a branch of the basal dendrites without forming collaterals. The axon was seen only in the vicinity of the cell body.

The cell had nearly no SpAc, and thus side-band inhibition of APs could not be detected. Because responses to noise were as strong as those to BF tones (7.15 kHz), the cell was classified as a type I/III unit. Samples of excitatory intracellular responses at and near BF and for noise are shown in Fig. 6B. These responses were similar in two ways. First, spike discharges occurred mainly within the first half of the stimuli. Second, a slow membrane depolarization, sometimes >10 mV, preceded most spikes. The ensuing repolarization process, however, quickly drove the membrane back toward resting potential. Finally, unlike fusiform/pyramidal cells with type III
unit responses, there was no obvious after-stimulus hyperpolarization in this cell, although the cell was strongly excited by both tones and noise bursts. Hyperpolarization was also not evident during the subthreshold stimuli.

The RSL curves for this cell are shown in Fig. 6C. For BF tones, the responses began at 10 dB SPL (threshold) and were slightly nonmonotonic. The discharge rate decreased slightly after exceeding 100 spikes/s at 25 dB SPL and then rose again to a new peak of 170 spikes/s at 80 dB SPL. Threshold for tones at BF ±0.7 octaves was much higher (40 dB SPL), and the responses were also nonmonotonic. In this case the driven rate simply decreased after reaching 95 spikes/s at 60 dB SPL. Responses to noise, however, were monotonic with a threshold of 25 dB SPL and a maximum rate of 105 spikes/s. The relative noise response, $r$, defined as the ratio of maximum noise-driven rate minus SpAc divided by maximum BF-driven rate minus SpAc (Davis et al. 1996; Young and Voigt 1982) was 0.96 for this cell.

Also shown in Fig. 6C are the RSL curves derived from intracellular responses to 100-ms tone and noise bursts applied immediately after neurobiotin injection. The labeling of the cell had almost no effect on these response properties because the RSL curves before and after the labeling were almost identical. During the labeling process, however, the membrane potential initially fluctuated and then decreased before finally showing a gradual increase (Fig. 6D). Nevertheless, these changes did not show a sudden upward shift of >50% from the resting potential. On the basis of this fact and the unchanged response properties, we conclude that there is a high confidence of the association between the morphology and the physiology of this cell.

Figure 7A is a digitized image of the other fusiform/pyramidal cell (Jo1895-9-1) with type I/III unit response properties. This cell had a typical fusiform-like cell body located in the fusiform cell layer. More apical dendrites were seen in this plane of section than basal dendrites. Unfortunately, no axon was found either near the cell body or in the DAS. Nevertheless, the cell’s identity is clear because of the soma’s location and shape and the structure of the dendrites.

Examples of intracellular responses to 100-ms tones and broadband noise bursts are shown in Fig. 7B. The resting potential was −65 mV, the AP amplitude reached 70 mV, SpAc was nil, and threshold for BF tones (9.74 kHz) was 5 dB SPL. Acoustic responses were excitatory for frequencies near BF and for noise. This cell was similar to the previous one with respect to AP features and membrane behavior. For example, there was often a slow depolarization immediately prior to the rising phase of each spike (e.g., 9.74 kHz, 20 dB SPL), and there were no after-stimulus hyperpolarizations, even after strong depolarizations and high discharge rates (e.g., 9.74 kHz,
The major difference between the two cells was that this cell’s responses were consistently stronger (spike discharge occurred throughout the stimulus). Accordingly, the cell’s monotonic RSL curves (Fig. 7C) achieved a higher maximum BF-driven rate (270 spikes/s). Noise responses were comparable with BF-tone responses but had a higher threshold and a lower maximum rate. Responses to BF – 0.7 octave tones had a much higher threshold (50 dB SPL), although the maximum rate was comparable with that at BF. Responses to BF + 0.7 octave tones were weak; the discharge rate never exceeded 35 spikes/s.

Responses to 200-ms BF tones and noise bursts were collected before and after neurobiotin injection. The resulting RSL curves are shown in Fig. 7D. These curves are very similar with only slight changes in the slopes and steady-state rate, providing strong evidence that the recordings were from the recovered cell.

**Fusiform/pyramidal cell with type IV-T unit response properties**

Figure 8A is a camera lucida of a fusiform/pyramidal cell (J61793-10-1) with type IV-T unit response properties. The cell body is located in the fusiform cell layer. A single dendritic trunk gave rise to both apical and basal dendrites (indicated by arrow head). Its axon was traced into the DAS without local branches.

Type IV-T units are distinguished from type III units by their highly nonmonotonic BF responses. Intracellular responses to 50-ms BF tones (5.66 kHz) at 10, 30, and 75 dB SPL in Fig. 8B show such nonmonotonicity. Although excited at all levels, the responses decline at midlevels (e.g., 30 dB SPL) from those at lower levels (e.g., 10 dB SPL) before increasing again at higher levels (e.g., 75 dB SPL). Membrane depolarizations were usually followed by after-stimulus hyperpolarizations, which would suppress the interstimulus activity (e.g., 5.66 kHz, 75 dB SPL and noise, 65 dB SPL). Side-band inhibition was seen at both lower and higher frequencies (e.g., 11.32 kHz, 75 dB SPL and 2.83 kHz, 25 dB SPL), and responses to noise were strictly excitatory (e.g., 65 dB SPL). Note the absence of sustained hyperpolarization when the cell was inhibited.

The RSL curve at BF is nonmonotonic with one local minimum and two local maxima (Fig. 8C). The first maximum of 170 spikes/s occurred at 10 dB SPL. The rate dropped to 69 spikes/s at 30 dB SPL before increasing again at higher levels. Note that the rate exceeded SpAc (~54 spikes/s) at all levels.

**FIG. 7.** A: digitized image of a fusiform/pyramidal cell (Jo1895-9-1). Inset: image at a lower magnification showing the location of the cell body (inside a circle) in the DCN. M, molecular layer; F, fusiform cell layer; D, deep layer. B: intracellular responses to 100-ms tones and broadband noise bursts. Numbers on top of each plot indicate stimulus frequency and level. Bottom bar indicates stimulus timing. C: RSL curves derived from intracelluar responses to 100-ms tones and noise bursts. SpAc calculated at BF. Data smoothed by a 3-point filter. D: RSL curves derived from intracellular responses to 200-ms BF-tones and noise bursts (0–50 dB SPL in 2-dB steps) before and after neurobiotin injection.
This cell is a type IV-T unit because the minimum occurred within 35 dB of the first peak and was less than half of the average of the first maximum and SpAc. At one octave below BF (BF – 1 octave), the discharge rate drops below SpAc between 20 and 30 dB SPL and then increases with increasing levels to a maximum of 170 spikes/s. One octave above BF (BF + 1 octave), however, the cell was not responsive until 45 dB SPL, when the driven rate decreased below SpAc. Responses to noise increased monotonically and exhibited sloping saturation beginning at 20 dB SPL.

The cell’s membrane potential was not monitored during HRP injection. After the injection, the SpAc decreased nearly to zero. The cell’s characteristic type IV-T RSL curve, however, remained nearly unchanged (Fig. 8C), although it was shifted downward due to the reduced SpAc. Type IV-T units were among the least common response types encountered in the decerebrate gerbil (Ding and Voigt 1997). The preservation of type IV-T unit response properties after HRP injection leads us to conclude that the cell labeled was the same cell from which data were recorded.

Giant cells with type IV-i unit response properties

Figure 9A shows a camera lucida of a giant cell (J22394-11-1) with type IV-i unit response properties. The cell’s large soma (~30 μm diam) was located in the deep DCN. Its multipolar dendrites extend isotropically in the deep DCN, and some were found well into the molecular layer. The axon was traced out of the DCN via the DAS toward the midline as far dorsal as the floor of the fourth ventricle without visible branches. This is the only cell in this study with APs <40 mV in size. Although it did not meet our criterion for an acceptable unit, the cell still is included because giant cells are among the least recorded and labeled cells in the study.

The giant cell was strongly inhibited by acoustic stimuli over a wide range of frequencies and levels, which is characteristic of type IV units. Figure 9B shows selected intracellular responses at various stimulus conditions. At BF (7.47 kHz), the responses were excitatory only for a small range of low levels (e.g., 7.47 kHz, 15 dB SPL). No depolarization was evident when the cell was excited. Inhibitory responses occurred at higher levels (e.g., 7.47 kHz, 40 dB SPL; 12.14 kHz, 35 dB SPL; and 4.35 kHz, 25 dB SPL). These responses consisted of onset spikes followed by membrane hyperpolarization, as strong as 15 mV (e.g., 12.14 kHz, 35 dB SPL). Responses to noise, however, were mostly excitatory and much stronger than the excitatory responses at BF (e.g., noise, 25 dB SPL). At higher levels, the noise responses became onset-like and inhibitory (e.g., noise, 70 dB SPL). Type IV units with this kind of noise rate versus level curve are subclassified as type IV-i.
units (Davis et al. 1996). Note there were no sustained hyperpolarizations during the inhibition by noise.

The RSL curves shown in Fig. 9C clearly show the type IV unit response properties. At BF, the excitatory response region was between 5 to 10 dB SPL, and the maximum driven rate was 110 spikes/s, indicating a weak excitation (average SpAc = 80 spikes/s; SpAc estimated at BF, 0 dB SPL). At higher levels, the driven rate dropped to zero, indicating strong inhibition. Responses to BF −0.7 octaves were similar to those at BF except the cell was excited again at very high levels. At BF +0.7 octaves, there was no excitatory region at all. The cell became responsive >30 dB SPL by quickly reducing the number of spikes to zero. Responses to noise were mainly excitatory and highly nonmonotonic. At 25 dB SPL, the noise-driven rate reached a maximum of 200 spikes/s, which is about twice the maximum BF-driven rate. The rate decreased gradually with increasing level, becoming inhibited at levels >55 dB SPL.

The giant cell was labeled with HRP, during which the resting potential slowly rose but remained negative throughout the injection (Fig. 9D). Responses to 200-ms BF tone bursts were collected for RSL curves before and after the HRP injection to assess its impact (Fig. 9E). The HRP injection did not change the cell’s SpAc or responses to BF stimuli, thus the confidence is high in associating this morphology to these response properties.

Figure 10A is a camera lucida of a second giant cell (J83095-3-1) with type IV-i unit response properties. The soma, ~25 μm in size, is located in the deep layer just below the fusiform cell layer. Several dendrites emanate from the soma in various directions, some extending into the molecular layer while others project to the deep layer. The axon originated from a dendrite rather than from the soma. Axon segments were found in the DAS.

Figure 10B shows examples of this cell’s intracellular responses to various stimuli. Responses to BF tones (8.79 kHz) were excitatory only at low levels (e.g., 8.79 kHz, 15 dB SPL). Strong depolarization and after-stimulus hyperpolarization were absent for BF excitatory stimuli. At higher levels, however, the responses consisted of onset spikes followed by sustained hyperpolarizations (e.g., 8.79 kHz, 40 dB SPL). When stimulated by either BF −0.7 octave tone bursts or noise bursts, the responses were inhibitory at low levels (e.g., 5.41 kHz, 15 dB SPL and noise, 30 dB SPL), and excitatory at high levels (e.g., 5.41 kHz, 65 dB SPL and noise, 70 dB SPL). The inhibitory responses for these stimuli at the lower sound levels
were similar to those at BF except that the inhibition seemed to last longer especially for the noise stimuli. The excitatory responses for the non-BF stimuli, however, were very different from those at BF. After an initial onset spike, a pause appeared, during which the membrane was hyperpolarized. This was followed by a burst of spikes with increasing interspike intervals. Such a discharge pattern would give rise to a pauser peristimulus time histogram. When the stimulus was completed, the membrane strongly hyperpolarized for ~100 ms. Not shown are responses to BF 0.7 octave tones, which were similar to the inhibitory responses at BF.

The RSL curves shown in Fig. 10C are highly nonmonotonic. The maximum driven rate of 122 spikes/s for BF tones was achieved within a narrow (5–15 dB SPL) excitatory region. The inhibition at higher levels was so strong that the driven rate was zero between 30 and 60 dB SPL. Some spikes occurred at still higher levels. Increases in driven rate at high levels were larger for the BF 0.7 octave tones and for noise, where the rate gradually exceeded SpAc and became excitatory. This nonmonotonic RSL curve for noise has not been seen before for type IV units. This giant cell therefore is classified as a variant of a type IV-i unit, which generally is inhibited by noise at all high levels.

The cell was labeled lightly by a brief injection of neurobiotin. Figure 10D shows the membrane voltage monitored during the injection. Approximately 5 s after the start of the injection, the electrode suddenly lost contact with the cell, as indicated by the sudden upward voltage shift. The labeled cell was most likely the same as the one from which the data were recorded because the membrane potential was stable during the initial 5 s, the injection was terminated immediately after the cell was lost, and it was the only cell labeled.

Cartwheel cells with weak acoustic responses

Figure 11A shows a camera lucida reconstruction of a cartwheel cell (Jo1493-8-1) with weak acoustic responses. The round cell body was in the fusiform cell layer, and its heavily spiny and recurving dendrites were confined to the fusiform and molecular layers. The axon originated from the cell body and ramified (not shown in the drawing) locally in the molecular and fusiform layers.

The cartwheel cell fired complex APs in combination with simple APs (Fig. 11B). Complex APs consist of bursts of simple APs, that usually increase in width and decrease in amplitude, superimposed on a slow, transient depolarization, lasting ~10–30

FIG. 10. A: camera lucida reconstruction of a giant cell (J83095-3-1). Inset: cell body (*) and its location in the DCN. B: intracellular responses of this cell to 100-ms tones and broadband noise bursts. Numbers on top of each plot indicate stimulus frequency and level. Bottom bar indicates stimulus timing. C: RSL curves derived from intracellular responses to 100-ms tones and noise bursts. SpAc calculated at BF. Data smoothed by a 3-point filter. D: RP vs. time during neurobiotin injection.
ms. Complex APs vary in appearance. For example, the depolarization could be as small as 10 mV (e.g., 1.23 kHz, 65 dB SPL) or as large as 30 mV (e.g., 2 kHz, 60 dB SPL), and the number of bursting spikes in a complex from this cell ranged from three (e.g., noise, 50 dB SPL) to five (e.g., 2 kHz, 60 dB SPL). Complex APs appeared randomly and were not acoustically responsive. Simple APs from this cartwheel cell were broader than those from pyramidal cells (average of 0.7 ms in half-size width) and had no undershoots (e.g., 2 kHz, 80 dB SPL). Simple APs outnumbered complex APs and responded weakly to acoustic stimulation at high levels, and some even rode on small (≤5 mV) depolarizations (e.g., 2 kHz, 60 dB SPL).

The RSL curves in Fig. 11C clearly indicate weak acoustic responses. The threshold for BF tones (2.00 kHz) was 35–40 dB, and the maximum BF-driven rate was 67 spikes/s, about half the lowest maximum rate for pyramidal cells. The receptive field was broadly tuned and it was difficult to find BF; i.e., for tonal stimuli spanning BF ±2 octaves, there were little differences in thresholds and maximum driven rates. SpAc was near zero, and there were virtually no responses to broadband noise.

The cell was labeled with HRP. During the labeling process, the membrane potential experienced some fluctuations (Fig. 11D). The changes, however, were small and at all times during the injection the membrane potential was up to −50 mV, indicating that the electrode remained intracellular and thus the confidence of associating this cell’s morphology to the recorded physiology is high. After labeling, however, the cell became silent and completely unresponsive.

A digitized image of another cartwheel cell (J91895-05-01) is shown in Fig. 12A. The round cell body was located in the fusiform cell layer. Thick, heavily spinous dendrites ramified within the molecular layer, some in a recurving pattern. The axon arose from the cell body and branched in the fusiform cell layer. The axon profile is not visible in this section because it is out of focus.

This cartwheel cell is similar to the previous cartwheel cell, Jo1493-8-1, in that it also fired both simple and complex APs. Figure 12B shows several examples of simple and complex APs (notice the time scale is longer in these panels). The large and overshooting simple APs had no undershoots and most of them rode on a small, transient depolarization (~10 mV, 15 ms). The complex APs, on the other hand, consisted of a burst of two to three simple spikes superimposed on larger depolarizations (20–40 mV, 30–50 ms). The size of successive simple spikes in a burst consistently decayed as the depolarization developed. The peak of the depolarization usually occurred after a burst of simple spikes (e.g., 5.48 kHz, 70 dB SPL), but also could
coincide with the last spike in a burst (e.g., noise, 70 dB SPL). Occasionally, there appeared to be a duplex of complex APs, in which two complexes partially overlapped each other (e.g., 5.48 kHz, 25 dB SPL and 3.37 kHz, 5 dB SPL).

This cell had a low average SpAc rate (12 spikes/s) and responded weakly to acoustic stimulation. The RSL curve for BF tones (5.48 kHz) showed a threshold between 25 and 30 dB SPL and a maximum driven rate of only \( \frac{60 \text{ spikes/s}}{} \); Fig. 12C). BF tones usually elicited simple APs, although sometimes, complex APs were generated. At BF –0.7 octave tones and broadband noise, the responses were even weaker with thresholds \( \geq 5–10 \text{ dB} \) higher and driven rates <50% of those at BF and comparable level. For BF +0.7 octave tones, the cell was unresponsive.

This cell was labeled by neurobiotin. As seen in Fig. 12D, the cell’s membrane potential remained negative throughout the current injection, rising slowing with time. Hence the confidence that the recorded responses are from this cartwheel cell is high. After the injection, the cell became silent and unresponsive, although the resting potential showed no further change.

**Electric response properties of identified DCN neurons**

Although responses of the identified DCN neurons to acoustic stimulation had a variety of different patterns, as shown in the preceding text, the responses of these cells to electric stimulation were less diverse both within and across cell type. Therefore the presentation of the responses to electric current is organized differently from the acoustic responses. Thus one example of intracellular current responses, the RCL, and the \( I-V \) curves derived from these, are presented for each cell type.

Figure 13A shows intracellular responses to 50-ms current pulses from a fusiform/pyramidal cell, a giant cell, and a cartwheel cell. The sudden shift in voltage seen at stimulus onset and offset is due to the voltage drop across the recording electrode. As a general feature, all identified cells were excited by depolarizing (positive values) currents and inhibited by hyperpolarizing (negative values) currents. The larger the current level, the stronger the excitation (inhibition) would be. Only simple spikes were elicited from fusiform/pyramidal cells and giant cells responding to depolarizing current pulses (Fig. 13A, left and middle). Cartwheel cells differed from these other two cell types in that high levels of depolarizing current elicited mainly complex APs (Fig. 13A, right). The response latency shortened and the shapes of the complex APs were distorted at higher current levels. Such distortion would reduce the size of the APs, making them more difficult to detect. There were no after-stimulus hyperpolarizations or anode break excitations.
from any of the cells. Sag phenomena was observed in only one cell, a fusiform/pyramidal cell with type I/III unit re-
sponse properties (Jo1895-9-1, not shown here).

Shown in Fig. 13B are the RCL curves for these three cells. The RCL curves from the fusiform/pyramidal cell and giant cell are qualitatively similar to each other: the driven rates increased monotonically with increasing current lev-
els. The threshold to current ($\theta_I$) was $-0.07$ nA for all cells (this was the smallest depolarizing current used), and the driven rate at 1.0 nA ranged from 140 to 380 spikes/s (also see Tables 2 and 3). Cartwheel cells, on the other hand, had nonmonotonic RCL curves as a result of shrinking complex-
APs at high current levels (see Table 4). Their I-V curves, however, were indistinguishable from other cell types (in-
sets in Fig. 13B). In fact, the I-V curves from all identified cells were approximately linear in the range from $-10$ to 
$-50$ mV below resting potential with input resistance rang-
ing from 16 to 117 M$\Omega$.

**DISCUSSION**

**Cell incidence**

Most of the identified cells in this study are fusiform cells (68%), followed by cartwheel cells (21%) and giant cells (10%), a 7:2:1 ratio. By comparison, Schwartz and her col-
leagues found in a morphological study of gerbil nongranule DCN cells, 4% fusiform cells, 20% cartwheel cells, and only 1% giant cells, a 4:20:1 ratio (personal communication). This anatomic study explains why encountering giant cells is such a rare event. There is still, however, a discrepancy in the ratio of fusiform/pyramidal to cartwheel cells. Complex-spiking cells (intracellular contact) and bursting-like units (extracellular contact) in fact were encountered most frequently; however, they were often either injured or lost. Although cartwheel cells are the second most common DCN cell type, second only to 
granule cells in gerbil (see preceding text) and rat (Wouterlood and Mugnaini 1984), their small size perhaps makes them more 
prone to damage by impaling microelectrodes. This may be 
why relatively few cartwheel cells were marked in this study.

**Associations between morphology and physiology**

Most of the identified gerbil fusiform/pyramidal cells are type III units, although some of them are type I/III units, type 
I/III units, and even type IV-T units. Identified giant cells are type IV units of a sort. Finally, cartwheel cells are difficult to 
classify according to the response map scheme because they have weak acoustic response properties (higher thresholds and 
lower driven rates). The confidence of these associations be-
tween cell morphology and physiology is high for all of these 
cells, as demonstrated by the examples in the preceding text. By providing records of the cells’ membrane potential while 
marking the cells and, in some cases, providing acoustic re-
sponses both before and after cell marking, we are assured that
the recovered cells are, in fact, the cells from which we recorded. Although the number of each cell type is limited, especially for giant cells, these results most likely reflect the general features of these cell populations. First, the distribution of intracellularly recorded type III units, type IV-like units, and complex-spiking cells with weak acoustic responses from a much larger sample of cells (49, 9, and 11%, see Table 1) is similar to the distribution of the identified fusiform/pyramidal cells, giant cells, and cartwheel cells (68, 10, and 21%). Second, the response characteristics of cartwheel and giant cells are fairly consistent. There is a diversity of response patterns associated with the fusiform/pyramidal cells, and this may be a reflection of the sensitivity of these neurons to particular assortments of excitatory and inhibitory inputs each cell receives. Although two fusiform/pyramidal cells were type III units and one was a type IV-T unit, these response types resemble type III units in several ways. What is remarkable is that the fusiform/pyramidal cells did not turn out to be type IV units. This was quite surprising because in cats, type IV units are associated with the fusiform and giant cell axons of the DAS (Young 1980). Davis et al. (1996) discuss various reasons why type IV units are less prevalent, and type III units are more prevalent in gerbils, compared with cat.

Finally, other physiological features of these intracellularly recorded type III units, type IV units, and complex-spiking cells with weak acoustic responses were similar to those of the fusiform/pyramidal cells, giant cells, and cartwheel cells, respectively. These features include membrane behavior, spontaneous activity, thresholds, and maximum discharge rate in response to acoustic/electric stimuli (e.g., compare Tables 4–6 of Ding and Voigt 1997 with Tables 2–4 in this study).

**Similarities and differences in responses across the cell types**

All three cell types in this study share certain membrane behaviors in response to acoustic and electric stimulation. For example, when responding to acoustic stimuli, all cell types exhibited membrane depolarization during excitation and/or hyperpolarization after excitation or during inhibition. In response to electric stimulation, all cell types were excited by depolarizing current and inhibited by hyperpolarizing current. Excitation thresholds (θₑ) were about the same for all cells (see Table 2–4).

There are also major differences across the cell types. None of the cartwheel cells and only one fusiform/pyramidal cells exhibited type IV or type IV-T unit response properties. Likewise, no giant cells fired complex APs or had type III, I/III, or IV-T unit responses. A single fusiform/pyramidal cell, however, did have complex APs, a feature common to all identified cartwheel cells. The cell, however, had relatively strong and classifiable (type III) response patterns, undershooting simple APs, and small complex APs (see Fig. 5B), whereas the cartwheel cells had weak and unclassifiable acoustic responses, simple APs without undershoots, and fired larger complex APs (see Figs. 11B and 12B). Overall, the preceding results suggest that different cell types are likely to have distinct acoustic responses characteristics. Parham and Kim (1995) reported that 20% of extracellularly recorded bursting units had strong acoustic responses and these were found deeper than the non-responsive bursting units. Extracellularly recorded (AC-coupled) complex spikes from either cartwheel cells or fusiform cells would look like bursts of action potentials without the slow depolarization. Perhaps bursters with strong acoustic responses correspond to complex-spiking fusiform cells and non-responsive bursters correspond to cartwheel cells.

Cartwheel cells also respond differently to electric stimulation than the other cell types. That is, cartwheel cells tended to discharge nonmonotonically to increasing current levels (due to distorted spikes of the complex APs at high depolarizing current levels), whereas fusiform/pyramidal cells and giant cells responded monotonically. Although a nonmonotonic RCL relationship for cartwheel cells may simply reflect the membrane properties that give rise to complex APs, for most fusiform/pyramidal cells and giant cells, a monotonic RCL curve suggests that the inhibitory behaviors of these cells to acoustic stimuli are likely the result of local intrinsic neural circuitry rather than simply the effect of membrane properties.

**Similarities and differences within individual cell types**

**MORPHOLOGY OF FUSIFORM/PYRAMIDAL CELLS.** All identified fusiform/pyramidal cells are located in the fusiform cell layer. Most of these cells have a typical fusiform-shaped cell body, although on two occasions a pyramidal shape (see Fig. 1A) and a round shape (see Fig. 4A) would be more descriptive. Apical dendrites are more plentiful than basal dendrites. They reach the surface of the DCN, close to the ependyma, and spines are found along parts of the apical dendrites distal to the cell body. Fewer basal dendrites are seen, and in many cases only a piece of the less-branched trunk is visible. Such differences in the appearances of apical and basal dendrites also were seen in fusiform/pyramidal labeled by Golgi impregnation (Schwartz et al. 1993), Lucifer yellow (Manis 1990), biocytin (Zhang and Oertel 1994), and HRP (Smith and Rhode 1985). Axons, when identified, do not have local collaterals. In most cases, axons can be traced directly to the DAS, some even farther (e.g., Fig. 3A), or found in segments in the DAS. The lack of local axonal collaterals of gerbil fusiform/pyramidal cells is consistent with results from guinea pig (Manis 1990) and mouse (Zhang and Oertel 1994) but contrary to results from cat (Smith and Rhode 1985).

There was no apparent correlation between the morphology and the response pattern among fusiform/pyramidal cells as demonstrated by the examples described in the preceding text for cells with type III (III-i), type I/III, and type IV-T unit responses. Different orientations of these cells with respect to the plane of section probably contributed to the variations in soma shapes and in the different appearances of apical and basal dendrites.

**MEMBRANE BEHAVIOR OF FUSIFORM/PYRAMIDAL CELLS IN ACOUSTIC/ELECTRIC RESPONSES.** Most of the identified fusiform/pyramidal cells fired simple APs with undershoots. This is consistent with reports by Zhang and Oertel (1994) and Manis (1990), who studied fusiform cells in slice preparations. When excited acoustically at high levels, most of our fusiform/pyramidal cells exhibited a small membrane depolarization, which usually was followed by a long-lasting after-stimulus hyperpolarization (e.g., Fig. 4B). Such membrane behavior in response to acoustic stimuli also was reported in fusiform cells of anesthetized cats (Rhode and Smith 1986b; Smith and Rhode 1985). After-stimulus hyperpolarization also was found.
in other intracellularly recorded units after responses to large depolarizing current (Ding and Voigt 1997), and may be a self-regulatory mechanism of the membrane. After-stimulus hyperpolarization, however, could also occur without an obvious preceding depolarization and strong excitation (e.g., Fig. 1B). Therefore this behavior also could be the result of some strong, sustained, and/or long-latency inhibitory inputs on these cells. When inhibited at off-BF frequencies, fusiform/pyramidal cells often were hyperpolarized during the stimulation. The sizes of these hyperpolarizations, however, were different among these cells, perhaps indicating various strengths of the side-band inhibition. It is interesting to note that neither of the type I/III fusiform/pyramidal cells exhibited hyperpolarizations at off-BF frequencies nor did they have an after-stimulus hyperpolarization after excitation (see Fig. 6B and 7B). This phenomenon was common for all unmarked intracellularly recorded type I/III units as well (Ding and Voigt 1997). It is unclear whether this was due to a disturbance in membrane regulatory mechanisms by the recording electrode or due to weakened inhibitory inputs onto cells that would otherwise have type III response properties.

One type III fusiform/pyramidal cell is distinguished from the others by firing complex-spiking APs. Fusiform/pyramidal cells with complex-spiking APs have not been reported before. The membrane behavior of this cell, however, was similar to its simple-spiking compatriots. For example, there was membrane depolarization (hyperpolarization) during the excitation (inhibition) and strong after-stimulus hyperpolarization after excitation. Complex-spiking cartwheel cells do not display these features. In addition, the complex APs of this cell differed from complex-spiking cartwheel cells by having smaller depolarizations and fewer bursts of simple spikes that were sharper and had undershoots (Fig. 5B vs. Fig. 12B). Overall, these results indicate that the cell may have similar membrane properties and/or similar pattern of synaptic inputs to other fusiform/pyramidal cells. An additional membrane mechanism probably accounts for the complex APs in this cell. Because only one such cell was recorded and labeled, it is not clear whether the cell belongs to a distinct group of fusiform/pyramidal cells. More recordings from this cell group are required.

ACOUSTIC RESPONSE TYPES OF FUSIFORM/PYRAMIDAL CELLS. The majority of recorded fusiform/pyramidal cells had type III unit response properties. Two of these cells also exhibited unique responses to broadband noise (see Figs. 3 and 4), which identifies them as type III-i units, a subclass of type III units recently found in decerebrate gerbils (Davis et al. 1996). Of the remaining cells, two had type I/III unit and one had type IV-T unit response properties. Type I/III units have very low SpAc. At off-BF tonal stimuli, there were no signs of membrane hyperpolarization (Figs. 6B and 7B) (see also Ding and Voigt 1997). Side-band inhibition, therefore could not be detected. Other than that, however, their tuning curves and responses to noise cannot be distinguished from type III units. In an intracellular recording study, a type I/III unit could possibly be derived from a type III unit. For example, if the impaling electrode alters the membrane properties and results in both a reduction in SpAc and strength of inhibition, then a type III unit would appear to be a type I/III unit.

Type IV-T units, on the other hand, may have a response map with a V-shaped excitatory region flanked by side-band inhibition and a strong, monotonic noise response (Davis et al. 1996). These are characteristics of type III units. The only difference between a type IV-T unit and a type III unit is the defining nonmonotonic RSL curve at BF for type IV-T units. Such nonmonotonicity, however, may also be seen in type III units, although to a lesser degree (e.g., unit J80295-11-1 from Davis et al. 1996).

Fusiform/pyramidal cells and giant cells are the major DCN projection neurons (Brawer et al. 1974; Kane 1977; Osen 1969). Type IV units were activated antidromically from the DAS in decerebrate cats (Young 1980), and so fusiform/pyramidal and giant cells have been hypothesized to be type IV units in decerebrate cats. In the present study, both giant cells were type IV-i units, but only 1 of the 13 fusiform/pyramidal was a type IV-T unit, which is only partially consistent with this hypothesis. Type III and type III-i unit responses (from the remainder of our fusiform/pyramidal cells) have not been attributed specifically to fusiform/pyramidal cells, although Davis et al. (1996) suggested that type III units may be the gerbil’s DCN projection neurons. This hypothesis is based on their recent extracellular single-unit study showing that the percentages of type IV and type II units are smaller, while the percentage of type III units is larger in decerebrate gerbils compared with decerebrate cats (Davis et al. 1996). They argue that type III units in gerbil are so because there is reduced inhibition to the projection cells from fewer type II units and/or weaker connections. Davis and Voigt (1997) showed that type III units in gerbil are inhibited by type II units in a way analogous to how type IV units in cat are inhibited by type II units (Voigt and Young 1980, 1990). The present study shows with no ambiguity that type III units in decerebrate gerbil DCN are fusiform/pyramidal cells.

ELECTRIC RESPONSES OF FUSIFORM/PYRAMIDAL CELLS. Fusiform/pyramidal cells respond to current injection monotonically. These results are consistent with other studies of this cell type in slice preparation (e.g., Manis 1990 in guinea pig; Hirsch and Oertel 1988a,b; Oertel and Wu 1989; Zhang and Oertel 1994 in mouse). The mean slope of the RCL curves for our fusiform/pyramidal cells, 258 ± 70 spikes/s/NA, is greater than that reported by Manis (1990) but comparable with Zhang and Oertel’s (1994) results.

Cell input resistances of our fusiform/pyramidal cells, on the other hand, were in the same range as those measured by Manis (1990) but were generally smaller than those reported by Zhang and Oertel (1994) and Golding and Oertel (1997). This difference may be the result of differences in the sizes, content, and coating of the recording electrodes. For example, electrodes used in this study were filled with 0.5 M potassium chloride together with HRP or neurobiotin, and they were beveled to reduce the impedance from >100 MΩ to between 40 and 80 MΩ before use. In contrast, Zhang and Oertel (1993a) used electrodes with larger impedances (between 120 and 250 MΩ), which presumably corresponds to smaller tips and/or to the different electrolyte used (KAc). Their electrodes also were coated with dichlorodimethyl silane, a chemical that facilitates sealing the membrane around the electrode penetration site and thus may prevent shunts, resulting in higher cell input resistances.

MORPHOLOGY OF GIANT CELLS. Both giant cells were found in the deep DCN. They have relatively large somata (>25 μm).
and multipolar dendrites extending in many directions. The giant cell axons, like those of fusiform/pyramidal cells, were seen in the DAS. In one case, the axon was traced out of the DCN through the DAS without DCN collaterals (Fig. 9A). The morphology of these cells is similar to those labeled by biocytin in mouse slice preparations (Zhang and Oertel 1993b).

**MEMBRANE BEHAVIOR OF GIANT CELLS IN ACOUSTIC RESPONSES.** Although one of the giant cells (J22394-11-1, Fig. 9) had a high resting potential (−47 mV) and smaller APs (34 mV), its membrane behavior in response to acoustic stimulation was similar to that of the other giant cell (J83095-3-1), which had a more normal resting potential and APs. Both cells fired only simple APs and showed sustained hyperpolarizations at high levels of BF-tone bursts where the cells were inhibited. Neither cell had strong membrane depolarization during BF-tone excitation.

Most APs from the ‘normal’ cell had undershoots, whereas APs from the other cell did not. Zhang and Oertel (1993b) showed several giant cells with simple APs with undershoots, which is consistent with cell J83095-3-1. This cell also had pause-like excitatory responses for a range of high level tones 0.7 octaves below BF and for noise. This was accompanied by strong after-stimulus hyperpolarization. The other cell had strong excitatory responses, but only for noise, and there was no after-stimulus hyperpolarization. These similarities and differences suggest that perhaps cell J22394-11-1 had a reduced resting potential and smaller APs because the electrode was inside a dendrite where spike-related phenomena were attenuated. Furthermore perhaps a minor injury caused by electrode impalement altered properties of the membrane ionic channels, causing a readjustment of the cell’s resting potential (and AP size), but not the acoustic response properties directly. Additional in vivo recordings and markings of giant cells are needed.

**RESPONSE TYPES OF GIANT CELLS.** Although both giant cells exhibited type IV unit responses to tonal stimuli, their noise responses were not typical of classic type IV units. One cell (J22394-11-1) was inhibited by noise at high levels and is therefore a type IV-i unit (Davis et al. 1996). The other cell (J83095-3-1), however, had a novel noise response that has not been described before. It was inhibited by noise only over a range of levels and as such showed a partial similarity to type IV-i units. Despite this difference in noise responses, the overall type IV unit character of both giant cells is consistent with the hypothesis that type IV responses are from some DCN projection cells (Young 1980).

In contrast to the nonmonotonic RSL curves, the RCL curves were generally monotonic for the giant cells. The RCL curves had similar slopes, and the driven rates at 1.0 nA were about the same, i.e., ~200 spikes/s, which yields a slope of ~200 spikes s\(^{-1}\) nA\(^{-1}\). This is comparable with the results obtained from mouse slice preparations (Zhang and Oertel 1993b). The largest input resistance of the two giant cells, however, was ~25 MΩ, which is smaller than most of the giant cells of Zhang and Oertel.

**MORPHOLOGY OF CARTWHEEL CELLS.** Labeled cartwheel cells were located either in the molecular layer or the fusiform cell layer. Cell bodies were usually round, and dendrites were confined within the two layers. A characteristic feature of rat cartwheel cells, recurring dendrites, were seen in all such identified cells (Wouterlood and Mognaini 1984). Heavy dendritic spines, another cartwheel cell feature, were observed in two darkly labeled cells (both shown in the preceding text). Axons often originated from the cell body and branched within the molecular layer and/or fusiform cell layer. These observations are consistent with the general descriptions of DCN cartwheel cells from other studies (e.g., Brawer et al. 1974; Manis et al. 1994; Oertel and Wu 1989; Schwartz et al. 1993; Wouterlood and Mognaini 1984; Zhang and Oertel 1993a), although Biocytin, used in one of the studies, usually does not label dendritic spines very well (Zhang and Oertel 1993a). One interesting finding by Manis et al. (1994) is that the recurring pattern of dendritic branches is not evident in guinea pig cartwheel cells.

**AP FEATURES AND MEMBRANE BEHAVIOR OF CARTWHEEL CELLS.** All identified cartwheel cells discharged a combination of simple and complex APs. Simple APs from cartwheel cells were the slowest (widest width, average ~0.7 ms) of all cell types and had no undershoots. There are variations in the profile of complex APs, such as the number of bursting simple spikes in a complex and the size and duration of the depolarization. These variations occurred not only from cell to cell but also within the same cell. The duration and firing pattern of cartwheel cells are Na\(^+\) and Ca\(^{2+}\) dependent (Golding and Oertel 1996). Whether spontaneously generated or in the presence of acoustic stimulation, complex APs were outnumbered by simple APs. The general profile of gerbil cartwheel cell complex APs is similar to that of mouse cartwheel cells (e.g., Zhang and Oertel 1993a) and guinea pig (Manis et al. 1994). There are, however, some differences in the physiological features of cartwheel cells among these studies. For example, Manis et al. reported that only a small portion (3/29) of the complex-spiking (cartwheel) cells also fired simple APs, whereas simple spikes were common in cartwheel cells of Zhang and Oertel (1993a), Golding and Oertel (1997), and in this study. Zhang and Oertel also reported that many simple spikes had double undershoots, which were absent in our cartwheel cells.

When presented with acoustic stimuli, none of the four cartwheel cells exhibited strong, sustained depolarizations, other than those forming complex APs. There were also no hyperpolarizations either during or after the stimuli. Such a nonacoustically active membrane suggests weak synaptic inputs from auditory sources, if any, onto cartwheel cells.

**RESPONSES OF CARTWHEEL CELLS.** Cartwheel cells responded weakly to acoustic stimuli without exception. BF thresholds were higher and the maximum BF rates were ~50% lower than those of most fusiform/pyramidal cells (Tables 2 and also 4). Usually only simple APs responded to acoustic stimuli. Complex APs appeared randomly and did not seem to be driven.

When depolarizing current pulses of low levels were injected, many cartwheel cells responded with simple spikes only. Depolarizing current pulses at high levels, however, triggered complex APs in all cases. The distortion in APs at high current levels is the main reason for the nonmonotonic RCL curves. Strong depolarizing current pulse stimuli also elicited complex APs from cartwheel cells in mouse slice preparations (Zhang and Oertel 1993a). Although their cartwheel cells had monotonic RCL curves, the maximum stimulus levels used were <1.0 nA, (<0.5 nA in many cases). Two of
our cartwheel cells have monotonic RCL curves for current levels between 0 and 0.5 nA.

On the basis of the fact that high-level depolarizing current pulses triggered complex APs primarily, the coexistence of simple and complex APs during acoustic stimulation suggests a difference in the strength of excitatory inputs. One hypothesis is that complex APs were triggered when strong excitatory inputs were integrated and the membrane potential reached well above the threshold, and simple APs were generated when weak excitatory inputs were integrated and slightly exceeded threshold. The strength of the excitatory input could be related to different synaptic contacts and proportional to the number of the excitatory synaptic inputs as well.

Cartwheel cells are thought to be inhibitory interneurons because they are labeled by glycine-like and GABA-like immunoreactivities (Adams and Mugnaini 1987; Mugnaini 1985; Osen et al. 1990). Golding and Oertel (1997) recently have shown that cartwheel cells are excited by glycinergic synaptic potentials from other cartwheel cells and that fusiform and giant cells are inhibited by such inputs. Little is known, however, about their response properties. No evidence of direct innervation by auditory nerve fibers has been reported. The dendritic spines of cartwheel cells are innervated by parallel fibers from granule cells (Osen et al. 1990; Wouterlood and Mugnaini 1984). It is likely that this input to cartwheel cells is excitatory (Manis 1989; Wouterlood and Mugnaini 1984). If this was the dominant input, the broad tuning of cartwheel cells would be due to an apparent absence of a tonotopic organization in granule cell inputs (Mugnaini et al. 1980a,b). The response to sound, if any, of granule cells remains unresolved.

In the present study, all of the cartwheel cells had high-threshold, weak responses to acoustic stimulation, which is in agreement with Zhang and Oertel’s (1993a) predication. In addition, the weak acoustic response may emphasize the importance of nonauditory input to cartwheel cells. Young et al. (1995) found that somatosensory stimulation can inhibit DCN principal neurons through interneurons other than type II units. The cartwheel cell is a potential candidate for this interneuron because it has inhibitory characteristics and, as mentioned in results and elsewhere, its axon terminates in the fusiform cell layer and in the upper region of the deep layer (Benson and Voigt 1995; Berrebi and Mugnaini 1991) where DCN principal neurons are located. Golding and Oertel (1997) report that clusters of inhibitory postsynaptic potentials (IPSPs) with temporal characteristics that are similar to complex APs are observed in recordings from both fusiform cells and giant cells. Similarly, clusters of excitatory postsynaptic potentials (EPSPs) are observed in cartwheel cells, suggesting that they too are targets of other cartwheel cells. In this study, clusters of EPSPs and IPSPs were not generally observed. Ostapoff et al. (1994) reported recording from a DCN cartwheel cell with chopper properties in an anesthetized gerbil.

ARE ALL COMPLEX-SPIKING, WEAK ACOUSTIC CELLS CARTWHEEL CELLS? The four identified cartwheel cells were all complex-spiking and responded weakly to acoustic stimuli. Of the seven other complex-spiking cells, which are reported in a separate paper (Ding and Voigt 1997), only one was a type III unit (J61793-8-), with relatively low threshold and high driven rate. The other six units had weak acoustic responses similar to those of the identified cartwheel cells. For example, there were no significant differences in resting potential, AP size, SpAc, thresholds and driven rates to acoustic/electric stimuli, and input resistances, although on average the AP size is smaller, SpAc and current-driven rates (at 1.0 nA) are higher for the six complex-spiking units (compare Table 4 with Table 6 of Ding and Voigt 1997). These differences are probably due to ‘less healthy’ membranes of some of these six units, which could not be held long enough for a successful marking. An increased discharge rate was common for many injured units.

On the basis of the similarities of physiological features of complex-spiking, weak acoustic units and those of identified cartwheel cells, we conclude that these units were likely cartwheel cells. A total of two complex-spiking cells have been classified as type III units, one from the Ding and Voigt (1997) study and the complex-spiking fusiform cell of this study. Further intracellular study is needed to determine the extent to which a subpopulation of complex-spiking fusiform cells contributes to DCN circuitry.

Functional role of the DCN

Investigators have been speculating for a long time that the DCN performs sophisticated information processing, partly because of the complexity of its architecture and neural circuitry. For example, Rhode and Kettner (1987) pointed out that DCN neurons are better suited for encoding spectral rather than temporal information. Nelken and Young (1994) suggested that DCN neurons may be responsible for extracting the notch frequencies and peaks of the pinna transfer functions. Young et al. (1995) also found that electric stimulation in the somatosensory spinal nuclei can inhibit spontaneous activity of DCN acoustic units, suggesting that DCN neurons also may be involved in coordinating or regulating some audio-somatosensory functions, such as pinna movement toward a sound source.

Although the results of this study do not directly resolve the functional role of the DCN in the central auditory pathway, they have demonstrated that morphologically distinct DCN cells can have different responses to acoustic stimuli and thus may play different roles in processing auditory information. The two distinct response patterns, type III and type IV, have been shown to be associated with the projection (fusiform/pyramidal and giant) neurons. Besides projecting to the contralateral inferior colliculus, as fusiform/pyramidal cells do, giant cells also project to the contralateral CN (Cant and Gaston 1982). It is possible that giant cells extract specific signal components needed for coordinated processing between the two sides of CN, whereas the majority of fusiform/pyramidal cells send other processed information about the acoustic environment to higher stages.

This study also has revealed the uniqueness of DCN cartwheel cells. The weak acoustic responses of these cells is consistent with a role for the DCN in processing multisensory information. Indeed, somatosensory inputs to DCN granule cells have been reported (Wright and Ryugo 1996), and these have been stimulated electrically to active the granule-cell associated circuitry of the DCN (Davis and Young 1997; Young et al. 1995). Cartwheel cells are inhibitory interneurons. Their complex APs provide a unique way to inhibit their targets, including fusiform/pyramidal cells. The processing of
nonauditory information therefore may undergo a different course than the processing of auditory information.

Because of the diversity of response types associated with fusiform/pyramidal cells and our failure, to date, to identify the neurons with either type II or classical type IV unit response properties, it is clear that additional in vivo experimentation is required.

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Present addresses: J. Ding, Guidant/CPI, 4100 Hamline Ave. North, MS E207, St. Paul, MN 55112; T. E. Benson, 5 Washington St., D5, Reading, MA 01867.

Address for reprint requests: H. F. Voigt, Dept. of Biomedical Engineering, Boston University, 44 Cummington St., Boston, MA 02215-2407.

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