Pharmacological Evidence of Inhibitory and Disinhibitory Neuronal Circuits in Dorsal Cochlear Nucleus

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INTRODUCTION

Principal cells in the dorsal cochlear nucleus (DCN) show responses to sound that are dominated by inhibitory effects (Evans and Nelson 1973; Young and Brownell 1976). For example, DCN principal cells often are excited by low-level best-frequency (BF) tones but inhibited by high-level BF tones (type IV units) (Young 1980). The current conceptual model of the DCN (Nelken and Young 1994) suggests that type IV unit properties are shaped by inputs from two inhibitory interneurons: type II units, thought to be recorded from DCN vertical cells, which inhibit for narrowband stimuli (Spirou et al. 1999; Voigt and Young 1990; Young 1980), and wideband inhibitors, thought to be recorded from onset-C neurons in the ventral cochlear nucleus (VCN), which inhibit for wideband stimuli (Nelken and Young 1994; Winter and Palmer 1995). In support of this model, immunocytochemical studies have shown that both of these interneurons are glycinergic (Doucet et al. 1999; Saint-Marie et al. 1991), and in vitro (Zhang and Oertel 1993b, 1994) and pharmacological studies (Caspary et al. 1987; Evans and Zhao 1993) have found that most inhibition in DCN principal cells is blocked by the glycine antagonist strychnine. Pharmacological blockade of GABA-α-mediated inhibition with bicuculline typically only increased the spontaneous rate of type IV units and had little effect on their responses to sound.

Several lines of evidence, however, suggest the potential for a more substantial role for GABAergic inhibition in shaping principal cell responses to sound. First, immunocytochemical studies have shown that the DCN is rich in GABA inhibitory systems and that the cell bodies and proximal dendrites of most DCN neurons are contacted by puncta that are labeled with antibodies against GABA or both GABA and glycine (Kolston et al. 1992; Moore et al. 1996; Osen et al. 1990; Saint-Marie et al. 1991). Consistent with these observations, recent in vitro studies have shown that pyramidal cells, one of the principal cell types, and cartwheel cells, an inhibitory interneuron in superficial DCN, both receive GABAergic inhibitory postsynaptic potentials (IPSPs) (Golding and Oertel 1996, 1997). Second, cartwheel cells recently have been shown to respond to sound (Davis and Young 1997; Ding et al. 1994b; Parham and Kim 1995) and make functional synapses on both types of principal cells (pyramidal and giant cells) (Golding and Oertel 1997). Because cartwheel cells receive prominent GABAergic inhibitory inputs and sometimes colocalize GABA in their synaptic terminals (Osen et al. 1990), they could mediate potent GABAergic effects on principal cells. Finally, DCN principal cells show a range of inhibitory responses varying from those that are strongly inhibited by BF tones (type IV) to those that are weakly (type IV-T) or not (type III) inhibited at BF (Ding et al. 1994a,b; Joris 1998; Young 1980). Previous studies using iontophoretic injection of inhibitory antagonists have restricted attention primarily to type IV units where glycinergic inputs appear to dominate; perhaps GABAergic inputs are more dominant in other DCN principal cell response types.

The goal of this study was to reassess the roles of both glycine and GABA in shaping the response properties of DCN principal cells. Responses were compared before and during application of their respective antagonists, strychnine and bicuculline. As in previous studies, strychnine eliminates near-BF
inhibition in type IV units, resulting in monotonic BF rate-level curves. Unexpectedly, bicuculline primarily enhances inhibition in principal cells; that is, type IV units are inhibited at lower sound levels and type III units are converted into type IV units. This enhancement of inhibition by bicuculline suggests a disinhibitory process involving GABA_2A_ action on a non-GABA_2ergic inhibitory pathway. The latter pathway is probably glycinergic and likely involves type II units and perhaps cartwheel cells because both of these unit types are disinhibited by bicuculline. Examination of the inhibitory effects of somatosensory inputs to the DCN, which have their effects by activating the superficial granule-cell associated circuitry, leads to the suggestion that superficial stellate cells are one source of the GABA_2A_ effects. These results are consistent with the hypothesis that glycinergic circuits mediate directly the signal-dependent inhibition of DCN principal cells (Caspary et al. 1987; Evans and Zhao 1993) but suggest that GABA_2ergic circuits modulate their strength.

**METHODS**

**Surgical procedures**

Experiments were conducted on 15 adult cats (3–4 kg) with infection-free ears and clear tympanic membranes. Animal use protocols were approved by the Johns Hopkins Animal Care and Use Committee (protocol CA96M481). A detailed description of the surgical procedures is provided in Young et al. (1995). Briefly, cats were premedicated with xylazine (2 mg im) and atropine (0.1 mg im) and anesthetized with ketamine (initial dose: 40 mg/kg im; supplemental dose: 15 mg/kg iv). Thereafter, core body temperature was maintained at 39°C using a regulated heating blanket. Cats were decerebrated by aspirating through the brain stem between the superior colliculus and the thalamus; anesthesia then was discontinued. The DCN was visualized by removing the skull about the nucal ridge and aspirating the underlying cerebellum. Cats were paralyzed with gallamine triethiodide (10 mg/h iv) and artificially respired at an end-tidal CO_2_ of 4%. Paralysis was never induced earlier than 4 h after the initial surgery to ensure that the decerebration was complete (as judged by lack of voluntary movements). At the end of the experiments, cats were euthanized with an overdose of pentobarbital sodium.

In some experiments, the somatosensory dorsal column and spinal trigeminal nuclei (together called MSN for medullary somatosensory nuclei) were exposed to permit orthodromic electrical activation of the superficial granule-cell circuitry in DCN (Davis and Young 1997; Young et al. 1995). Access to the MSN was obtained by enlarging the dorsal aspect of the foramen magnum (exposing the underlying obex) and removing the meninges between the skull and atlas; a thin layer of agar was placed over the exposure to prevent drying. A bipolar tungsten electrode (Micro Probe) was advanced into the MSN until manual somatosensory stimulation indicated that the receptive field of the neural activity was centered on the pinna.

**Stimulating and recording protocols**

Recordings were made in a sound-attenuating chamber. Acoustic stimuli were delivered via a calibrated electrostat speaker coupled to a hollow ear bar. All test stimuli were 200 ms in duration, gated on and off with a 10-ms rise/fall time, and presented once per second. Electrical stimuli (for MSN experiments) were applied once per second as a train of four bipolar pulses (100 μs per phase) spaced at 50-μs interpulse intervals; current amplitudes were 10 or 20 μA.

“Piggy-back” multibarreled electrodes were used in DCN to record unit activity and deliver pharmacological agents (after Havey and Caspary 1980). These electrodes were made by attaching (at a 10–15° angle) a three-barrel glass micropipette (10- to 15-μm tip) ~10–15 μm behind the tip of a platinum-iridium metal recording electrode. Two barrels of the pipette were filled with strychnine hydrochloride and bicuculline methiodide solutions (each 10 mM, pH 3.5–4.0, Sigma). The third, balancing or sum, barrel was filled with a pH-balanced buffer (pH 4.0, potassium hydrogen phthalate, CMS). The barrels were connected via silver-silver chloride wires to two microiontophoresis constant current generators (WPI, Model 260) that were used to generate and monitor retention (20 nA, electrode negative) and ejection (50 nA, electrode positive) currents.

**Experimental protocol and unit identification**

When a single unit was isolated, its BF and threshold were determined manually, and its response type was determined from responses to 200-ms BF-tone and broadband noise bursts presented across a range of sound levels, usually 100 dB in 1-dB steps. Units were classified as type IV, IV-T, III, or II using the standard criteria (e.g., Shofer and Young 1985). Units were considered to be cartwheel cells if they were located within ~600 μm of the DCN’s surface (Berrebi and Mugnaini 1991) and also showed bursts of two to four action potentials the amplitude of which decreased during the burst (so called complex-spike discharges) (Davis and Young 1997; Manis et al. 1994; Parham and Kim 1995; Zhang and Oertel 1993a). Response maps were constructed from responses to tone bursts swept across a four-octave range of frequencies, logarithmically spaced about BF; these frequency sweeps were presented at multiple sound levels, ranging from ~10 dB below to 70 dB above threshold. In some experiments, unit responses to the four-shock electrical stimulation paradigm of the MSN were acquired (100–400 trials), followed by digitization and averaging of the potential evoked at the recording site in response to the same stimulus.

Responses to stimuli were recorded before, during, and after antagonist application. After the ejection current was turned on, a minimum of 5 min was allowed for the drug concentration to build up and its effects to stabilize before data were taken; antagonist was applied continuously during data acquisition. Antagonist application was then discontinued and the cell allowed to recover, which usually required 20–30 min. In some cases, the other antagonist then was applied (or occasionally both antagonists simultaneously) and the process repeated. To ensure that response changes were due to the antagonists and not to the applied current or the low pH of the solutions, the buffer was sometimes ejected onto units.

**RESULTS**

**Effects of strychnine and bicuculline on DCN principal cells**

The effects of strychnine (n = 36) and/or bicuculline (n = 18) were studied on 41 type IV units. All units exhibited drug effects, but unexpectedly, the primary effect of each drug was different: strychnine released type IV units from inhibition, whereas bicuculline enhanced inhibition. Figure 1 shows a series of BF rate-level functions for representative type IV units before, during, and after iontophoretic application of strychnine and bicuculline. Under control conditions (thin solid lines), type IV units have highly nonmonotonic input-output functions; they give excitatory responses to low-level BF tones, but give inhibitory responses to BF tones at higher levels (starting at ~20–40 dB above threshold). In the presence of strychnine (heavy lines), the near-threshold portions of these functions were largely unaffected; however, in each case, the unit lost entirely its inhibitory responses to high-level tones with the result that the functions became monotonic. In contrast, type IV units retained their nonmonotonic rate-level
functions in the presence of bicuculline (Fig. 1, A–C; dashed lines); however, all units showed lower thresholds of inhibition (by an average of 3 dB; Fig. 9B) defined as the level at which the response declines rapidly. Some units also showed effects near threshold, for example, increased driven activity (n = 6; Fig. 1B) or lowered excitatory thresholds (n = 2; Fig. 1C). Twenty to 30 min after termination of either antagonist, the rate-level functions assumed their original nonmonotonic shapes (dotted lines). The heavy dotted line in Fig. 1D shows a case in which only buffer was ejected as a control for current and pH effects.

Both strychnine and bicuculline sometimes increased the spontaneous rate (SR) of type IV units (e.g., the heavy gray bars vs. the light gray bars in Fig. 1, A and C). The percent change in SR is plotted against the control SR for each unit in Fig. 2A. Rate changes in excess of two standard deviations of the mean are considered significant and are shown as the square symbols that lie above the dashed line. Because changes in SR often required 10 min to stabilize, units held for shorter durations are marked with crosses to indicate that their SR changes may be underestimated. Increases in SR were observed in over one-half of the units under both strychnine (19/36 cases) and bicuculline (11/18 cases) and the average increases were similar (48 vs. 55%). Interestingly, changes in SR sometimes lagged changes in rate-level shape. Examples of this phenomenon for two units in response to strychnine and bicuculline are shown in Fig. 2, B and C, respectively. Here, the unit in strychnine showed the typical release from tone-evoked inhibition and the unit in bicuculline showed the usual enhancement of tone-evoked inhibition after 5 min of drug application (dotted lines), with little change in SR (light gray bars). However, after 20 min, the rate functions shifted toward higher discharge rates (heavy lines) as the SR in both cases increased by ~50% over the original value (heavy gray bars).

Frequency response maps for type IV units before (left) and during (right) application of strychnine and bicuculline are shown in Figs. 3 and 4, respectively. These plots show discharge rate versus frequency functions at a series of sound pressure levels, given as attenuation values at the right of the plots. Excitatory areas (black fill) are defined as stimulus conditions that elicited responses consistently above SR (horizontal lines) and inhibitory areas (gray fill) are regions of rate below SR. Vertical bars indicate the units’ BFs. In control conditions (Figs. 3 and 4, A and B), type IV response maps exhibit an island of excitation around BF at levels near threshold and predominantly inhibition at higher sound levels. This inhibition has been separated into two areas (Spirou and Young 1991): a central inhibitory area (CIA), centered just below BF, that extends from just above BF downward in frequency, and an upper inhibitory sideband (UIS) at frequencies well above BF (these regions are clearly separated in Figs. 3B and 4A by small inhibitory areas). Strychnine eliminated the CIA in type IV units and largely replaced it with excitation (Fig. 3, C and D); in each of the six cases tested, the width of this excitation at 40 dB above threshold was within the Q40 values reported for auditory nerve fibers (Liberman 1978). Strychnine also reduced but did not eliminate, the UIS. In contrast, bicuculline strengthened the CIA such that the upward extent of the excitatory island at BF was reduced (Fig. 4, C at 70 dB attn and D at 90 dB attn). The discharge rate also was reduced more uniformly to zero at frequencies within and slightly beyond the original CIA (e.g., Fig. 4, C and D at 60 dB attn). In units like the ones in Fig. 4, A and C, bicuculline also tended to reduce or eliminate the UIS and enhance excitatory responses outside the CIA. Strychnine did not enhance excitation at frequencies outside the original CIA.

The largely opposite effects of strychnine and bicuculline on the response properties of type IV units to narrowband stimuli also are observed in response to wideband stimuli. Figure 5 shows rate-level functions for responses to broadband noise of four type IV units before and during strychnine and/or bicuculline application. Type IV units are usually weakly excited by noise at all suprathreshold levels, and the functions tend to be nonmonotonic (thin solid lines); the response of the unit in Fig. 5D is unusual in that the unit is inhibited by high-level noise (~5–10%) (Nelken and Young 1994).
Thus make their functions more monotonic. Conversely, bicuculline (dashed lines in Fig. 5, B–D) enhanced the high-level inhibition in type IV units, even in units already inhibited, so that the functions all became more nonmonotonic. In addition, bicuculline always noticeably increased the discharge rate at low levels, just above threshold.

The effects of antagonists on type IV-T and type III units are shown in Fig. 6, left and right, respectively. The contrasting effects of strychnine and bicuculline were observed readily in the responses of a small population of type IV-T units (n = 3) and were similar to effects in type IV units. In control conditions, type IV-T units have nonmonotonic BF rate-level functions like those of type IV units (thin solid lines in Fig. 6, A and B) except that the rate does not drop below the spontaneous rate at high sound levels. In response to strychnine (heavy lines), this nonmonotonicity was eliminated. Responses under bicuculline (dashed lines) showed lower inhibitory thresholds, a rate increase at low levels, and a decrease in rate at high levels. These effects are seen for both tones (Fig. 6, A and B) and noise (Fig. 6C).

In control conditions, type III units (n = 15) show monotonic or near-monotonic rate-level functions to BF tones (thin solid lines, Fig. 6, D–F). Strychnine (n = 7) had the usual effect of releasing the units from inhibition (heavy solid lines, Fig. 6, E and F). However, the effect was less pronounced in type III units probably because they are near their saturation rates in control conditions. The effect of bicuculline (n = 9) was dramatic as it converted type III rate functions into highly nonmonotonic curves resembling those of type IV units (heavy dashed lines in Fig. 6, D and F). For the unit in Fig. 6F, bicuculline was applied first, giving the unit a type IV rate-level curve (heavy dashed line). While the unit was still recovering from the bicuculline (dotted line), strychnine was applied, resulting in abolition of on-BF inhibition and a monotonic rate-level curve (heavy solid line). This result suggests that the bicuculline-enhanced inhibition in these units is mediated by a glycinergic interneuron.

The examples shown in Figs. 1–6 are typical of the behavior of all the type IV, IV-T, and III units encountered in this study (41, 3, and 15 units, respectively). There were no trends with BF over a range of 1.9–39.9 kHz in type IV units and 1.7–26.1 kHz in type III units.

**Effects of strychnine and bicuculline on DCN interneurons**

The effects of pharmacological manipulation were studied on two types of glycinergic inhibitory interneurons in DCN known to contact principal cells: type II units (vertical cells) (Rhode 1999; Voigt and Young 1990; Young 1980) and complex-spiking units (presumed cartwheel cells) (Manis et al. 1994; Zhang and Oertel 1993a, 1994). In total, the effects of strychnine (n = 7) and bicuculline (n = 7) were studied on eight type II-like units with zero SR: five of these units met the conservative definition of type II units described in Spirou et al. (1999), the three others had larger-than-usual noise responses but otherwise resembled and responded like type II units. Figure 7, A–C, shows BF-tone and noise rate-level functions for two type II units before, during, and after iontophoretic application of strychnine and bicuculline. Under control conditions (thin solid lines), type II units characteristically have no SR, give excitatory responses to BF tones at all sound levels, and respond weakly, if at all, to broadband noise. In the
presence of either strychnine (heavy lines) or bicuculline (dashed lines), the units showed lower thresholds to BF tones and achieved higher discharge rates. Neither drug alone or in combination (not shown) endowed type II units with SR. Both drugs also reduced the threshold and strongly enhanced type II unit responses to noise.

Comparing the two drugs, units usually exhibited greater increases in tone-driven activity under bicuculline than strychnine (median increase of 100 vs. 64 spike/s), whereas units usually exhibited greater increases in noise-driven activity under strychnine (median increase of 296 vs. 203 spikes/s). As a result, the average relative noise to tone maximum response was greater under strychnine than under bicuculline (0.75 vs. 0.65) (see Fig. 8 in Spirou et al. 1999).

Response maps for two type II units before and during strychnine and bicuculline application are shown in Fig. 7, D and E. In both cases, both agents expanded the excitatory response region of type II units, particularly toward lower frequencies. Typically, the discharge rate increased at all frequencies within the map (Fig. 7D). The only exception to this rule is shown in Fig. 7E where the rates near BF are actually slightly lower in response to strychnine.

Strychnine ($n = 5$) and bicuculline ($n = 9$) also disinhibit complex-spiking units ($n = 10$). The BF tone rate-level responses of two representative units are shown in Fig. 8, A and B, where the thin solid lines show the responses under control conditions, the heavy lines show the responses in strychnine, and the dashed lines show the responses in the presence of bicuculline. As is usual for this unit type (Davis and Young 1997; Parham and Kim 1995), both of these examples showed little or no SR and weak, variable responses to BF tones. In response to either strychnine or bicuculline, there was a decrease in unit threshold and an increase in driven rate and sometimes an increase in SR. Similar drug effects were observed on the responses of complex-spiking units to noise (Fig. 8, C and D).

**Behavior of thresholds**

The bicuculline-induced changes of the tone-evoked thresholds in type II and complex-spiking units and of the inhibitory
thresholds of type IV units are in the same direction (toward lower values), suggesting a causal relationship. To examine this relationship on a population basis, Fig. 9A shows histograms of the excitatory thresholds of type II and complex-spiking units and of the inhibitory thresholds of type IV units. Thresholds were computed relative to the lowest threshold of auditory nerve fibers at the same BF (from Miller et al. 1997) to allow the data to be combined across frequency. The histograms for type II and type IV units show extensive overlap; that is, both distributions are clustered mainly between 0 and 20 dB and have similar median values (2 at 10.5 dB for type II units and at 9 dB for type IV units). By contrast, the thresholds of complex-spiking units are distributed broadly across level and the median threshold (2 at 17 dB) for this unit type is significantly higher than that for type IV units (10 dB; P < 0.01, Mann-Whitney U test).

The histograms in Fig. 9B compare the bicuculline-induced decrease in type II (square) and complex-spiking (diamond) unit thresholds to the bicuculline-induced shift in the inhibitory thresholds of type IV units (triangle). The shifts for type II and type IV units cover roughly the same range, suggesting that disinhibition of type II units is likely to be the source of the decrease in type IV inhibitory threshold following bicuculline. The median shift exhibited by complex-spiking units is 5 dB larger than that exhibited by type IV units. This difference approximately compensates for the higher median threshold of complex-spiking units compared with type IV units before drug application, suggesting that complex-spiking units might contribute more strongly to the inhibition of type IV units after bicuculline.

**Effects of strychnine and bicuculline on granule-cell circuit mediated activity in DCN**

Two potential local sources of GABAergic inhibition within the DCN are the Golgi and stellate cells in superficial DCN (Mugnaini 1985). The superficial DCN circuitry was studied by electrically stimulating the somatosensory (MSN) inputs to DCN, which are known to contact the granule cells (Wright and Ryugo 1996) and to activate the granule-cell associated circuitry in superficial DCN (Davis and Young 1997; Davis et al. 1996b; Young et al. 1995). Figure 10 shows the typical
effects of MSN stimulation on DCN type IV and complex-spiking units. For each plot, the top panel shows the extracellular evoked potential (EP) at the recording site in the DCN elicited by a sequence of four 10- or 20-μA shocks delivered to the MSN, and the bottom panel shows a histogram of the responses (400 trials) of a single unit that was otherwise firing spontaneously. The response of the type IV unit in Fig. 10A shows three components (Davis et al. 1996b): a short-latency inhibitory component that precedes the onset of the EP (the sharp decrease in spontaneous rate before the dashed lines which mark the onset of the EPs); a transient excitatory component (bold) just after the onset of the EP; and a long-latency inhibitory component that follows the excitatory component. The response of the type IV unit in Fig. 10B shows only the third, long-latency inhibitory component. Note that the amplitude of the long-latency inhibitory response component adapts as a function of the stimulus pulse number; that is, it is largest at the first pulse, smallest at the second pulse, and then tends to increase (Davis et al. 1996b). Complex-spiking neurons are excited by MSN stimulation (Fig. 10C) and the latency and pattern of four-pulse amplitude change of this excitation are appropriate to account for the long-latency (component 3) inhibition exhibited by type IV units (Davis and Young 1997).

Figure 11 shows the responses of two DCN type IV units and one complex-spiking unit before and during pharmacological blockade. For most type IV units (30/34 cases; Fig. 11, A and C), strychnine (heavy lines) reduced or eliminated both the short- and long-latency inhibitory components and revealed the underlying excitatory component, which is presumed to reflect direct granule cell input to the principal cell. In contrast, bicuculline produces little, if any, effect on type IV unit responses to MSN stimulation (15/15 cases; Fig. 11B). Most commonly seen are small decreases in the long latency inhibitory component, particularly at the fourth pulse (curved arrow) and small increases in the excitatory component (not shown). The type IV unit shown in Fig. 11C is one of two cases where both strychnine and bicuculline were applied simultaneously. This unit showed only a long-latency inhibitory component under control conditions (thin solid line). Under strychnine, an excitatory component is revealed, and the long-latency inhibitory component is reduced slightly. Subsequent addition of bicuculline abolishes the inhibitory component entirely (dotted line). In contrast to type IV units, both strychnine and bicuculline usually produce strong release from inhibition in complex-spiking neurons (6 cases; Fig. 11D). Bicuculline increases the spontaneous and driven firing rates of complex-spiking neurons by an average of 270%; the average increase for strychnine is 170%.

**DISCUSSION**

**Nature of glycinergic and GABAergic inputs onto DCN principal cells**

The results described above show that DCN principal cell responses to sound are, for the most part, oppositely affected by the iontophoretic application of strychnine and bicuculline. In particular, strychnine eliminates the stimulus-evoked inhibition at frequencies near BF in DCN principal cells, whereas bicuculline predominantly enhances this inhibition. The effects are powerful: strychnine can convert a neuron formerly exhibiting type IV or type IV-T unit properties into one showing type III or type IV-T properties (Figs. 1, 3, 5, and 6), whereas bicuculline can change a type III or type IV-T unit into a type IV unit (Fig. 6). These latter observations have not been reported before and provide a direct demonstration that DCN principal cells can exhibit a continuum of responses dependent on the balance of their excitatory and inhibitory inputs. These data suggest that the inhibition of DCN principal cells is mediated predominantly by glycinergic inputs and that the strength (activity) of these inputs is modulated by GABAergic inputs.

Multiple lines of evidence are consistent with a larger role for glycine than GABA in shaping directly the response properties of DCN principal cells. First, immunocytochemical studies have shown that glycine-positive terminals predominate over GABA-positive terminals on the somata and proximal dendrites of pyramidal and giant cells (Kolston et al. 1992; Osen et al. 1990; Saint-Marie et al. 1991). Second, in vitro
intracellular studies in mouse have reported that most IPSPs in pyramidal and giant cells are blocked by strychnine and not bicuculline (Golding and Oertel 1996, 1997; Zhang and Oertel 1993b, 1994). Finally, the results of this and previous in vivo pharmacological studies (Caspary et al. 1987; Evans and Zhao 1993) have found that strychnine and not bicuculline releases type IV (or type IV-like) units from most of their tone-evoked inhibition.

The data are also clear on the role of GABAergic inputs as modulators of the activity of the glycinergic interneurons that contact DCN principal cells. The decreased inhibitory thresholds exhibited by type IV units (Figs. 1, 4, and 9) under bicuculline, and the conversion of the more monotonic BF rate-level functions of type III and type IV-T units into highly nonmonotonic functions typical of type IV units (Fig. 6) show that application of the GABA\textsubscript{A} antagonist enhances the strength of inhibitory inputs to these unit types, all associated with DCN principal cells (Ding et al. 1994a,b; Joris 1998; Young 1980). The example shown where strychnine converted the partially-recovered type III unit back into a type III unit (Fig. 6F) suggests that these enhanced inhibitory inputs are glycinergic. Consistent with this result, application of bicuculline releases presumed inhibitory interneurons (type II and complex-spiking units) from inhibition (Figs. 7 and 8) (Caspary et al. 1987). Type II units are known to inhibit type IV units (Voigt and Young 1980, 1990) and have been asso-

![Graphs showing effects of strychnine and bicuculline on BF-tone and noise rate-level functions of type IV-T and type III units.](image-url)
associated with vertical cells (Young 1980) which immunostain for glycine (Saint-Marie et al. 1991). Complex-spiking units are thought to inhibit type IV units (Davis and Young 1997; Golding and Oertel 1997) and have been associated with cartwheel cells (Manis et al. 1994; Zhang and Oertel 1993a), which also seem to be glycineergic despite double-labeling for GABA (Gates et al. 1996; Golding and Oertel 1997; Osen et al. 1990). In addition to vertical and cartwheel cells, the D-stellate or radiate neurons of the VCN provide inhibitory inputs to DCN principal cells (Doucet and Ryugo 1997; Oertel et al. 1990; Zhang and Oertel 1993b, 1994); these neurons are also glycine-immunoreactive (Doucet et al. 1999) and receive GABAergic input (Ferragamo et al. 1998).

Thus we are proposing that the effect of bicuculline on principal cells is primarily indirect and reflects the spread of antagonists to nearby glycinergic inhibitory interneurons, blocking their inhibitory inputs. It is worth considering which of the preceding inhibitory interneurons are likely affected by bicuculline iontophoresed near a principal cell. The distance through which molecules spread during iontophoresis is difficult to estimate accurately. However, the type II-type IV connection is strongest for type II units located near type IV units.
cartwheel cell axons spread predominantly within the cell’s isofrequency sheet (Berrebi and Mugnaini 1991; Zhang and Oertel 1993a). Thus it is possible that iontophoresis at the recording site of a type IV unit would spread to the locations of both of these interneurons. Here, the facts that the enhanced inhibition in type IV unit response maps is restricted to the CIA (Fig. 4) and that the lowering of the inhibitory thresholds of type IV units is matched by the lowering of type II unit excitatory thresholds (Fig. 9) suggest that type II units are being affected by the bicuculline applied on their target type IV neurons. Further support for this conclusion is provided by the dramatic increase in the strength of inhibitory responses to noise produced by bicuculline (Fig. 5, B–D), which matches the significant increase in noise responsiveness in type II units (Fig. 7). On the other hand, the fact that type IV units do not show increased long-latency inhibition in response to MSN stimulation (Fig. 11B), whereas cartwheel cell excitation is strongly enhanced (Fig. 11D), suggests that the bicuculline applied at a principal cell does not reach cartwheel cells. It is also unlikely that the D-stellate or radiate neurons connected to a principal cell would be affected because these neurons are in the VCN.

Although GABAergic effects on DCN principal cells seem to be mainly indirect, there is evidence for some direct inhibition of these units. For example, type IV units under bicuculline may show an enhancement in the magnitude of their near-threshold responses or lower thresholds to BF tones (Fig. 1) or noise (Fig. 5), a release from inhibition in their response maps at frequencies well above and well below BF (outside the CIA; Fig. 4), or an increase in their SR (Fig. 2). Consistent with these observations, immunocytochemical studies have shown that GABA-positive terminals exist on both pyramidal and giant cells (Kolston et al. 1992; Osen et al. 1990; Saint-Marie et al. 1991), in vitro studies have reported GABAergic IPSPs in pyramidal cells (Golding and Oertel 1997), and previous pharmacological studies have shown principal cell sensitivity to GABA (Caspary et al. 1987) or bicuculline (Evans and Zhao 1993).

Interestingly, the changes in a unit’s SR (whether under bicuculline or strychnine) occasionally lagged in time the changes in the shape of a unit’s input-output function (Fig. 2, B and C). This suggests that the driven and SR aspects of a unit’s response are controlled independently and that the inputs affecting SR are comparatively more diffuse or distant. In the case of bicuculline, immunocytochemical studies have shown that single-labeled GABA-positive terminals on pyramidal cells are usually located on more distal parts of the apical dendrites (Osen et al. 1990).
IV units, and a wideband inhibitor (WBI) (Nelken and Young 1994). The type II unit, with a BF slightly below that of the type IV unit, strongly inhibits the type IV unit to narrowband stimuli (Voigt and Young 1980, 1990) thereby producing the CIA in its response map (Fig. 4A) (Spirou and Young 1991). The WBI receives input from a wide range of BFs and is weakly driven by tones and strongly excited by noise. The WBI strongly inhibits the type II unit and weakly inhibits the type IV unit; thus it can account for the weak responses of type II units to noise (Fig. 7) (Young and Voigt 1982), the inhibitory responses of type IV units to notch noise (Nelken and Young 1994), and the UIS in type IV response maps (Fig. 4A) (Spirou and Young 1991). Computer models based on this network configuration (Blum and Reed 1998; Hancock and Voigt 1999; Hancock et al. 1997) account for all the known response properties of type IV units before this study.

The data in this study impact on this basic model in several ways. First, the model suggests that DCN type IV units receive excitatory input from a narrowly tuned array, for example, from auditory nerve fibers (ANF) and/or VCN T-stellate cells with the same BF (Oertel et al. 1990; Osen 1970; Ryugo and May 1993; Zhang and Oertel 1993b, 1994). When the CIA was eliminated in type IV units with strychnine (Fig. 3), the result-

**FIG. 9.** A: comparison of type II and complex-spiking unit BF-tone excitatory thresholds to the levels at which type IV unit BF rate-level curves exhibit their sharp decline (the so-called inhibitory threshold). Thresholds were computed relative to the lowest thresholds of auditory nerve fibers at the same BF for animals from the same supplier (Miller et al. 1997). †, median values for each group. All units encountered in the present experiments are included, regardless of whether pharmacological data were acquired; the type II and complex-spiking groups were augmented by data from Spirou et al. (1999) and Davis and Young (1997), respectively. Note the good correspondence of type II excitatory and type IV inhibitory thresholds. B: histograms of bicuculline-induced changes in type II (●) and complex-spiking unit (□) excitatory thresholds and in type IV unit inhibitory thresholds (○). †, data for 2 type II-like units with noise responses stronger than the cutoff in the conservative definition of type II units (Spirou et al. 1999). †, median threshold change for each group.

**FIG. 10.** Responses of DCN type IV (A and B) and complex-spiking units (C) to electrical stimulation at a pinna site in the medullary somatosensory nuclei (MSN). Top: evoked potential (EP) at the recording site. Bottom: peristimulus time (PST) histogram of the responses. PST histograms show the effects of the stimuli on the SR of the units in the absence of acoustic stimuli. Arrows at the top show the times of the 4 electrical pulses spaced 50 ms apart, presented once per second; current level is given above the histograms. Vertical dashed lines are placed at the onset of the EP in response to all 4 pulses. Response in A has 3 components, described in the text. Histograms were constructed from 400 repetitions of the stimulus using a binwidth of 1 ms. Unit BFs and depths are as follows: A, 14.1 kHz, 0.59 mm; B, 12.5 kHz, 0.92 mm; and C, 4.4 kHz, 0.35 mm.
ing excitatory areas were indeed as sharply tuned as single ANFs at 40 dB re threshold, consistent with the model. In contrast, Zhao and Evans (1992) reported that in chloralose-anesthetized guinea pigs the excitatory bandwidths of type IV units at 10 dB re threshold were two to three times greater under strychnine than those of single ANFs. The source of this disparity is unclear. Second, the model predicts that application of strychnine should abolish all inhibition in the responses of type IV units because both type II units (vertical cells) and the WBI (D-stellate cells) are glycinergic. Most, but not all, inhibition in type IV units is eliminated by application of strychnine (Figs. 3 and 5) (Evans and Zhao 1993). The residual inhibition at high frequencies in response maps appears to be GABAergic in origin (Fig. 4), and there appears to be a GABAergic inhibition for noise stimuli which is apparent at low sound levels (Fig. 5). Thus GABAergic inhibitory sources terminating on the type IV unit need to be added to the model. Finally, the model predicts that type II units should show greater increases in noise- than tone-evoked discharge rates under strychnine because of the loss of inhibition from the WBI. Consistent with this, the relative increase in type II unit discharge rate is greater for noise than tones by a nearly five to one margin (Fig. 7) (Spirou et al. 1999).

Several lines of evidence support two additions to the model. The first addition is a cartwheel cell. These cells in the superficial DCN have been shown to respond (weakly) to sound (Davis and Young 1997; Ding et al. 1994b; Parham and Kim 1995), to provide glycinergic inhibitory input to both principal cell types (Golding and Oertel 1997), and to have response properties appropriate to account for the third, long-latency inhibitory component in type IV unit responses to MSN stimulation (Davis and Young 1997). Consistent with the latter two observations, iontophoretic application of strychnine largely eliminated the long-latency component in the MSN responses of type IV units (Fig. 11). The role of cartwheel cells in the sound-driven responses of DCN principal cells is less clear. Compared with type II units, the excitatory thresholds of complex-spiking units do not correspond well to the inhibitory thresholds of type IV units (Fig. 9). Nevertheless, cartwheel cells are activated by sound and may contribute to the acoustically evoked inhibition of type IV units.

FIG. 11. Effects of strychnine and bicuculline on the responses of DCN type IV units (A–C) and a complex-spiking unit (D) to electrical stimulation of the MSN. Plots arranged as in Fig. 10. Thin solid lines are control responses; heavy solid lines are strychnine-affected responses; and dashed lines are bicuculline-affected responses. In C, the dotted line indicates the response of the unit under simultaneous application of strychnine and bicuculline. Strychnine blocks both the short- and long-latency inhibitory components in type IV unit responses, whereas bicuculline has little effect. Both agents enhance the activity of complex-spiking units. Unit BFs and strychnine and/or bicuculline application times are as follows: A and B, 14.1 kHz, 16 min, 16 min; C, 12.5 kHz, 23 min, 12 min (both); and D, 4.4 kHz, 10 min, 10 min.

FIG. 12. Model of the DCN circuitry; the shaded components comprise the previous model (Nelken and Young 1994). Filled ovals are excitatory connections and empty ovals are inhibitory connections. Putative glycinergic connections are heavy solid lines and GABAergic connections are dashed lines. Size of a synapse is proportional to its strength. Horizontal line at the bottom is the input tonotopic array, e.g., auditory nerve fibers. Input connections to CN units are shown originating at points corresponding to their BFs. gr, granule cells; MSN, medullary somatosensory nuclei. In this figure, the same GABAergic source is shown inhibiting type IV units, type II units, and cartwheel cells; however, there may be different and/or other GABAergic populations connected to each cell type.
The most significant change to the model is the addition of a GABAergic source, represented by the GABA cell in Fig. 12. The preceding results show that all principal cell response types, particularly type III units, exhibit strongly enhanced inhibition under bicuculline (Figs. 1, 4, 5, and 6), whereas type II (Fig. 7) and complex-spiking units (Figs. 8 and 11D) are strongly disinhibited by bicuculline. These data suggest the presence of functional GABAergic inputs on the inhibitory interneurons; this is consistent with anatomic studies reporting the presence of GABA-positive boutons on the somata and dendrites of these cell types (Osen et al. 1990; Saint-Marie et al. 1991). Direct GABAergic inhibition of principal cells is also suggested by the data in Figs. 1, 4, 5, 6, and 11, B and C, although this source seems to be weaker than the glycineergic sources. In turn, the GABAergic source needs both auditory and somatosensory inputs to produce the full range of disinhibitory effects observed in its targets. The importance of the GABAergic source in the model is clear: that is, with increasing levels of GABA input to the inhibitory interneurons, principal cells can exhibit a continuum of responses from type IV, through type IV-T, to type III properties.

Previous pharmacological studies have not reported significant effects of bicuculline on the stimulus-evoked activity of DCN principal cells (Caspy et al. 1987; Evans and Zhao 1993). Indeed, Caspary et al. (1987) reported none, whereas Evans and Zhao (1993) found that bicuculline affected primarily the SR of type IV units (although some disinhibitory effects were noted in the lateral inhibitory areas of these units). The differences among the studies likely result from the use of anesthetics, which are known to affect DCN response properties (Evans and Nelson 1973) and should particularly affect GABAergic properties (e.g., Richter and Holtman 1982), and the fact that the previous studies focused attention mainly on type IV (or type IV-like) units’ responses to sound where the effects of bicuculline are more subtle (especially if SR increases; Fig. 2C). On the other hand, Caspary et al. (1987) did find that units in deep DCN with monotonic rate-level curves (likely type II units) showed a large release from inhibition under bicuculline.

There are several potential sources of GABAergic inhibitory terminals within the CN (Kolston et al. 1992; Mugnaini 1985; Osen et al. 1990; Roberts and Ribak 1987; Saint-Marie et al. 1991) as well as commissural inputs from the contralateral CN (i.e., not all are glycineergic) (Doucet et al. 1999), and descending inputs from higher-order auditory structures, primarily the periolivary nuclei (Ostapoff et al. 1990, 1997). The possibility that the inhibitory inputs to the DCN’s principal cells and interneurons involve sources outside the DCN cannot be discounted; however, not enough is known about these inputs to evaluate their possible contributions, so they will not be considered further here. Of the local inhibitory sources, superficial stellate cells are the most likely candidate to be the GABAergic source. Stellate cells are GABA-immunopositive and are known to contact both pyramidal and cartwheel cells (Mugnaini 1985; Osen et al. 1990; Wouterlood et al. 1984); whether they contact vertical cells is unknown. In vitro studies have shown that stellate cells are spontaneously active (Golding and Oertel 1996; Zhang and Oertel 1993a), therefore blocking their activity with bicuculline could result in the increased SRs observed in principal and cartwheel cells. Further, the in vitro studies found that stellate cell responses to electrical stimulation of granule cell axons were appropriate to account for the pattern of GABAergic PSPs observed in both pyramidal and cartwheel cells. Finally, stellate cells should be activated by orthodromic activation of the granule-cell circuit via MSN stimulation. Although bicuculline does not affect the first, short-latency inhibitory component of type IV unit responses (the source of this component is still unknown), it does block some of the third, long-latency inhibitory component in their responses and disinhibits complex-spiking units (Fig. 11).

The only other known GABA-positive neurons in DCN are cartwheel and Golgi cells. However, most evidence suggests that cartwheel cells are functionally glycineergic, including the data shown here (Fig. 11) (also Golding and Oertel 1997; Zhang and Oertel 1994). Golgi cells are thought to receive input from and provide input to granule cells (Mugnaini 1985; Mugnaini et al. 1980; Osen et al. 1990). Bicuculline’s effect on the Golgi cell circuit would be to potentiate responses to MSN stimulation. This could be a factor in the strong disinhibitory effect seen in complex-spiking units (Fig. 11D) and the weak enhancement of the excitatory component observed in the responses of some principal cells.

**Functional significance**

Our results suggest that adjustment of the glycineergic and GABAergic systems in DCN allows the auditory system to control information processing within this nucleus. In particular, low levels of GABAergic activity in the DCN results in principal cells exhibiting type IV unit properties, whereas high levels of GABAergic activity results in principal cells exhibiting type III properties.

Type IV and type III responses differ in their degree of sensitivity to sharp spectral features in a stimulus. By definition, type IV neurons are inhibited strongly by narrowband stimuli (tones or narrow noise bands), whereas type III units are excited by the same stimuli. Although the difference is not as great, type IV units are more strongly inhibited by notches in the spectrum of a broadband sound than are type III units (Young et al. 1992a,b). Thus type IV units give a strong inhibitory response to the kinds of spectral features that carry information in sounds (e.g., the formants of speech or the spectral notches of head-related transfer functions). Type III units, on the other hand, appear to be sensitive primarily to spectral energy at BF (Yu and Young 1999) and thus may be involved in a rate-place representation of spectral information.

It is interesting to speculate that the basic response properties of the DCN’s principal cells are shaped over time by behavioral experience. For example in cat, a predator species with a highly mobile pinna, type IV units predominate in the DCN (Shofner and Young 1985). By contrast in gerbil, a prey species that does not appear to use pinna movements, type III units predominate (Davis et al. 1996a; Ding et al. 1994a). The possible connection between the behavioral auditory needs and experiences of an animal and the response properties of its DCN cells remains to be studied.

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