Temporal Properties of Chronic Cochlear Electrical Stimulation Determine Temporal Resolution of Neurons in Cat Inferior Colliculus

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Temporal properties of chronic cochlear electrical stimulation determine temporal resolution of neurons in cat inferior colliculus. J. Neurophysiol. 82: 2883–2902. 1999. As cochlear implants have become increasingly successful in the rehabilitation of adults with profound hearing impairment, the number of pediatric implant subjects has increased. We have developed an animal model of congenital deafness and investigated the effect of electrical stimulus frequency on the temporal resolution of central neurons in the developing auditory system of deaf cats. Maximum following frequencies (Fmax) and response latencies of isolated single neurons to intracochlear electrical pulse trains (charge balanced, constant current biphasic pulses) were recorded in the contralateral inferior colliculus (IC) of two groups of neonatally deafened, barbiturate-anesthetized cats: animals chronically stimulated with low-frequency signals (≤80 Hz) and animals receiving chronic high-frequency stimulation (≥300 pps). The results were compared with data from unstimulated, acutely deafened and implanted adult cats with previously normal hearing (controls). Characteristic differences were seen between the temporal response properties of neurons in the external nucleus (ICX; ~16% of the recordings) and neurons in the central nucleus (ICC; ~81% of all recordings) of the IC. 1) In all three experimental groups, neurons in the ICX had significantly lower Fmax and longer response latencies than those in the ICC. 2) Chronic electrical stimulation in neonatally deafened cats altered the temporal resolution of neurons exclusively in the ICC but not in the ICX. The magnitude of this effect was dependent on the frequency of the chronic stimulation. Specifically, low-frequency signals (30 pps, 80 pps) maintained the temporal resolution of ICC neurons, whereas higher-frequency signals significantly improved temporal resolution of ICC neurons (i.e., higher Fmax and shorter response latencies) compared with neurons in control cats. Furthermore, Fmax and latencies to electrical stimuli were not correlated with the tonotopic gradient of the ICC, and changes in temporal resolution following chronic electrical stimulation occurred uniformly throughout the entire ICC. In all three experimental groups, increasing Fmax was correlated with shorter response latencies. The results indicate that the temporal features of the chronically applied electrical signals critically influence temporal processing of neurons in the cochleotopically organized ICC. We suggest that such plastic changes in temporal processing of central auditory neurons may contribute to the intersubject variability and gradual improvements in speech recognition performance observed in clinical studies of deaf children using cochlear implants.

INTRODUCTION

For >20 years cochlear implants (CIs) have been used successfully in the rehabilitation of adult individuals with profound sensorineural hearing loss. Most implant users demonstrate gradual improvement in speech recognition with increasing (electrical) auditory experience (Cowan et al. 1993; Dorman et al. 1990; Dowell et al. 1992; Waltzman et al. 1992; Zwolan 1995), although the extent of improvement varies markedly among subjects and among devices. Many CI users, however, are able to understand open set speech without additional visual cues (lip reading) (e.g., Dowell et al. 1986; Doyle et al. 1991; Schindler and Kessler 1989; Spivak and Waltzman 1990; Wilson et al. 1991).

Because of the substantial benefits seen in adult CI subjects and improvements in CI technology, the number of pediatric implant subjects (including very young and congenitally deaf children) has increased dramatically over the past 10 years. An important rationale for the implantation of young children is based on data suggesting that the acquisition of speech and language in humans has a critical period (Eggermont and Bock 1986; Ruben and Rapin 1980) occurring within the first few years of life. According to this view, earlier and more extensive auditory deprivation produces greater deficits in speech and language acquisition. The greatest deficits are observed when hearing loss occurs around birth with diminishing consequences if impairment occurs toward the end of the second year (Ruben 1986). In addition, the hypothesis of a critical period for auditory system development is supported by a number of animal studies suggesting that auditory deprivation during the early postnatal period induces more profound degeneration (Blatchley et al. 1983; Coleman and O’Connor 1979; Coleman et al. 1982; Evans et al. 1983; Trune 1982; Webster 1983, 1988; Webster and Webster 1977, 1979) and reorganization in the developing auditory system (Harrison et al. 1993, 1996; Kitzes 1996; Knudsen et al. 1984; Moore and Kitzes 1985; Moore and Kowalchuk 1988; Nordeen et al. 1983; Rubel et al. 1984; Silverman and Clopton 1977).

There is some evidence indicating that early chronic electrical stimulation of the developing auditory system can ameliorate or prevent some of the detrimental effects of auditory deprivation. For example, a number of quantitative histological studies in animals have shown that chronic intracochlear electrical stimulation delays or prevents the degeneration of spiral ganglion cells that occurs after deafness (Hartshorn et al. 1991; Leake and Snyder 1994; Leake et al. 1991, 1992, 1995, 1999; Loutsteau 1987) and ameliorates degenerative changes in the cochlear nucleus resulting from deafness (Lustig et al. 1994; Matsushima et al. 1991). In addition, clinical studies in young deaf children have shown a trend for better performance in children implanted at an earlier age. Children implanted <4–6
yr of age demonstrate better speech discrimination performance than those implanted at an older age (Osberger 1995). These findings suggest that the immature auditory system might be more adaptable to or better able to interpret the electrical information provided by CIs. However, relatively little is known about the encoding of electrical stimuli in the central auditory system. Moreover, even less is known about the functional consequences of this highly artificial stimulation on the developing auditory system and potential changes in signal processing which may underlie individual differences and gradual changes in speech discrimination performance in young CI subjects.

It is widely accepted that the temporal properties of the electrical stimulation pattern play a critical role in the transfer of information that enables CI subjects to recognize and discriminate speech. Moreover, it has been suggested that temporal resolution is critical for the perception of temporal pitch, prosody, and speech (Eddington et al. 1978; Shannon 1983, 1985, 1992; Townsend et al. 1987; Wilson et al. 1991). These observations suggest that the manner in which central auditory neurons encode the temporal information of the electrical stimulus is a particularly important aspect of the physiological response that may provide specific clues as to how experience with electrical stimulation affects central auditory processing.

To address these issues, the present study has focused on the temporal response properties of IC neurons in an animal model of congenital or early acquired bilateral deafness. Neonatally deafened animals were implanted with a round window or scala tympani electrode and chronically stimulated. After chronic stimulation, each animal was anesthetized, and the contralateral IC was mapped using tungsten microelectrodes. In these acute electrophysiological studies, the temporal resolution of single neurons was estimated by quantifying the onset latencies and the ability of IC neurons to follow repetitive signals (pulse trains of increasing frequency). The shorter the latencies and the higher the repetition rate that a neuron can follow (Fmax), the greater is its temporal resolution.

In a previous report Snyder and colleagues (1995) investigated the ability of IC neurons to follow electrical pulse trains of increasing frequency. They demonstrated that 1) the temporal resolution of IC neurons responding to electrical stimulation was comparable with that observed with acoustic stimulation, 2) the maximum frequency following rates of IC neurons to electrical signals are within the range of the psychophysically estimated temporal resolution of normal hearing and cochlear implant subjects, and 3) the temporal response properties of IC neurons were only marginally decreased by complete auditory deprivation, but temporal resolution was significantly enhanced by chronic electrical stimulation of the developing auditory system.

The present study extends the investigations of Snyder and colleagues and focuses on three important additional objectives: first, previous (acoustic and electrical) studies of temporal resolution in the IC either did not distinguish between the responses of external nucleus (ICX) versus central nucleus (ICC) neurons (Snyder et al. 1995) or were focused exclusively on the response properties of neurons in the ICC (e.g., Langner and Schreiner 1988). In view of differences in the physiological response properties between ICX and ICC neurons (e.g., Aitkin et al. 1975, 1994), an important goal of the present study was to characterize the temporal response properties of neurons separately for the two nuclei.

Second, from previous studies it is not clear whether the capacity of the developing auditory system for functional plasticity to electrical stimuli is dependent on the frequency of the peripheral stimulation. Because newer CI speech processing strategies use increasingly higher stimulation rates, a major focus of the present study was to investigate the effect of chronic stimulation using electrical signals with different temporal characteristics (e.g., low-frequency unmodulated pulse trains vs. higher-frequency amplitude-modulated pulse trains) on the temporal resolution of IC neurons. One hypothesis addressed was that higher stimulus frequencies (≥500 pps) were temporally more challenging for the auditory system and would result in increased temporal resolution of IC neurons.

A third objective of the present study was to determine whether Fmax and response latencies of IC neurons are related systematically to the tonotopic frequency organization. Acoustic studies have shown that the characteristic frequencies (CF) of ICX neurons are usually easy to define although units are broadly tuned (e.g., Aitkin 1978). However, there is only a vague indication in the published literature that the CF of ICX neurons are tonotopically organized (Aitkin et al. 1994; Binns et al. 1992). In contrast, it is well known that the ICC exhibits a precise cochleotopic frequency gradient that is related systematically to ICC depth in normal hearing cats (e.g., Brown et al. 1997; Merzenich and Reid 1974; Oliver 1987; Oliver and Morest 1984; Rose et al. 1966). ICC depth also is related systematically to the intracochlear electrode location in deafened, implanted cats (Snyder et al. 1990, 1991, 1995). An hypothesis addressed in the present study is that chronic stimulation primarily affects neurons in the high-frequency region of the central nucleus, i.e., neurons that normally encode higher-frequency auditory signals (Snyder et al. 1995). Alternatively, electrical stimulation might alter responses uniformly in the entire population of neurons throughout the ICC.

The systematic characterization of changes in the temporal response properties of IC neurons after chronic electrical stimulation should provide a better understanding of the efficacy of this highly artificial mode of stimulation to modify signal processing capacity and the overall functional status of the developing auditory system.

**METHODS**

**Deafening, implantation, chronic stimulation**

Experiments were conducted in 29 adult cats: Fifteen animals were neonatally deafened and chronically stimulated (“stimulated cats”). Fourteen cats were acutely deafened, previously normal adults that served as control animals. These animals were deafened and implanted on average 2 wk before the final electrophysiological experiment.

Procedures of deafening, implantation, chronic stimulation, surgical preparation, and recording techniques in the physiological experiment have been described in detail in previous reports (Snyder 1990, 1991, 1995). All procedures followed National Institutes of Health guidelines for care and use of laboratory animals.

To briefly summarize: kittens were deafened neonatally by daily intramuscular injections of neomycin sulfate at a dosage of 50–60 mg/kg body wt beginning 24 h after birth and continuing for a total of 16–25 days (Table 1). It is reported that acoustic thresholds in kittens...
at 1 wk postnatally are ~120 dB, subsequently improving ~10 dB per day and achieving adult-like levels at ~20 days postnatally (Brugge 1992; Walsh and McGee 1986). At 16–18 days of age, auditory brain stem responses (ABRs) to clicks (200 μs/ph, 20 pps) and 500-Hz tone-evoked frequency following responses (FFRs) were measured under ketamine/acepromazine tranquilization. Profound hearing loss was confirmed by the absence of responses to both stimulus conditions up to equipment limits (~108 dB SPL). If acoustically evoked responses still were present, the administration of neomycin was continued until thresholds were 2–3 h after deafening until thresholds were >108 dB SPL. Neomycin was injected (25–100 mg/kg). ABRs and FFRs were monitored for 2–3 wk as a minimum from 60–100% from the cochlear base which is equivalent to 6.5 mm or 16.1 kHz. Chronic stimulation of the neonatally deafened cats was delivered by the apical electrode pair 1,2.

A monopolar round window electrode was used for chronic stimulation in two animals (K71 and K76) and consisted of a single Teflon-coated platinum-iridium wire ending in a 400-μm ball-shaped contact near the round window. The reference contact for this monopolar electrode was an identical electrode located beneath the temporalis muscle. During the acute physiological experiment the monopolar electrode was replaced by an intracochlear implant to allow the recordings of responses to intracochlear stimulation.

Chronic stimulation of the animals was delivered 4 h/day, 5 day/wk for periods of 9–46 wk until 1–3 days before the acute electrophysiological study. To study the effects of temporally different stimuli on the temporal resolution of IC neurons, the chronically stimulated animals were divided into “low”- and “high”-frequency stimulation groups. The choice of the stimulus frequencies for each group was based on previous studies with acoustic and electrical stimulation of the cochlea that have shown that the majority of IC neurons followed stimulus frequencies up to ~100 Hz (e.g., Batra et al. 1989; Langner and Schreiner 1988; Snyder et al. 1995), and only a small percentage of neurons followed frequencies up to or >300 Hz (e.g., Batra et al. 1989; Rees and Möller 1987; Snyder et al. 1995). Therefore the group of low-frequency-stimulated animals (n = 5) was stimulated chronically with unmodulated pulse trains of 30 or 80 pps, i.e., with frequencies that are well below the average maximum following rate of IC neurons (~100 pps). All stimuli were capacitively coupled, charge balanced, biphasic square-wave pulses (0.2 ms/phase), and the intensity was set at 2 dB above the electrically evoked auditory brain stem response (EABR) threshold. The duration of chronic stimulation for these animals was on average 16.2 ± 5.9 (SD) wk. During the

<table>
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<tr>
<th>Cat</th>
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<th>Age at Surgery, wk</th>
<th>Age at Initial Stimulus, wk</th>
<th>Stimulus Current, μA</th>
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<td>300/30, Beh.</td>
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*Round window electrode. **Beh., behavioral training. †SP, analogue speech processor. ‡300/30, 300 pps amplitude modulated at 30 Hz.
period of chronic stimulation, one animal in this group (K83) received additional behavioral training with low-frequency signals (80 pps). Behavioral training (cf. Beitel et al. 1999) was performed once per day, 5 day/wk for a period of 3.5 wk. The total duration of supra-threshold stimulation per training session was on average ~20 s and therefore very short compared with the duration of chronic (passive) stimulation (4 h/day).

The group of animals with higher-frequency stimulation (n = 10) was stimulated chronically with frequencies at or above the previously estimated maximum frequency following capacity of IC neurons (~300 pps). This group included five animals that received chronic stimulation with 100% sinusoidally amplitude modulated pulse trains (300 pps carrier/30 Hz AM; Table 1). The other five animals in the higher-frequency group were stimulated chronically with an analogue processor that transduced environmental sounds. The frequency spectrum of the analogue stimulation was band-pass filtered from 250 Hz to 3 kHz with a roll-off at the shoulder frequencies of 6 dB/octave.

Chronic stimulation with 100% sinusoidally amplitude modulated pulse trains between 2 and 1,000 pps and/or with AM pulse trains (carrier frequency: 100–500 pps, modulation frequency: 8–30 Hz). The periods of behavioral training for these animals ranged between 5 wk (K91) and 26.6 wk (K86). Again, with an average suprathreshold stimulus duration of ~20 s per training session (1 training session/day, 5 day/wk), the stimulation during the behavioral training was very limited as compared with the chronic passive stimulation (4 h/day at suprathreshold level).

All stimuli were delivered using an optically isolated, constant current stimulator (Vureck et al. 1981). EABRs were determined after implantation and consecutively at intervals of 3–4 wk, and the stimulus level was adjusted if necessary. The stimulation histories of all cats are presented in detail in Table 1.

Acute electrophysiological experiment

Anesthesia was induced with ketamine/acepromazine and maintained with pentobarbital sodium (see Deafening, implantation, chronic stimulation). During the entire final physiological experiment, the cats regularly received dexamethasone sodium phosphate (1 mg/kg every 12 h sc) and mannitol (1–2 g/kg iv, as required) to prevent brain edema, atropine (0.045 mg/kg every 12 h sc) to decrease salivation, and prophylactic antibiotics (cefazolin 22–33 mg/kg every 8 h sc). The temperature of the animal was monitored continuously with a rectal temperature probe and kept constant at 37.5°C by a feedback-controlled heating water blanket. Reflexes were tested regularly, and oxygen saturation and heart rate were monitored continuously.

After sedation, the animals head was stabilized in a mouth bar head holder (KOPF). The right temporalis muscle was reflected, and a craniotomy was performed in the right parietal bone just anterior to the tentorium. The occipital cortex was aspirated, and an opening was made in the tentorium to provide access to the dorsal and dorsolateral surface of the right inferior colliculus.

Parylene-coated tungsten microelectrodes (impedances of 0.8–1.5 MΩ at 1 kHz) were used to record neuronal responses from single IC neurons. To minimize electrical artifact, the responses were recorded differentially using two microelectrodes matched in impedance. The reference electrode was positioned in the surrounding tissue (e.g., in the cerebellum or the cerebrospinal fluid), and the active recording electrode was mounted on a remotely controlled hydraulic microdrive (KOPF), which was held in a micromanipulator. The trajectory of the recording electrode was in the coronal plane and tilted laterally at an angle of ~45° from the vertical plane. The electrode was advanced in the IC from dorsolateral to ventromedial orthogonal to the orientation of the cochleotopically arranged isofrequency laminae (Brown et al. 1997; Oliver and Morest 1984). On this axis, the electrode passed through the full range of frequencies represented in the IC. Moreover, penetration depth corresponded to relative CF (Rose et al. 1966), with low frequencies represented most superficially within the central nucleus of the IC and progressively higher frequencies at progressively deeper sites.

Single-neuron responses were recorded at any location along the penetration where they could be isolated. Neural activity was band-pass filtered (10 Hz to 10 kHz) and amplified (total amplification: 100,000 times) using a battery-powered preamplifier (DAM 50) and a second-stage amplifier (Tektronix 3A90). The activity was displayed on an oscilloscope (Tektronix 565). Spike activity was isolated from background noise and artifact with a window discriminator (BAK-DIS-1). The number of spikes and the time of occurrence of each response after the stimulus onset were stored in an IBM PC and displayed on a monitor.

Biphasic square-wave pulses of nonalternating polarity were used as a search stimulus to isolate single neurons. Threshold levels for single neuron responses to pulses (0.2 ms/ph, 3–10 pps) were determined audiovisually. The stimulus intensity then was set at 2–6 dB above threshold, and single-neuron responses to pulse trains of increasing frequency (beginning at 10 pps, increments between 5 and 20 pps) were recorded until the neuron responded to the stimulus with only an onset response. The duration of the recording window was 320 ms after stimulus onset followed by an interstimulus interval of 1,000 ms. Responses were collected for 20 repetitions of each stimulus condition, and poststimulus time histograms (PSTHs, Fig. 2) were constructed (binwidth 33.3 μm) during the recording.

For each isolated neuron, the maximum stimulus frequency to which the neuron responded in a synchronized manner was determined. This was accomplished by constructing period histograms for the entire recording window (320 ms) excluding the onset response and determining whether the phase locked response at a given stimulus frequency was significant (P < 0.01, Rayleigh test) (Mardia 1972). Neurons with maximum following frequencies <10 pps were excluded from this study. In addition, first spike latencies were measured using stimuli of 20 pps. Neurons with latencies <4.5 ms were excluded from further analysis because their responses could be confused with the synchronized afferent input.

RESULTS

The primary goal of the following analyses is to compare the effects of low-frequency (30 pps, 80 pps) and temporally challenging higher-frequency (~300 pps) stimulation on the temporal resolution (maximum following frequencies, first-spike response latencies) of IC neurons. In addition, neurons were classified as belonging to either the ICX or the ICC, and the differences in temporal response characteristics of the two nuclei are described.

Differentiation of ICX and ICC recording locations

To relate the temporal response properties of IC neurons to a given subdivision of the IC, minimum response threshold levels for three cycles of a 100-Hz sinusoidal signal (Kiang and Moxon 1972) and for pulses (0.2 ms/ph, 3–10 pps) were determined for either single- or multiple-neuron responses at intervals of 100 μm along each penetration. Thresholds were plotted as a function of IC depth to obtain a spatial tuning curve (STC) (Snyder et al. 1990) (Fig. 1). A typical spatial tuning curve has a W-shape with two locations of minimum threshold,
For each group of animals, the ranges of recording depths for ICX and ICC neurons were documented. Neurons from incompletes STCs were included as ICX neurons if their recording depth was less than 1,354 μm and included as ICC neurons if their recording location was greater than 1,880 μm. Neurons recorded between 1,355 and 1,880 μm were not assigned to either nucleus and were excluded from the analyses.

Maximum following frequencies (Fmax)

Figure 2 illustrates examples of PSTHs reconstructed for two single neurons responding to intracochlear electrical stimulation with pulse trains (0.2 ms/ph, biphasic pulses) of increasing frequencies. The number of spikes per pulse (normalized spike rate) and the vector strength are noted at the top right of each histogram. The frequency was increased stepwise, and responses were recorded until each neuron ceased to respond to the sustained stimulus or produced only an onset response. Period histograms were used to determine the phase-locked capacities of neurons to given frequencies. The maximum frequency to which the neurons responded in a synchronized manner ($P < 0.01$) was assessed and referred to as the maximum following frequency (Fmax). Figure 2A shows the responses of a neuron with low temporal resolution. Fmax of this neuron was about 35 pps; it responded only to the onset of the stimulus at higher frequencies ($\geq 50$ pps). In contrast, Fig. 2B illustrates an example of a neuron with high temporal resolution. It clearly responds vigorously at much higher pulse rates, and the Fmax of this neuron was about 320 pps.

In the present study, the responses of 676 single neurons to pulse trains of increasing frequencies were recorded in the IC of 254 neurons in 29 cats. Fmax for a total of 254 neurons were determined in 14 control animals, Fmax for 104 neurons were studied in 5 animals that had been stimulated chronically with low-frequency signals, and Fmax for 296 neurons were evaluated in 10 animals that had been stimulated with high-frequency signals. As assessed by the spatial tuning curves constructed for each penetration (Fig. 1), about 16% of all recording sites (a total of 108 single neurons) were located in the ICX, and about 81% (a total of 546 single neurons) were located in the ICC. In total, 22 neurons (3% of the number recorded) were excluded from this analysis because they could not be classified as either ICX or ICC neurons.

**Quantitative distribution of Fmax.** The means and standard deviations of Fmax estimated for the individual animals within each group are presented in Table 2. Figure 3 illustrates the distributions of Fmax of all IC neurons for the three experimental groups. Separate distributions are shown for neurons in the ICX (●) and those in the ICC (□). In each histogram, the mean Fmax for the two nuclei is indicated (†). The statistical comparisons of mean Fmax for the different experimental groups and the two nuclei (t-test) are summarized in Table 3.

Generally, Fmax from ICX neurons in all three experimental groups covered a broad range of frequencies. While the lower frequency limits were roughly equal for both ICX and ICC neurons, the distributions of Fmax for the ICC neurons extended to higher frequencies. In all three groups, responses of neurons in the ICX had significantly lower Fmax than those in the ICC (all $P < 0.02$; Table 3A). In the control animals (Fig. 3A), the observed Fmax for ICX neurons ranged from 25 to
More than 50% of these neurons had \( F_{\text{max}} \) between 20 and 60 pps, and the average \( F_{\text{max}} \) calculated for all single ICX neurons was \( 58 \pm 6 \) (SE) pps. In the ICC of control animals \( F_{\text{max}} \) ranged from 10 to 330 pps. About 50% of these estimates fell in the range between 60 and 120 pps, and the mean \( F_{\text{max}} \) of ICC neurons was \( 102 \pm 3.8 \) (SE) pps.

In animals stimulated with low-frequency pulses (Fig. 3B), \( F_{\text{max}} \) of ICX neurons ranged from 10 to 100 pps. For \( \approx 50\% \) of these recordings \( F_{\text{max}} \) was between 40 and 80 pps. The mean \( F_{\text{max}} \) for ICX neurons was \( 58 \pm 11 \) (SE) pps and did not differ significantly from the controls. In the ICC \( F_{\text{max}} \) ranged from 10 to 250 pps. Around 50% of the \( F_{\text{max}} \) from ICC neurons fell in the range between 60 and 120 pps, and the mean \( F_{\text{max}} \) was \( 109 \pm 5.8 \) (SE) pps. Thus the mean \( F_{\text{max}} \) in the ICC was slightly higher than in control animals, but this difference did not achieve statistical significance (\( P > 0.05 \)).

In high-frequency-stimulated animals (Fig. 3C), \( F_{\text{max}} \) for ICX neurons ranged from 10 to 190 pps. More than 50% of these recordings had maximum following rates ranging between 40 and 80 pps, and the average \( F_{\text{max}} \) for ICX neurons was \( 74 \pm 5.3 \) (SE) pps. Thus ICX neurons in the high-frequency-stimulated group showed a somewhat higher average frequency following compared with both control and low-frequency-stimulated animals, but these differences were not significant (\( P > 0.05 \), Table 3B). In the ICC, the estimates for \( F_{\text{max}} \) ranged from 20 to 324 pps. More than 50% of the \( F_{\text{max}} \) ranged between 80 and 180 pps, and the mean \( F_{\text{max}} \) was \( 134 \pm 4.1 \) (SE) pps. Thus ICC neurons from high-frequency-stimulated animals demonstrated a marked increase in average temporal resolution that significantly exceeded the temporal resolution of ICC neurons in both control and low-frequency-stimulated animals (both \( P < 0.001 \), see Table 3B). However, the peak \( F_{\text{max}} \) of high-frequency-stimulated animals (324 pps) was nearly identical to that measured in the control animals (330 pps).

The results suggest that there are characteristic differences in the temporal resolution between neurons in the ICX and ICC. In each of the three groups, ICX neurons had significantly lower \( F_{\text{max}} \) than neurons in the ICC. Chronic electrical stimulation maintains normal temporal resolution but does not significantly increase the average \( F_{\text{max}} \) of neurons in the ICC of neonatally deafened cats. However, in the ICC chronic stimulation clearly can increase the ability of neurons to follow repetitive signals. Moreover, the magnitude of this effect is dependent on the temporal properties of the chronically applied electrical signal. Whereas low-frequency stimulation maintains or slightly increases temporal resolution, high-frequency stimulation results in significantly increased average \( F_{\text{max}} \) of ICC neurons.

**TOPOGRAPHIC DISTRIBUTION OF FMAX.** An additional purpose of the present study was to determine whether the distribution of \( F_{\text{max}} \) is related to the tonotopic frequency representation of the IC and whether changes in temporal resolution following
chronic electrical stimulation occur preferentially in specific regions of the ICC.

The spatial distributions of Fmax relative to the border between ICX and ICC are shown in Fig. 4 for single neurons from the three experimental groups. The numbers of neurons are smaller than in Fig. 3 because some neurons for which the nucleus could be classified but the depth of the border between the nuclei could not be determined precisely were excluded. The recording locations of all single neurons were normalized to the border between ICX and ICC for each penetration. The border is marked in each graph (- - -).

When normalized in this fashion, the most superficial neuron was recorded at 2,148 ± 5 mm in the ICX, and the deepest ICC recording location was at 4,200 ± 5 mm. Both extreme values were derived from recordings in the high-frequency-stimulated animals for which the majority of data were available.

As mentioned previously, the tonotopic gradient of ICX neurons is not clearly defined. It is suggested that the characteristic frequencies of ICX neurons may have a tendency to decrease with the passage of the electrode from the surface of the ICX to increasingly deeper recording locations (e.g., Aitkin 1975). In contrast, ICC neurons have demonstrated a clear cochleotopic organization with increasing recording depth corresponding to higher CFs (e.g., Brown et al. 1997; Merzenich and Reid 1974). If the frequency-following ability of ICC neurons was related to the tonotopic frequency gradient, we

<table>
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<tr>
<th>Cat</th>
<th>External Nucleus</th>
<th>Central Nucleus</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>CH105</td>
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<tr>
<td>CH168</td>
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<td>CH188</td>
<td>73 ± 60.8</td>
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<td>CH138</td>
<td>60 ± 23.1</td>
<td>114.6 ± 50.0</td>
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<td>CH958</td>
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<td>148.8 ± 62.8</td>
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<td>CH518</td>
<td>67.5 ± 53</td>
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<td>63 ± 21.4</td>
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<tr>
<td>CH228†</td>
<td>42.5 ± 10.6</td>
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<td>CH257</td>
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<td>CH242</td>
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<td>72.1 ± 24.8</td>
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Low-frequency stimulation

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<td>73.3 ± 20.2</td>
<td>113.1 ± 36</td>
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High-frequency stimulation

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<tr>
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<th>Central Nucleus</th>
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<tbody>
<tr>
<td>K62</td>
<td>50 ± 35.4</td>
<td>151.9 ± 56.8</td>
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<td>80.9 ± 48.4</td>
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<td>K99</td>
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<td>K91</td>
<td>55 ± 44.5</td>
<td>150.6 ± 65.7</td>
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<td>K92</td>
<td>78.3 ± 83.9</td>
<td>115.6 ± 55.3</td>
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<td>57.2 ± 15.6</td>
<td>173.4 ± 55.1</td>
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<td>K94</td>
<td>55.3 ± 31.1</td>
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<td>K99</td>
<td>85 ± 38.8</td>
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</tr>
<tr>
<td>K102</td>
<td>41.3 ± 16.5</td>
<td>128.6 ± 48.9</td>
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</table>

Values are means ± SD. * NA, not applicable. † Round window electrode.

As mentioned previously, the tonotopic gradient of ICX neurons is not clearly defined. It is suggested that the characteristic frequencies of ICX neurons may have a tendency to decrease with the passage of the electrode from the surface of the ICX to increasingly deeper recording locations (e.g., Aitkin 1975). In contrast, ICC neurons have demonstrated a clear cochleotopic organization with increasing recording depth corresponding to higher CFs (e.g., Brown et al. 1997; Merzenich and Reid 1974). If the frequency-following ability of ICC neurons was related to the tonotopic frequency gradient, we

FIG. 3. Distributions of Fmax for all single ICX (●) and ICC (○) neurons in the control (A), low-frequency stimulation (B), and high-frequency stimulation (C) group. ▼, mean Fmax for neurons in the ICX and ICC. N = number of neurons.
would expect $F_{\text{max}}$ to increase with recording depth. Consequently the regression lines for $F_{\text{max}}$ would be expected to rise along the CF gradient.

In Fig. 4 the slopes of the regression lines for $F_{\text{max}}$ of ICX neurons were all positive, but the correlation coefficients were relatively small. Although the ICX data in the low-frequency-stimulated animals presented a relatively high correlation coefficient ($R = 0.77$), the number of recording locations was very small ($n = 6$). Thus data from ICX neurons indicated only a slight tendency toward higher $F_{\text{max}}$ with increasing depth.

In the ICC, only the regression line for $F_{\text{max}}$ in low-frequency-stimulated animals had a slightly positive slope. In contrast, the slopes for the regression lines in both control animals and high-frequency animals were slightly negative. Moreover, in all three groups the slopes of the regression lines and the correlation coefficients for the ICC measures were very low, suggesting that $F_{\text{max}}$ of ICC neurons did not change systematically with increasing depth, i.e., along CF gradient. Instead, ICC neurons exhibited a broad range of $F_{\text{max}}$ at all locations throughout the entire nucleus. In the control and high-frequency groups, in which the most ICC neurons were available, the majority of neurons with higher $F_{\text{max}}$ appear to be located in the center of the nucleus (at ~1,000 – 2,000 μm re internuclear border). Consequently, at recording locations around the center of the ICC the encountered $F_{\text{max}}$ also showed the widest ranges. For example, at ~2,000 μm neurons from high-frequency-stimulated animals presented $F_{\text{max}}$ ranging from 50 to 240 pps. The frequency ranges decreased toward the more ventral and more dorsal areas of the ICC. Conversely, lower $F_{\text{max}}$ (<100 pps) could be found in neurons at nearly all depths along the CF gradient.

To better illustrate the area of maximum increase in temporal resolution in relation to the gradient of frequency representation, the average $F_{\text{max}}$ for given ranges of IC depth along the CF gradient (normalized to the border) are illustrated in Fig. 5. The horizontal line marks the average $F_{\text{max}}$ of ICC neurons recorded in control animals. For comparison, this line is repeated in each graph. Again, in the ICX the average $F_{\text{max}}$ of neurons appeared to increase with depth. In the ICC, the average $F_{\text{max}}$ in neurons from control and high-frequency-stimulated animals showed a broad maximum around the center of the nucleus with a decline in temporal resolution toward both the more dorsolateral (superficial) and more ventromedial (deep) regions. The limited number of neurons from low-
frequency-stimulated animals did not allow a final conclusion about changes in temporal resolution along IC depth in this group. ICC neurons recorded in high-frequency-stimulated animals showed an increase in Fmax across the entire nucleus. The results support the hypothesis that Fmax does not correlate with the tonotopic gradient in the ICC. Rather, high-frequency stimulation markedly increases the Fmax of neurons throughout the entire ICC.

Response onset latencies

Quantitative distribution of onset latencies. Onset latencies were determined in a total of 698 single neurons in response to 20-pps pulse trains presented at an average of 4 dB above threshold. About 16% (110 neurons) of the first spike latencies were recorded in the ICX, and ~81% (564 neurons) of the latencies were recorded in the ICC. Latencies for a total of 262 neurons were determined in control animals; latencies for 110 neurons were determined in low-frequency-stimulated animals; and latencies for 302 neurons were evaluated in animals that received chronic high-frequency stimulation. About 3% of all response latencies (24 neurons) were excluded from the study because it was not possible to classify the recording location as ICX or ICC.

Because latencies are typically not normally distributed, the central tendency of the data is expressed best by the median response latency. Table 4 shows the medians and quartile deviations (Q) of latencies for the individual animals within each group. The distributions of all onset latencies for the three animal groups are illustrated in Fig. 6. Onset latencies of neurons in the ICX (■) and the ICC (●) are displayed separately. The median latencies for the two nuclei are indicated (↓). The statistical comparisons for the latencies measures (Mann-Whitney U test) are given in Table 5.

Overall, responses from neurons in the ICX of all three groups had significantly longer latencies than those in the ICC (all *P* < 0.009, Table 5A). In control animals (Fig. 6A), ICX latencies ranged between 5.52 and 35.15 ms. More than 50% of these latencies were between 7 and 11 ms, and the median latency calculated for all single neurons in this group was 8.9 ± 1.8 (Q) ms. The ICC latencies of control animals ranged from 4.52 to 11.88 ms. More than 50% of the values were between 6–8 ms, and the median latency was 6.9 ± 0.8 (Q) ms.

In the low-frequency-stimulated animals (Fig. 6B) the ICX latency data were limited by the small number of neurons. Nevertheless, the latencies in this group demonstrated a distribution similar to that seen in control animals. ICX latencies ranged from 5.86 to 12.38 ms. About 50% of the measures fell in the range from 9 to 11 ms, and the median ICX latency was 9.1 ± 1.3 (Q) ms. ICC neurons from low-frequency-stimulated animals ranged from 5.02 to 11.72 ms. More than 87% of the latencies were nearly evenly distributed between 5 and 9 ms, and the median ICC latency in this group was 7.4 ± 1.1 (Q) ms. Overall, ICC neurons from low-frequency-stimulated animals demonstrated longer latencies than those measured in the control animals.

In the high-frequency-stimulated animals (Fig. 6C) onset latencies of ICX neurons covered a range from 4.69 to 19.08 ms. Fifty percent of these latencies fell in the range of 7–9 ms,
Control animals

The latencies of low-frequency-stimulated animals suggested a redistribution of latencies along IC depth (i.e., CF gradient) also was investigated. In Fig. 7 onset latencies of ICX (■) and ICC (●) neuron responses to pulse trains of 20 pps are plotted as a function of normalized depth (depth at border = 0 μm) for the three experimental groups. The number of ICX and ICC neurons in each group is smaller than in Fig. 6 because only neurons for which the border between the two nuclei could be determined unambiguously were included. The border between ICX and ICC is marked (---). The range of recording locations fell between −1485 and 4,200 μm re border. Recordings from both extreme locations were achieved in the high-frequency group, for which the majority of data were collected.

The slopes for the linear regression lines for latencies in the ICX were all negative. These results suggest a tendency for increasingly shorter latencies with increasing depth of the recording locations in the ICX, but the correlation coefficients were very small. In the ICC both the slopes of the regression lines and the correlation coefficients of all three experimental groups were very small. Therefore onset latencies did not appear to be systematically related to the penetration depth along the tonotopic gradient in the ICC.

In addition, at given recording locations, the responses of ICC neurons in the control and the high-frequency-stimulated animals generally had narrower latency ranges that also included shorter latencies than responses of neurons in the ICX. The latencies of low-frequency-stimulated animals suggested a similar tendency, although the number of neurons studied in the ICX was very small (n = 6). Most of the latencies in the ICC of all three groups (>90%) were found to vary between 4.7 and 10 ms across the entire ICC. That is, the same range of latencies was observed throughout most of the ICC.

To relate changes and differences in onset latencies to the spatial tonotopic or frequency organization in the IC, median response latencies are plotted for given ranges of depth normalized to the border between ICX and ICC in Fig. 8. All three groups of animals demonstrated a relatively flat distribution of average latencies across IC depth with only a weak tendency toward shorter median latencies in the center of the ICC surrounded by slightly increasing median latencies toward the more dorsal and more ventral regions of the ICC. The effect of high-frequency stimulation in reducing latency did not appear to be localized to a specific region of the ICC. The average ICC latencies of high-frequency-stimulated animals clearly decreased uniformly across the entire central nucleus. These results suggest that neuron responses throughout the IC cover a broad range of onset latencies; onset latencies are not correlated to the tonotopic gradient, that is, the shortest latencies are not observed selectively in regions of highest frequency representation; and changes (reduction) in onset latencies following high-frequency stimulation occur uniformly across the entire ICC.

**Covariation of Fmax and response latency**

In normal hearing cats, Langner et al. (1987) described onset latencies of IC neurons responding to amplitude modulated signals of ICC neurons, whereas high-frequency stimulation results in significantly shorter latencies and increased Fmax in ICC neurons.
tones as being significantly correlated with the best modulation frequency (modulation frequency with the strongest neuronal response; BMF) using an equation based on a multiple linear regression analysis [onset latency = (7.1 ± 0.9) ms + (1.2 ± 0.2) · 1/CF + (0.16 ± 0.03) · 1/BMF]. In contrast to acoustic stimulation, electrical pulses have an instantaneous onset and are independent of any cochlear delays. Therefore Snyder et al. (1995) modified the equation for electrical stimulation by reducing the asymptotic value for latency from 7.1 to 5.1 ms. This correction accounts for the on-ramp of the acoustic signal and a number of cochlear delays including conduction delay, travel time delay, transduction delay, and synaptic transmission delay (Ruggero and Rich 1987). Further, Snyder and colleagues replaced the period of the BMF by the period of Fmax (see equation at bottom of Fig. 9C). Using this modified equation for electrical stimulation, they found a modest correlation between onset latencies and Fmax for IC neurons responding to intracochlear electrical stimulation with pulse trains in acutely deafened adult cats.

Figure 9 shows the relationships between onset latencies and Fmax (plotted on a logarithmic scale) for ICX and ICC neurons in the three experimental groups investigated in the present paper. The curves in each graph encompass onset latencies predicted by the modified equation (Fig. 9C, bottom): top curves predict the latencies for neurons with a CF of 2 kHz (equivalent to a cochlear position of 66% or 16.5 mm from the cochlear base) (Liberman 1982) and the variables in the equation set to a maximum. Bottom curves approximate the latencies for neurons with a CF of 60 kHz (representing nerve fibers at the cochlear base) and the variables set to a minimum. The frequency values of the curves were selected to broadly encompass the regions along the spiral ganglion that might be activated by electrical stimulation with pulsatile stimuli in the present experiments. As illustrated in Fig. 1, chronic stimulus levels set at 2 dB above EABR thresholds for electrical pulses often appear to activate neurons over a relatively broad range of frequencies. A CF of 2 kHz (equivalent to 66% of the average 23.9 mm basilar membrane, see METHODS) was chosen for the upper limit curve to encompass frequencies that might be excited by a spread of current generated by the apical

| TABLE 5. Statistical comparisons of IC response latencies (Mann-Whitney U test) |
|---------------------------------|-----------------|-----------------|
| A. Experimental groups          | ICX | ICC | P value for ICX vs. ICC |
| Normal                          | 8.9 | 6.9 | 0.001 |
| Low frequency                   | 9.1 | 7.4 | 0.008 |
| High frequency                  | 8.0 | 5.9 | 0.001 |

B. Experimental groups |

<table>
<thead>
<tr>
<th>P value</th>
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<tr>
<td>ICX</td>
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<tr>
<td>Normal vs. low frequency</td>
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<tr>
<td>Normal vs. high frequency</td>
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<td>Low vs. high frequency</td>
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FIG. 6. Distributions of first spike latencies for ICX (●) and ICC (○) neurons for groups A–C. □, median latencies for neurons in the ICX and ICC. N = number of single neurons.
electrode pair with a maximum insertion depth of electrode 1 at 55% of the basilar membrane (equivalent to 3.6 kHz) and stimulus intensities of 2–6 dB above single IC neuron thresh-

**FIG. 7.** Response latencies of neurons in the ICX (○) and ICC (●) as functions of normalized depth for A–C. ---, borders between the two nuclei. Linear regression lines are shown for ICX and ICC neurons in each panel. Numbers of neurons (N) and the correlation coefficients for ICX and ICC are noted (top right).

**FIG. 8.** Distributions of median response latencies for given depth ranges in the IC for A–C. Number of latencies measured at each depth range is shown in the bars. ---, borders between ICX and ICC. — in A delineates the median response latency of ICC neurons in control animals and is repeated in B and C for comparison. Total number of recorded neurons (N) and the median latencies (MDN) for each nucleus are shown in the graphs.
A CF of 60 kHz (corresponding to the basal extreme of the basilar membrane) was selected for the bottom limit curves to fully encompass frequencies that might be excited by an electrical field generated by the basal electrode pair with a minimum insertion depth of electrode 4 at 21% (equivalent to 20.5 kHz) based on the assumption that the 60-kHz location might be activated due to the compression of the frequency map of the spiral ganglion at the cochlear base (Keithley and Cronin-Schreiber 1987).

Although there were some outlying data points, the majority of both ICX and ICC neurons in each group fell into the predicted range of latencies. These observations suggest that 1) for intracochlear electrical stimulation onset latencies were correlated inversely with the Fmax of neurons both in the ICX and the ICC, i.e., neurons with higher stimulus following rates demonstrated shorter response latencies, and that 2) this correlation was maintained despite characteristic changes in the temporal resolution of IC neurons following chronic low- and high-frequency stimulation. Thus the present results may reflect the existence of intrinsic coding mechanisms in the temporal processing of periodic signals in central auditory neurons (Langner et al. 1987; Snyder et al. 1991, 1995).

**DISCUSSION**

**Frequency following to acoustic and electrical stimulation**

Previous electrophysiological studies have compared the responses of single neurons to acoustic and electrical stimulation and have reported similar temporal resolution of IC neurons to both stimulus conditions. Using acoustic signals, temporal resolution has been determined in different species by analyzing the ability of single- and multiple-IC neurons to follow fluctuations in the envelope of amplitude-modulated tones or broadband noise (Batra et al. 1989; Langner and Schreiner 1988; Rees and Møller 1983, 1987; Rees and Palmer 1989; Schreiner and Langner 1988). Most relevant to the present investigation are acoustic studies in cats by Langner and Schreiner (1988), who used amplitude-modulated tones to determine the BMF of single- and multiple-neuron responses in the central nucleus of the IC. The upper limit of BMFs reported by Langner and Schreiner exceeded the upper limit of Fmax observed in the present study: ~8% of the recordings had BMFs >300 Hz including rare examples of responses with BMFs as high as ~1,000 Hz. In comparison, in the present study <1% of all IC neurons from control animals had Fmax >300 pps, and the highest Fmax was 330 pps. It should be noted, however, that most of the high BMFs (>300 Hz) reported by Langner and Schreiner (1988) were derived from multiple-neuron recordings, whereas the present study was limited to well-isolated single neurons. Further, Langner and Schreiner suggested that neurons with high BMFs may be harder to isolate than low-frequency neurons perhaps due to morphological differences. It is also possible that the recorded high BMFs predominantly originate from afferent or input fibers rather than from central nucleus neurons per se (Langner and Schreiner 1988). On the other hand, for >80% of the recording locations, Langner and Schreiner reported BMFs <120 Hz. These data are in close agreement with the present study and confirm the finding that the great majority of neurons

**FIG. 9.** Response latencies as functions of Fmax for single neurons in control (A), low-frequency- (B), and high-frequency-stimulated (C) animals. Curves encompass the latencies predicted by the equation in C. Top curves: approximate response latencies for neurons with a characteristic frequency (CF) = 2 kHz with all variables set to a maximum. Bottom curves: approximate latencies for fibers with a CF = 60 kHz and all variables set to a minimum.
preferentially followed modulation frequencies well below those found in the auditory nerve (e.g., Evans 1978; Hartmann and Klinke 1989; Javel 1980; Joris and Yin 1992; Palmer 1982) or the cochlear nucleus (Gersuni and Vartanyan 1973; Moller 1972, 1974, 1976; Nelson et al. 1966; Vartanyan 1969).

In fact, most of the acoustic studies in mammals have described BMFs in IC neurons that were comparable with the Fmax observed in the present study: Rees and Moller (1987) reported that for IC neurons in rats the most effective modulation frequencies were between 100 and 120 Hz, and the highest maximum following frequency they recorded was 320 Hz. Batra et al. (1989) recorded single- and multiple-neuron responses to amplitude-modulated tones in normal hearing unanesthetized rabbits and measured an average BMF of 87 Hz and a maximum BMF of 250 Hz.

In a previous study, Snyder et al. (1995) examined the temporal resolution of IC neurons in cats using electrical cochlear stimulation. They found that Fmax in “normal,” acutely deafened adult animals ranged from 10 to 340 pps with an average of 93 pps. In the present study, Fmax of all IC neurons from control cats was virtually identical, ranging from 10 to 330 pps with an average temporal resolution of 97 pps.

In summary, despite the different stimulus conditions (acoustic vs. electrical stimulation), different animal species and different criteria used for the determination of temporal resolution (BMF vs. Fmax), similar estimates of temporal resolution of IC neurons are reported for both acoustic and electrical stimulation, and most estimates are clearly below the frequency following capacity of the auditory nerve.

Effects of chronic stimulus frequency on Fmax

The main goal of this study was to examine the effect of temporally different types of chronic electrical stimuli on the temporal resolution of IC neurons. The selection of the specific signals for chronic stimulation was based on earlier acoustic and electrical studies of the temporal resolution of IC neurons. As mentioned in the preceding text, most studies report an average frequency following of IC neurons in normal cats of ~100 pps and a maximum frequency following of ~300 pps. In respect to these findings, animals chronically stimulated with frequencies below the average frequency following capacity of IC neurons (<100 pps) therefore were categorized as the “low-frequency stimulation” group. This group included animals stimulated with continuous unmodulated pulse trains at 30 or 80 pps. Animals chronically stimulated with frequencies around or above the maximum frequency following capacity of IC neurons (≥300 pps) were categorized as the temporally challenging, “high-frequency stimulation” group. This group included animals stimulated with continuous 300 pps/30 Hz AM signals and those stimulated with an analogue speech processor (SP; band-passed filtered 250 Hz to 3 kHz). Besides the higher frequency components of these signals, one rationale for combining the results from both AM and SP stimulated animals was that Fmax of neither ICX nor ICC neurons varied significantly among the two populations. Another rationale for the selection of 300 pps/30 Hz AM and analogue SP was to model speech processing strategies that are currently used in human CI subjects. Specifically, stimulation with 300 pps/30 Hz AM modeled speech processing strategies that employ higher-frequency, amplitude-modulated pulsatile stimulation (CIS, MSPEAK, SMSP) (e.g., McDermott et al. 1992, Wilson et al. 1991); and stimulation with the analogue speech processor modeled the analogue waveform used in compressed analogue (CA) speech processing strategies.

The present study has shown that chronic stimulation of neonatally deafened cats resulted in marked alterations in the frequency following capacity (Fmax) of neurons in the auditory midbrain. The magnitude of this effect was related to two specific factors: 1) the frequency of the chronically applied electrical signal critically influenced the temporal responses of IC neurons. That is, low frequency stimulation maintained the normal frequency following capacities of neurons; in contrast, high-frequency stimulation markedly enhanced the temporal resolution of IC neurons. 2) However, significant changes in temporal resolution following chronic electrical stimulation were observed only for neurons in the ICC and not for neurons located in the ICX.

Snyder et al. (1995) previously reported a significant increase in temporal resolution in IC neurons from chronically stimulated animals as compared with normal control cats. As stated in the preceding text, they estimated a mean Fmax of 93.2 pps (range 10–340 pps) in control animals, whereas in stimulated animals, they reported an increase in average Fmax to 139.5 pps (range 10–710 pps). This increase in average Fmax is comparable with the average frequency following capacity of ICC neurons from high-frequency stimulated animals of the present report (134.2 pps). However, Snyder et al. did not distinguish between low- and higher-frequency stimulated animals: four of the six stimulated cats included in this previous study had a history of low-frequency stimulation (30 or 80 pps), one cat received high-frequency stimulation with an analogue processor, and another cat (K90) received chronic passive stimulation with 30 pps followed by extensive behavioral training with predominantly 100-Hz sinusoidal stimuli. Moreover, responses from both ICX and ICC neurons were included in the estimates. Both of these conditions would suggest that Fmax should have been lower than in the present observations. Instead, IC neurons from stimulated animals reported by Snyder et al. (1995) had higher average and peak Fmax than corresponding measures in the present study (low-frequency group: mean Fmax for all IC neurons = 103 pps, range = 10–250 pps; high-frequency group: mean Fmax for all IC neurons = 119 pps, range = 10–324 pps). The individual variation of frequency following among the animals included in Snyder et al. (1995) might have contributed to this difference. One of their animals (K90) demonstrated an average Fmax for all IC neurons (166 pps) that markedly exceeded the average following capacities of IC neurons in high-frequency stimulated cats reported in the present study (119 pps). Thus given the limited number of animals (n = 6) in their group of combined low- and high-frequency-stimulated animals, this animal clearly increased the average temporal resolution of neurons reported by Snyder and colleagues. In addition, one animal of the present study (K98) failed to show increased temporal resolution following higher frequency stimulation. In fact, the average Fmax of this animal for ICC neurons (82.9 pps; Table 2) was well below that of control animals (101.6 pps) and thus reduced the average Fmax for IC neurons in the higher-frequency group. Review of the stimulation history
revealed no features that might explain the low temporal resolution of this animal. Although we have emphasized the role of stimulus rate, further investigations are required to understand any additional factors (e.g., stimulus waveform complexity, behavioral training) that may contribute to the variability in temporal resolution among individual animals.

The mechanism(s) underlying the observed changes in temporal resolution of ICC neurons following chronic high-frequency stimulation are not known. On the basis of studies using acoustic stimulation, it has been proposed that the duration of inhibition for cortical neurons is directly related to the period of the BMF (Eggermont 1992; Krueger and Schreiner 1994; Schreiner and Joris 1986). Thus the ability of cortical neurons to follow higher frequencies suggests that the inhibitory period ends earlier. This earlier termination of the inhibitory period may effectively reduce the temporal scatter of the inhibitory inputs to IC neurons and could be explained by either a shorter duration of inhibition and/or by an earlier onset of inhibition, as suggested by Schreiner and Raggio (1996).

Assuming a similar relationship between the duration of inhibition and the period of Fmax for neurons at the level of the IC, the results from the present study suggest that chronic electrical stimulation of the cochlea leads to long term changes in the inhibitory circuits. Specifically, higher-frequency chronic stimulation may be more effective in modulating the inhibitory mechanisms, whereas low-frequency stimulation at least maintains or regenerates the “normal” degree of inhibitory contributions in neonatally deafened animals. Results from animals that were neonatally deafened for prolonged periods (>2 yr) and not chronically stimulated showed a significant degradation in the temporal resolution of central nucleus neurons, thus supporting this hypothesis (Vollmer et al. 1998a,b).

Further, nothing presently is known about the specific nature or extent of structural changes that may underlie the observed long-term changes in temporal resolution of central auditory neurons following chronic high-frequency stimulation. There are at least two different ways in which structural modifications in afferent projections might occur prior to and/or at the level of the IC (e.g., Eysel et al. 1981; Kaas 1996; Keller et al. 1990). First, “strengthening” of already existing (either previously active or ineffective) connections may occur as a consequence of chronic electrical stimulation. For example, synapses may undergo modification in size to become persistently more effective or synapses may move to more effective locations on the target neurons. Second, “sprouting” of new axonal and dendritic connections may result in an increased number of synapses and/or more effectively located synapses. Both mechanisms would lead to increased synaptic efficacy, a higher synchrony in the neuronal excitation pattern, and, consequently, in increased temporal resolution of IC neurons.

It should be noted that low-frequency-stimulated animals had a mean duration of chronic stimulation of 3.8 ± 1.4 (SD) mo that was markedly shorter than the mean duration of stimulation in high-frequency-stimulated animals of 7.6 ± 1.7 mo. Thus it is not clear at present if the duration of chronic stimulation also played a role in the observed differences in temporal resolution between the two groups. However, a comparison of data from age matched individuals in low (K63 and K83)- and high (K62 and K92)-frequency stimulation groups showed the same results as in the overall comparisons, with markedly lower average Fmax (114 pps) and a significantly longer median latency (7.87 ms; P < 0.05) in low-frequency-stimulated animals than in high-frequency-stimulated animals (136 pps and 7.36 ms, respectively). Further, although stimulation periods in the low-frequency stimulation group ranged between 9 and 23 wk, the average Fmax for the individual animals showed only a relatively small variability (mean Fmax = 109.9 ± 4.37 pps). These observations suggest that at least for stimulation periods ≥9 wk, the frequency of the chronically applied signal is more important in determining the temporal response properties of ICC neurons than the duration of chronic stimulation per se.

In contrast to results in the ICC, the temporal resolution of ICX neurons was not significantly different in any of the experimental groups, regardless of the stimulation history. There is no clear explanation for this finding. One possibility is that additional somatosensory input (e.g., Aitkin 1986) modulates or maintains the temporal response properties of ICX neurons such that they are less sensitive to deprivation or modulation of auditory inputs. Furthermore the ICX is distinguished from the ICC by its major descending inputs from both primary and nonprimary auditory cortex (e.g., Andersen et al. 1980; Coleman and Clerici 1987; Faye-Lund 1985; Gonzalez-Hernandez et al. 1987; Oliver and Huerta 1992; Willard and Martin 1983). Because Fmax of auditory cortical neurons are approximately an order of magnitude lower than those of neurons in the ICC (Schreiner and Raggio 1996), the cortical projections to the ICX may modulate the temporal resolution of ICX neurons and may therefore explain the overall lower-frequency following capacity observed in ICX neurons compared with neurons in the ICC. Furthermore, cortical projections to the ICX may prevent or counteract the influence of high-frequency stimulation on long-lasting functional or structural changes of afferent connections as suggested for ICC neurons. Clearly, additional studies are required to more fully understand the role of ICX neurons in the processing of auditory signals.

**Topographic distribution of Fmax**

Another major goal of this study was to investigate the topographic distribution of temporal response properties along IC depth and to determine if temporal resolution was related systematically to the cochleatopically organized frequency gradient of the ICC (e.g., Brown et al. 1997; Merzenich and Reid 1974), in which increasing penetration depth corresponds to increasingly higher CF and to more basal intracochlear electrode locations in implanted animals (Snyder 1990). Specifically, we investigated how selective changes in temporal resolution following low- versus high-frequency chronic electrical stimulation are distributed along the CF gradient of the IC.

In all three experimental groups Fmax did not vary systematically with IC depth. That is, neither average nor peak maximum following frequencies increased along the gradient of increasing CF. Moreover, there was a slight tendency for the average Fmax to reach a maximum in the center of the ICC. It is interesting to note that Rees and Møller (1987), using amplitude-modulated noise in rats, also did not see a consistent relationship with characteristic frequency of either peak MTF (modulation transfer function) or the high-frequency cutoff of
the MTF. In contrast, other studies report a systematic increase of BMF or Fmax with the tonotopic (CF) gradient (Langner and Schreiner 1988; Snyder et al. 1995).

One possible explanation for the differences between the present and some previous reports is a difference in sampling strategies. Langner and Schreiner (1988) attempted to achieve a high sampling density in certain CF areas. Furthermore, as mentioned previously, the inclusion of single- and multiple-neuron responses by these investigators may have played a role in defining the distributions of temporal resolution across the tonotopic gradient. The majority of high BMFs were obtained from multiple-neuron recordings and might reflect characteristics of neural inputs to the IC rather than response properties of IC neurons per se.

Snyder et al. (1995) also reported increased average and peak Fmax with increasing depth, with a steeper slope of the regression lines especially for chronically stimulated animals, but the correlation coefficients for these measures were small ($R < 0.3$). Moreover, in that study responses were not separated between ICX and ICC. As demonstrated in the present study, neurons from the ICX have significantly lower Fmax than neurons from the ICC. Thus including neurons from both nuclei in the same correlation analysis likely explains the correlation between increasing Fmax with the tonotopic gradient observed by Snyder and colleagues.

In the present study, the sample size of single neurons in the most superficial and deepest recording locations was smaller than in more central locations of the IC (Fig. 5), and this might have influenced the distribution of Fmax averaged over given depth ranges. Regardless of the smaller samples in the two extreme depths, however, the average Fmax in the two animal groups with the overall highest number of recorded neurons (control and high-frequency-stimulated animals) clearly showed a comparable distribution, indicating that temporal resolution across IC depth does indeed reach a broad maximum in the center of the ICC.

Finally, changes in temporal resolution of IC neurons following high-frequency stimulation were not restricted to circumscribed regions (e.g., the best location for the chronically stimulated electrode pair or region of highest CF) but occurred broadly across the entire ICC. The broad increase in Fmax of single neurons across the entire ICC following high-frequency stimulation may be related to the relatively broad current spread of the electrical pulsatile stimuli. The chronic stimulus level was set at 2 dB above EABR threshold. It has been shown that the EABR threshold is on average $\sim 4$–6 dB above psychophysical and minimum single IC neuron threshold (Abbas and Brown 1991; Beitel et al. 1999; Smith et al. 1994). Because the dynamic range of IC neurons for electrical pulsatile stimulation (0.2 ms/ph) is usually $< 10$ dB (unpublished observations), a stimulus intensity of $\sim 6$–8 dB above minimum IC threshold is likely to activate a broad region of the IC (Fig. 1) and may explain the broad distribution of increased temporal resolution following higher-frequency stimulation.

**Response latencies**

Neurons in central auditory nuclei receive their inputs via multiple pathways that differ in length, number of synapses, and intrinsic timing properties of the afferent neurons (De Ribaupierre et al. 1980; Møller 1975). Therefore response latencies of neurons may vary considerably at different locations in the same nucleus (Langner et al. 1987). The findings of the present study confirmed the large variation of neural response latencies in the inferior colliculus. Latencies in all three animal groups ranged between 5 and 10 ms at most locations throughout the entire IC (Fig. 7).

It should be noted that studies using acoustic stimulation have shown that latency to the stimulus onset decreases with increasing sound intensity (Anderson et al. 1971; Møller 1975). This effect also was observed to some degree with intracochlear electrical stimulation. Thus depending on the separation of the neuronal response and the electrical artifact, our approach in the acute electrophysiological experiments was to consistently select stimulus intensities between 2 and 6 dB above single-unit threshold. Quantitative analysis has shown that recordings made at the upper and lower limit of our intensity range (i.e., 2 and 6 dB above threshold, respectively) vary in onset latencies by $\pm 0.5$ ms (unpublished observations). Because this effect was relatively small and applied similarly to the single neuron recordings of all three experimental groups, it is assumed that any possible intensity dependent variation in latencies did not affect the overall conclusions. In fact, the presented range and distribution of onset latencies from the control animals were in close agreement with results from normal animals reported in previous studies using both electrical (Snyder et al. 1991, 1995) and, adjusted for the lack of cochlear delay, acoustic stimulation (Irvine and Gago 1990; Langner and Schreiner 1988).

Regardless of the stimulation history, ICX neurons generally had significantly longer average response latencies than neurons in the ICC, and chronic electrical stimulation did not significantly influence the latencies of ICX neurons. In contrast, response latencies of neurons in the ICC were affected by the frequency of the stimulus in two ways. First, low-frequency stimulation did not maintain temporal resolution of ICC neurons, i.e., average latencies in the low-frequency group were significantly longer than those in the control group. Secondly, high-frequency stimulation resulted in significantly shorter latencies for ICC neurons compared with control animals and appeared to be the more effective stimulus for maintaining or enhancing temporal resolution.

In the high-frequency group, neurons from animals stimulated with 300 pps carrier/30 Hz AM demonstrated significantly shorter latencies ($P < 0.05$) than those from animals stimulated with an analogue processor. The underlying mechanisms for this difference in latency are presently unknown, but it is tempting to speculate that the sharp onset of the square pulse waveform might have produced greater synchrony (resulting in shorter latency) than the less abrupt onset of analogue stimuli. However, the relatively broad range of frequencies delivered by the speech processor (band-pass filtered from 250 Hz to 3 kHz, see METHODS), additional behavioral training with a variety of different frequency signals and the possible behavioral relevance of auditory information delivered by a speech processor make it impossible to draw definite conclusions relating the differences in average latency between the two high-frequency-stimulated subpopulations (300 pps/30 Hz AM vs. analogue processor) to a specific characteristic of the stimulus. Also, further studies are required to understand the
extent to which different time patterns of the chronic high-frequency stimuli (i.e., periodic pulsatile AM stimulation with a constant time pattern vs. analog broadband stimulation with changing, frequency-modulated time patterns) may contribute to specific differences in the frequency-encoding capability of ICC neurons. However, it should be noted that ICC latencies from both high-frequency-stimulated subpopulations were significantly shorter than those from normal and low-frequency-stimulated animals, and no differences were observed in the Fmax between the two populations. Therefore the results from both populations were summarized in the high-frequency stimulation group.

Finally, in the present study onset latencies did not vary systematically with IC depth (i.e., CF gradient). These results were in close agreement with earlier studies using acoustic stimulation that found only a weak correlation between onset latency and CF (Langner et al. 1987). In the present study, average latencies were relatively uniformly distributed across the entire ICC with only a slight minimum in the center of the nucleus in all three experimental groups. In fact, we observed a correlation between response latency and Fmax that was independent of the frequency characteristics of the chronic stimulation or the recording location (i.e., CF). These findings support the suggestion of Langner and Schreiner that the co-variation of frequency following and latencies may reflect the existence of intrinsic neuronal mechanisms in the temporal processing or coding of periodic signals in central auditory neurons (Langner and Schreiner 1988; Langner et al. 1987; Schreiner and Langner 1988). The present study suggests that 1) these intrinsic mechanisms are applicable to both acoustic and electrical signals, 2) not altered by the temporal properties of the chronically applied peripheral stimulation pattern, and 3) valid for neurons in both ICX and ICC.

**Implications for future studies**

The ability to encode and resolve the temporal patterns of the electrical signal is crucial for the speech recognition performance of cochlear implant subjects (e.g., Eddington et al. 1978; Shannon 1992; Townsend et al. 1987; Wilson et al. 1991). In psychophysical studies on rate pitch discrimination, Shannon (1983 1985, 1992) has reported that implant subjects can discriminate repetition or modulation frequencies up to ~300 pps. This upper frequency roughly reflects the upper limit in frequency following for ~90% of IC neurons (Langner and Schreiner 1988; Snyder et al. 1995; present study). On the other hand, Wilson et al. (1991) demonstrated that modulated carrier frequencies of ≤800 pps resulted in improved speech recognition when compared with slower pulse frequencies. Because none of the IC neurons in this study was able to follow such high frequencies, it raises the question how this improvement can be explained. Presumably once the carrier frequency exceeds Fmax, the neurons begin to follow the modulation frequency instead, and (depending on the relation between modulation and carrier frequencies) carrier frequencies that are well above the frequency following capacities of central auditory neurons are able to shape the modulation frequency in more detail. To better understand the encoding of high-frequency amplitude-modulated signals, one important goal of further studies is to investigate the response characteristics of IC neurons to different combinations of carrier and modulation frequencies.

Evidence from several laboratories is now available that indicates that the neural representations of behaviorally important stimuli in the mammalian auditory forebrain can be modified by behavioral training with acoustic (Lennartz and Weinberger 1992; Recanzone et al. 1993; Scheich 1991; Weinberger and Diamond 1987) and electrical signals (Raggio et al. 1995). Furthermore Snyder et al. (1990, 1991, 1995) have shown that chronic electrical stimulation of the cochlea can induce spatial (spectral) and temporal plasticity in the inferior colliculus of neonatally deafened cats. The present study has established that high-frequency electrical stimuli more effectively increase temporal resolution in the tonotopically organized central nucleus of the inferior colliculus than low frequency stimulation. Although the mechanisms involved in representational plasticity are not well understood, there is evidence that plasticity of the sensory system can be based e.g., on local (auditory) Hebbian-type synaptic processes (Cruikshank and Weinberger 1996, Diamond et al. 1993), changes in the topographic and synaptic organization of afferent connections (e.g., Eysel et al. 1981; Kaas 1996; Keller et al. 1990), diffuse neuromodulatory systems that project to the auditory system (Barkin and Weinberger 1996; Kilgard and Merzenich 1998), and behavioral context (Ahissar et al. 1992). The animal model described allows us to control the entire “hearing” history of neonatally deafened cats. In future experiments, behavioral training with well defined electrical signals will be combined with chronic and acute electrophysiological recordings to understand the contribution of behaviorally relevant electrical stimuli for representational plasticity in the temporal processing of signals in the central auditory system.

Further experiments are required to determine whether the observed plasticity in temporal resolution is limited to the immature, early deafened auditory system or to what extent chronic electrical stimulation can modulate the functional status of neurons in the adult auditory system.

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