Unilateral cochlear ablation produces greater loss of inhibition in the contralateral inferior colliculus

Carmen Vale,[1] José M. Juíz,[1] David R. Moore[2] and Dan H. Sanes[3]

[1]School of Medicine, and Centro Regional de Investigaciones Biomedicas (CRIB), University of Castilla-La Mancha, Spain
[2]MRC Institute of Hearing Research, University Park, Nottingham, UK
[3]Center for Neural Science, 4 Washington Place, New York University, New York, NY 10003 USA

Abstract

Bilateral cochlear ablation leads to a profound weakening of synaptic inhibition within the inferior colliculus (IC) of gerbils [Vale & Sanes (2000) *J. Neurosci.*, 20, 1912–1921]. To examine whether unilateral deafening leads to similar functional alterations, we studied the effect of unilateral cochlear ablation on inhibitory synaptic properties both ipsilateral and contralateral to the deafened ear. Lateral lemniscal and commissure of the IC-evoked inhibitory postsynaptic currents (IPSCs) were recorded in an IC brain slice preparation using whole-cell and gramicidin perforated-patch electrodes in the presence of kynurenic acid. Unilateral cochlear ablation led to a 23 mV depolarizing shift in the IPSC equilibrium potential for IC neurons contralateral to the deafened ear, but only a 10 mV depolarization in the ipsilateral IC. Lateral lemniscal-evoked inhibitory synaptic conductance declined significantly in the ipsilateral and contralateral IC, whereas commissural-evoked inhibitory synaptic conductance declined only contralateral to the ablated cochlea. An analysis of paired-pulse facilitation showed that inhibitory transmitter release was more affected ipsilateral to the ablated cochlea. Thus, unilateral cochlear ablation modifies inhibitory synapses in the inferior colliculus, but these changes appear to be dominated by postsynaptic alterations in the contralateral IC, and by presynaptic changes in the ipsilateral IC.

Introduction

Spontaneous and evoked synaptic transmission can influence the maturation of central neuronal circuits. In the auditory pathway, decreased cochlear activity during early development may alter the metabolism, morphology, and survival of central neurons (reviewed in Rubel, 1978; Moore, 1992; Parks, 1999). However, *in vivo* recordings from deafened adult animals strongly suggest that changes in physiological properties may have an impact on auditory processing (Syka, 2002). For example, when animals are unilaterally deafened as neonates, acoustically evoked activity in the ipsilateral inferior colliculus was found to have increased in adult animals (Kitzes & Semple, 1985; McAlpine *et al.*, 1997). A similar effect was observed following acute unilateral ablation in adult animals (McAlpine *et al.*, 1997). As this effect comes about rapidly, it has been suggested that excitatory inputs are 'unmasked' by decreasing inhibitory drive from the deafened ear. In fact, the level of glutamic acid decarboxylase (GAD), the synthesizing enzyme for GABA, declines during the 24-h period following acute ablation in adult animals (Mossop *et al.*, 2000). Recent findings, in developing and young animals, make clear that synaptic and membrane properties are, in fact, altered following deafness (Kotak & Sanes, 1996, 1997; Francis & Manis, 2000; Vale & Sanes, 2000, 2002; Oleskevich & Walmsley, 2002; Vale *et al.*, 2003). In the present study, we asked whether an adjustment of inhibitory synaptic strength was observed in unilaterally deafened animals, and

whether alterations differed ipsilateral and contralateral to the deafened ear.

The inferior colliculus (IC) is the site of convergence for nearly all ascending and descending auditory pathways. Two major sources of inhibitory and excitatory projections to the IC arise from the lateral lemniscus (LL) and the commissure of the inferior colliculus (CIC) (Adams, 1979; Nordeen *et al.*, 1983a; Coleman & Clerici, 1987; Smith, 1992; Oliver *et al.*, 1994; Wagner, 1996; Wu & Kelly, 1996; Kuwada *et al.*, 1997; Lo *et al.*, 1998; Moore *et al.*, 1998; Reetz & Ehret, 1999; Vale & Sanes, 2000). Anatomical studies have shown that neonatal unilateral cochlear ablation disrupts the normal development of afferent projections to the IC (Nordeen *et al.*, 1983b; Moore & Kitzes, 1985; Moore, 1994; Gabriele *et al.*, 2000). However, unilateral deafness does not alter synaptic density in the IC of cats (Hardie *et al.*, 1998). In fact, retrograde tracing studies show a significant increase in cochlear nucleus (CN) projections to the IC ipsilateral to the remaining cochlea (Nordeen *et al.*, 1983b; Moore & Kowalchuck, 1988; Moore, 1994). Taken together, these observations suggest that dissimilar synaptic physiological changes may occur within the ipsilateral and contralateral IC following unilateral cochlear ablation.

We have previously shown that bilateral cochlear ablation profoundly alters both inhibitory and excitatory synaptic strength in IC neurons (Vale & Sanes, 2000, 2002). Evoked inhibitory synaptic responses are weaker, as assessed by their ability to block action potentials (Vale *et al.*, 2003). The decreased inhibitory strength is due, in part, to a depolarizing shift in the IPSC equilibrium potential that stems from dysfunction of the chloride extrusion mechanism (Vale *et al.*, 2003). The present study was designed to explore the synaptic properties contributing to novel IC responses following unilateral

deafening. Towards this end, the evoked inhibitory synaptic responses of the LL and CIC projections to the IC were recorded in a brain slice preparation following unilateral cochlear ablation prior to the onset of hearing. Our results identify different effects in the ipsilateral and contralateral IC, and help to explain the novel auditory coding properties that are observed in unilaterally deafened animals.

Materials and methods

Cochlear ablation

All protocols were reviewed and approved by the New York University Institutional Animal Care and Use Committee. At postnatal day (P) 7, gerbil (*Meriones unguiculatus*) pups were anaesthetized with hypothermia until respiration temporarily ceased and there was a complete absence of response to nociceptive stimuli. The right cochlea was exposed and extirpated with a fine forceps as described previously (Sanes *et al.*, 1992). The cochlear cavity was filled with gelfoam and the wound was closed. Following surgery, the pups were revived slowly on a heating pad and returned to the litter when respiration and motor activity recovered. Successful ablations were confirmed prior to each brain slice experiment by opening the inner wall of the cochlea under a dissecting microscope and observing the absence of cochlear tissue and the presence of a gelfoam insert. Unilateral cochlear ablated animals were studied from 1 to 7 days following surgery. To exclude an effect of anoxia during surgery, we previously examined IPSC equilibrium potential (E_{IPSC}) in the IC of P10–11 gerbils that were anaesthetized with hypothermia at P7 until respiration stopped, but no cochlear ablation was performed. No difference was found as compared to unanaesthetized controls (Vale & Sanes, 2000).

Brain slice preparation

Control and unilaterally ablated P8–14 gerbils were anaesthetized with chloral hydrate (350 mg/kg). Following decapitation, the brain was rapidly removed from the skull and placed in cold oxygenated artificial cerebrospinal fluid (ACSF). The brain was blocked at the level of the thalamus and the caudal hindbrain. The ventral surface of the brain was affixed to an agar block (cyanoacrylate glue), and the block was secured to the stage of a vibratome (Leica). Frontal slices of 300 μm were obtained in cold oxygenated ACSF containing (in mM): 123 NaCl, 4 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 28 $NaHCO_3$, 15 glucose, 2.4 $CaCl_2$, and 0.4 L-ascorbic acid (pH 7.3 when oxygenated with 95% O_2, 5% CO_2). Slices were maintained in an incubation chamber at room temperature for 2 h. Slices containing the rostral IC were placed in a recording chamber and superfused with oxygenated ACSF (7 mL/min) at room temperature.

Electrophysiology

Whole-cell voltage-clamp recordings (Warner Instruments PC-501A, Hamden, CT) were obtained as described previously (Moore *et al.*, 1998; Vale & Sanes, 2000). Recording electrodes were fabricated from borosilicate glass microcapillaries (1.5 mm OD), and the tip resistance was 5–10 MΩ. Access resistance was balanced throughout the recordings. For whole-cell recordings, the internal pipette solution contained (in mM): 127.5 caesium gluconate, 0.6 EGTA, 10 HEPES, 2 $MgCl_2$, 5 KCl, 2 ATP, 0.3 GTP, 5 QX-314 (pH 7.2). Series resistance was less than 20 MΩ, and was compensated by 60–80%. Recordings were also performed using the perforated-patch technique as described previously (Rhee *et al.*, 1994; Vale & Sanes, 2000, 2002).

Briefly, gramicidin (Sigma, St. Louis) was used as the membrane-perforating agent, to permit the recording of IPSCs without influencing the cytoplasmic chloride concentration. Gramicidin was dissolved in dimethylsulfoxide (DMSO; 2–5 mg/mL) then diluted in the pipette solution to a final concentration of 2–5 μg/mL (0.2% DMSO). For perforated-patch recordings, KCl replaced caesium gluconate in the pipette solution, but QX-314 (Alamone, Jerusalem) was retained to confirm that the membrane did not rupture. The presence of depolarization-evoked breakaway action potentials was taken as indication of the integrity of the gramicidin perforation. The progress of perforation was evaluated by monitoring the decrease in membrane resistance. After the membrane resistance had stabilized (between 5 and 40 min after obtaining the GΩ seal), data were obtained. Series resistance ranged between 30 and 50 MΩ and was compensated by 60–80%.

Stimuli were delivered and data sampled via a ITC−18 Computer Interface (Instrutech Corporation, Port Washington, NY). Data were collected using a Macintosh PC running a custom-designed IGOR macro (WaveMetrics, v3.14; WaveMetrics Inc, Lake Oswego, OR), as described previously (Kotak *et al.*, 2001). Extracellular stimuli (200-μs pulses) were delivered through paired Teflon-insulated platinum electrodes driven by isolated biphasic stimulators (Intronics Technologies, Ontario, Canada). Analyses of peak PSC amplitudes and reversal potentials were performed off-line using a second IGOR macro.

Stimulating electrodes were placed in the afferent pathways from the commissure of the inferior colliculus (CIC), and the lateral lemniscus (LL). The maximum amplitude of evoked synaptic currents was obtained with the whole-cell recording configuration at membrane holding potentials (V_{HOLD}) between −80 and −20 mV. Inhibitory synaptic conductance was calculated from the slope of the fits of IPSC amplitude vs. membrane holding potential. Measurements of E_{IPSC} were obtained with the gramicidin-perforated patch recording configuration. The E_{IPSC} was calculated from linear fits of IPSC amplitude vs. membrane holding potential.

The presynaptic release characteristics were evaluated by delivering paired stimulus pulses to the LL pathway and measuring the amplitude of the second IPSC relative to the first. Two pulses of equal strength were delivered at interpulse intervals of 200, 100, 50 and 33 ms. This analysis was performed in kynurenic acid (KYN), using stimuli that elicited a minimum IPSC amplitude (presumed to reflect the evoked responses of one or a few inhibitory afferents). Trials in which the first IPSC failed were excluded from analysis. The amplitude of the second IPSC was taken from the 'local minimum' just prior to the second stimulus artifact. Therefore, temporal summation did not contribute to the measurement. In general, a high probability of release is associated with a paired-pulse depression because the large quantal content released on the first pulse depletes the vesicle pool available for release when the second action potential arrives at the terminal. A low probability of release is associated with paired-pulse facilitation, with more neurotransmitter released on the second pulse, due to residual calcium in the terminals. In hippocampal cultures, changes in the size of the readily releasable pool are directly correlated with release probability (Goda & Stevens, 1998). Therefore, any manipulation that alters the probability of release or the number of release sites would result in a change in the paired-pulse ratio. There are several models of depletion (Zucker & Regehr, 2002) that attempt to explain which parameters are responsible for short-term depression or facilitation, which is on the scale of milliseconds and seconds. These models generally incorporate two basic parameters; the probability of release (*p*) and the number of active release site (*n*). Therefore, any manipulation that selectively increases either *p* or *n*, should enhance

the first response of a pair and reduce the second one, due to a greater amount of depletion. The degree of paired-pulse depression (short-term depression) observed is a function of how fast the readily releasable vesicle pool becomes replenished. For example, at the Calyx of Held synapse onto the MNTB, the time needed to fully replenish releasable vesicles is more than 15 s (Wu & Borst, 1999).

Biocytin (0.2%) was also added to the internal pipette solution for whole-cell recordings. The slices were fixed in 4% paraformaldehyde, and a subset was processed to visualize the IC neurons using an avidin-biotin complex coupled to horseradish peroxidase (Vector Laboratories, Burlingame, CA).

Ionotropic glutamate receptors were blocked by adding a broad-spectrum ionotropic glutamate receptor antagonist, 5 mM kynurenic acid (KYN; Fluka Chemical, Sigma-Aldrich Corp, St. Louis, MO) to the ACSF. The total inhibitory postsynaptic current was blocked by the sequential addition of 2 μM strychnine (SN; Sigma-Aldrich Corp, St. Louis, MO), a glycine receptor antagonist, and 10 μM bicuculline methobromide (BIC; RBI-Sigma, Natick, MA), a GABA$_A$ receptor antagonist, to the ACSF.

Multiple analysis of variance (MANOVA) followed by pairwise comparisons (Student's t-test) were used to assess whether significant differences existed between neurons from control and unilaterally ablated animals. All values are expressed as mean ± standard error of the mean, with the number of observations in parentheses.

Results

Recordings in deafened animals were performed in the inferior colliculus ipsilateral (ICA) or contralateral (CCA) to the ablated cochlea. Of the total number of IC neurons recorded in whole-cell voltage clamp mode from control and experimental animals ($n = 68$), 43 were recovered histologically and were assigned to locations in the rostrocaudal plane and the central or lateral positions. Twenty-seven cells were located in central positions, and the remaining cells were located either in lateral positions of the IC or in the border between the central and external cortex of the IC. Neurons that were located within the putative central nucleus of the IC generally exhibited the largest postsynaptic currents. A total number of 34 control animals, 25 CCA animals and 23 ICA animals were used for this study. All recorded IC neurons displayed a synaptic response to independent stimulation of the LL and the CIC afferent pathways. Unilateral cochlear ablation did not significantly modify the resting membrane potential, as measured with the perforated patch configuration (control, −52 ± 1.6 mV, $n = 10$; CCA, −52 ± 1.3 mV, $n = 19$; ICA, −54 ± 1.1 mV, $n = 24$).

The stimulus required to produce maximal afferent-evoked currents, was first determined by stimulating the afferents with pulses of 5 to 100 V in 5 V steps, at a holding potential of −80 mV, before the addition of pharmacological agents. Figure 1A shows
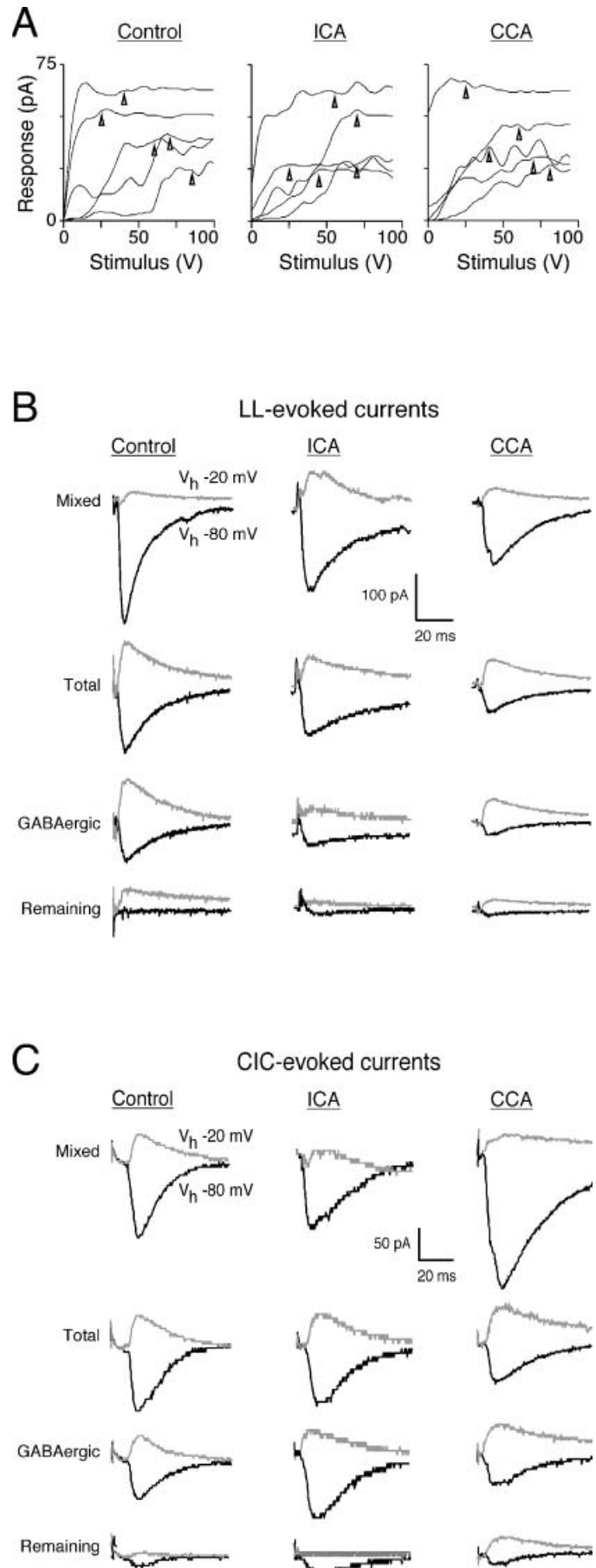

FIG. 1. LL-evoked and CIC-evoked postsynaptic currents recorded in IC neurons from control, ipsilateral cochlear ablated (ICA) and contralateral cochlear ablated animals (CCA). Recordings were performed in whole-cell voltage clamp mode. (A) Representative stimulus–response curves are shown for representative neurons in each experimental group. The stimulus intensity chosen for collection of quantitative data is indicated by a triangle for each cell. (B) LL-evoked synaptic currents in ACSF (Mixed PSC) were generally inward at V$_{HOLD}$ of −80 mV and outward at V$_{HOLD}$ of −20 mV. Pure inhibitory synaptic currents (Total IPSC) were elicited in the presence of kynurenic acid. The inhibitory currents were reduced with the addition of strychnine (GABAergic IPSC), and bicuculline (Remaining IPSC). (C) A similar progression of synaptic current amplitudes were observed for CIC-evoked IPSC as each antagonist was added to the ACSF.

representative stimulus-response functions for each experimental group. The mean stimulus intensities used to produce maximum LL-evoked responses in control, ICA and CCA neurons where 45 ± 4 V ($n = 27$), 54 ± 6 V ($n = 20$), and 49 ± 6 V ($n = 17$), respectively. Multiple ANOVA of these data showed no significant difference between groups ($F = 1.20$; $P = 0.27$). The mean stimulus intensities used to produce maximum CIC-evoked responses in control, ICA and CCA neurons were 47 ± 4 V ($n = 26$), 55 ± 6 V ($n = 16$), and 56 ± 4 V ($n = 18$), respectively. Again, no significant differences were found between the three groups. The maximum LL-evoked and CIC-evoked amplitudes were in the same range as reported previously (Vale & Sanes, 2000).

Figure 1B and C show examples of LL-evoked and CIC-evoked postsynaptic currents in control, ICA and CCA neurons under each pharmacological condition. Pure outward currents were observed at V_{HOLD} of -20 mV in the presence of KYN. Although strychnine and BIC eliminated most of the IPSC amplitude, a significant component did remain in all neurons in the presence of all three antagonists, as reported previously (Vale & Sanes, 2000).
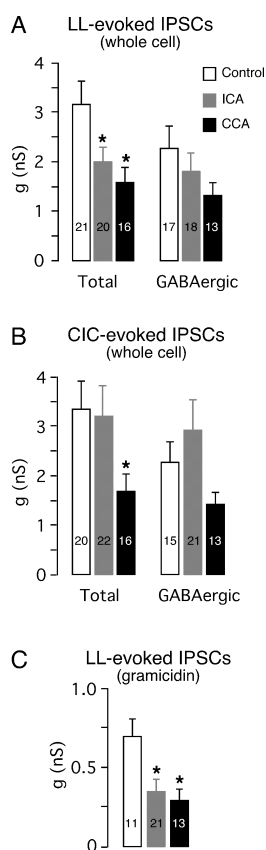
FIG. 2. Conductances of LL-evoked and CIC-evoked total inhibitory and GABAergic synaptic currents in control (open bars), ICA (grey bars) and CCA neurons (black bars). (A) In recordings performed in whole-cell voltage clamp mode, total LL-evoked inhibitory conductances were found to be significantly smaller in ICA and CCA neurons. Although there was a trend for smaller GABAergic conductances in the experimental neurons, this was not significant. (B) The inhibitory synaptic conductance of the CIC pathway declined significantly in CCA neurons, but was at the control amplitude in the ICA group. This trend was also observed for GABAergic synaptic conductances. (C) In recordings performed with gramicidin perforated-patch electrodes, total LL-evoked inhibitory conductances were again found to be significantly smaller in both ICA and CCA neurons. Values are means ± SEM with the number of observations shown in each bar. *$P < 0.05$ vs. control neurons.

Effect of unilateral ablation on LL- and CIC-evoked inhibitory conductance

Because the driving force influences IPSC amplitude, the inhibitory postsynaptic conductance (IPSG) was calculated from the slope of the inhibitory current-holding voltage curves obtained with the whole-cell recording configuration. Figure 2A summarizes the magnitude of LL-evoked IPSGs in each group. Multiple ANOVA of these data indicate a significant affect of experimental condition ($F = 5.83$; $P = 0.006$), but no effect of age ($F = 0.44$; $P = 0.51$). LL-evoked total IPSGs were 50% smaller than controls contralateral to the deafened ear (control, 3.2 ± 0.5 nS, $n = 21$; CCA, 1.6 ± 0.3 nS, $n = 16$; d.f. = 35, $t = -2.61$, $P = 0.013$). The LL-evoked total IPSG declined by 40% ipsilateral to the deaf ear (control, 3.2 ± 0.5 nS, $n = 21$; ICA, 2.0 ± 0.4 nS, $n = 20$; d.f. = 39; $t = 2.08$; $P = 0.044$). Although a similar trend was observed for GABAergic IPSGs obtained in the presence of 2 µM strychnine (Fig. 2A), the ipsilateral and contralateral values were not significantly different from controls. To verify that the effect of treatment was similar during the postsurgical period studied, the data were divided into two age groups (P8–10 and P11–14), and mean total LL-evoked inhibitory synaptic conductance values were calculated. A similar trend was obtained at both survival intervals in that control values were larger than either the ICA or the CCA groups (P8–10, control 3.7 ± 0.6, ICA 1.9 ± 0.6, CCA 1.9 ± 0.7; P11–14, control, 2.8 ± 0.4, ICA 2.0 ± 0.4, CCA 1.3 ± 0.5). Finally, an independent set of conductance values was obtained from gramicidin perforated-patch recordings, and these data confirmed the findings from whole-cell recordings (Fig. 2C).

The effect of unilateral ablation was different quantitatively for the inhibitory projection via the commissure (Fig. 2B). Multiple ANOVA showed a significant affect of experimental condition ($F = 4.9$; $P = 0.04$), but not age ($F = 0.49$; $P = 0.5$). CIC-evoked total IPSGs declined by approximately 50% contralateral to the deaf ear (control, 3.3 ± 0.6 nS, $n = 20$; CCA, 1.7 ± 0.36 nS, $n = 16$, d.f. = 34; $t = -2.36$; $P = 0.024$). However, the inhibitory conductance did not change ipsilateral to the ablated cochlea (control, 3.3 ± 0.6 nS, $n = 20$; ICA, 3.2 ± 0.6 nS, $n = 22$, d.f. = 40; $t = 0.165$; $P = 0.87$). A similar trend was evident for CIC-evoked GABAergic conductances (control vs. CCA: control, 2.3 ± 0.3 nS, $n = 15$; CCA, 1.4 ± 0.4 nS, $n = 13$; control vs. ICA: control 2.3 ± 0.3 nS, $n = 15$; ICA, 2.9 ± 0.6 nS, $n = 21$).

Effect of unilateral ablation on inhibitory equilibrium potential

We reported previously that bilateral cochlear ablation at P7 or P9 decreases inhibitory synaptic strength in IC neurons partly due to a postsynaptic alteration in the transport mechanisms that regulate chloride homeostasis (Vale & Sanes, 2000, 2002; Vale *et al.*, 2003). As caesium-containing whole-cell electrodes block potassium-dependent transport mechanisms (Figs 1 and 2), we did not obtain reversal potential data from these recordings. To determine whether unilateral ablation alters chloride homeostasis in IC neurons, the E_{IPSC} was measured using the gramicidin-perforated patch recording configuration, with potassium in the pipette solution. Figure 3A shows IPSCs obtained at several holding potentials in control, ICA, and CCA neurons. Multiple ANOVA of these data showed a significant effect of experimental condition ($F = 8.77$; $P = 0.0007$), but not age ($F = 0.08$; $P = 0.77$). IPSC reversal potentials were obtained from the linear portion of the I–V plots (Fig. 3B). As shown in Fig. 3C, the E_{IPSC} measured in the presence of KYN was depolarized by 23 mV in neurons recorded contralateral to the ablated cochlea, and this was significantly different from the control value (d.f. = 26, $t = -5.09$,
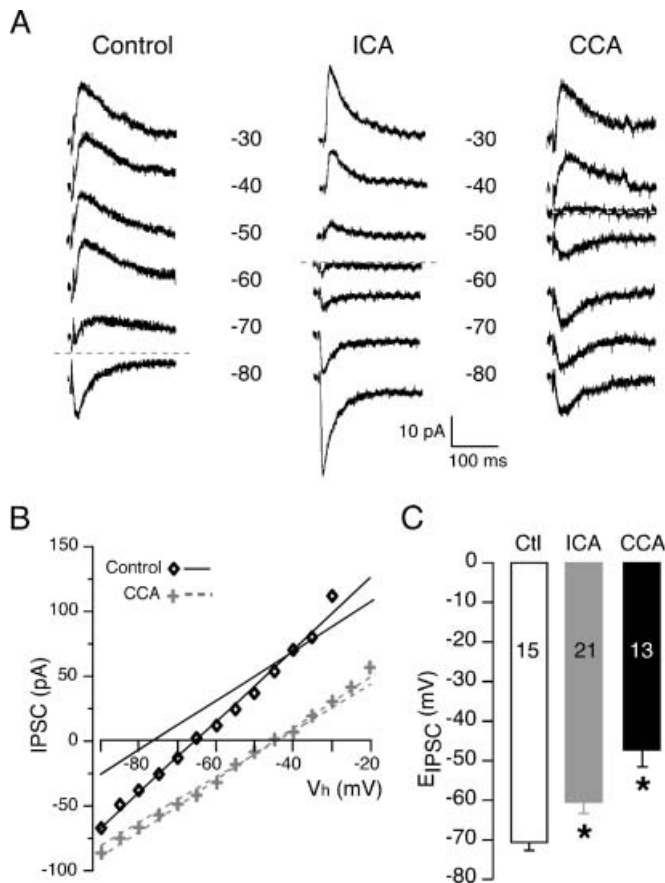
FIG. 3. Effect of unilateral cochlear ablation on IPSC equilibrium potential was assessed with gramicidin-perforated patch recordings. (A) LL-evoked IPSCs are shown for a control, an ICA, and a CCA neuron at a range of holding potentials (in mV). (B) Two representative $I–V$ plots are shown for both control and CCA neurons. Symbols show the data points for only one control and one CCA linear fit. IPSC reversal potentials were obtained from the linear portion of the $I–V$ plots. (C) The average IPSC equilibrium potential is shown for each group. ICA neurons displayed a 10 mV depolarizing shift in the mean E_{IPSC} and CCA neurons displayed a mean E_{IPSC} shift of 23 mV. Values are means ± SEM with the number of observations shown in each bar. *$P < 0.0001$ vs. control neurons.

$P < 0.0001$). In contrast, the E_{IPSC} was depolarized by only 10 mV ipsilateral to the ablated cochlea, but this was still significantly different from controls (d.f. = 34, $t = -2.54$, $P < 0.05$). The difference between the ICA and CCA groups was also significant (d.f. = 32, $t = -2.94$, $P = 0.006$).

Effect of unilateral ablation on paired-pulse facilitation

To determine whether unilateral cochlear ablation altered presynaptic function, paired-pulse responses were recorded as an indirect measure of inhibitory release properties, an accepted index of presynaptic function. For central synapses, neurotransmitter release probability across all release sites is inversely related to the amount of facilitation or depression that is observed with paired-pulse stimulation (Manabe *et al.*, 1993; Dobrunz & Stevens, 1997). As shown in Fig. 4A, control neurons displayed an increase in the amplitude of the second IPSC, as compared to the first, at all intervals tested. This facilitation was virtually absent ipsilateral to the deaf ear at all intervals. Multiple ANOVA showed a significant effect of experimental condition ($F = 10.27$; $P < 0.0001$), but not of age ($F = 2.35$; $P = 0.13$), or
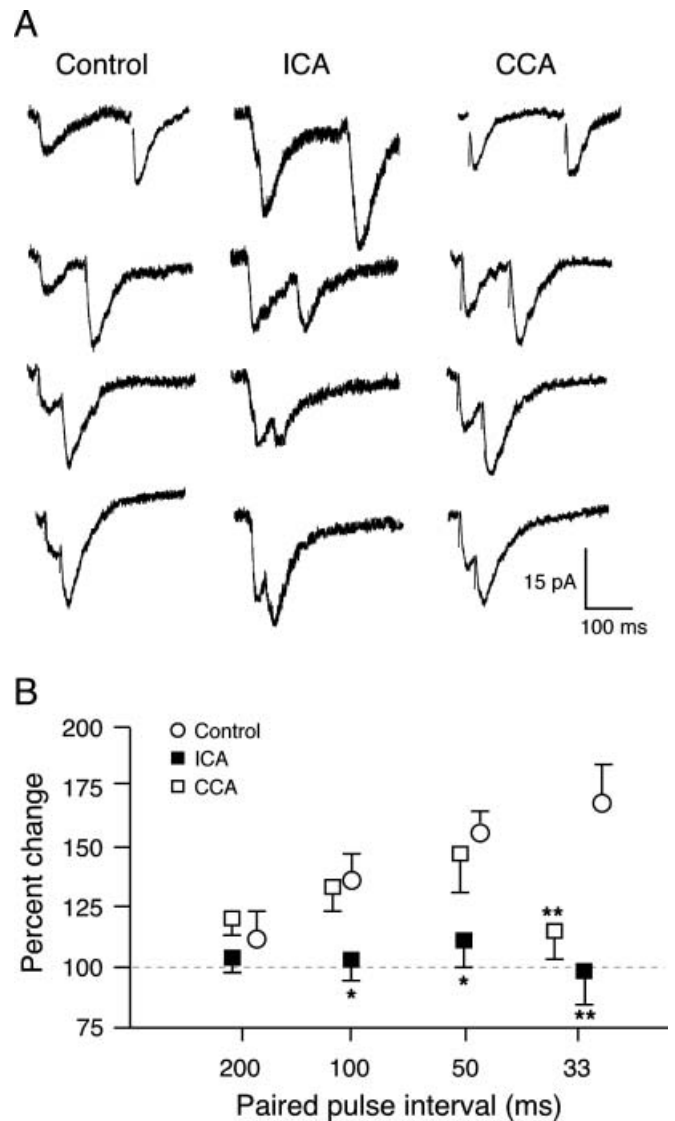

FIG. 4. Analysis of inhibitory neurotransmitter release using the paired-pulse protocol. (A) Minimum IPSCs were obtained by stimulation of the LL-pathway at intervals of 200, 100, 50 and 33 ms (from top to bottom) in a control, an ICA, and a CCA neuron. (B) Summary of the effect of paired-pulse stimulation in control neurons (circle), ICA neurons (black squares) and CCA neurons (open squares). The holding potential was −20 mV. Data were obtained in the presence of 5 mM KYN. (control $n = 19$; ICA $n = 21$; CCA $n = 19$) *$P < 0.05$ vs. control neurons, **$P < 0.01$ vs. control neurons.

paired pulse frequency ($F = 1.96$; $P = 0.12$). Significant differences between control and ICA neurons were found at intervals of 100 ms (d.f. = 36, $t = 2.63$, $P = 0.013$), 50 ms (d.f. = 38, $t = 2.81$, $P = 0.008$), and 33 ms (d.f. = 38, $t = 3.87$, $P = 0.004$). However, facilitation was absent only at an interval of 33 ms contralateral to the deaf ear (d.f. = 36, $t = -3.22$, $P = 0.003$). The mean values for each interpulse interval are shown in Fig. 4B.

Discussion

The major finding of this study was that unilateral deafening altered inhibitory synaptic function by different mechanisms and different magnitudes in the ipsilateral and contralateral IC. First, the maximum inhibitory synaptic conductance of commissural afferents was reduced

significantly only in the contralateral IC lobe (Fig. 2). Second, the inhibitory synaptic equilibrium potential was far more depolarized in neurons contralateral to the deafened ear (Fig. 3). Third, short-term facilitation was completely disrupted ipsilateral to the deafened ear, but remained intact for longer interpulse intervals in the contralateral IC (Fig. 4).

The distinct effects of unilateral cochlear ablation could stem from the prominent decrease in excitatory drive to contralateral IC, relative to the ipsilateral side. Experiments performed on invertebrate circuits and, more recently, on dissociated cortical neurons show that synapses and ion channels are each regulated by electrical activity (Marder & Prinz, 2002). Thus, cortical neurons that are cultured with the sodium channel blocker, TTX, increase their sodium channels and decrease their potassium channels (Desai *et al.*, 1999). In a similar fashion, excitatory synaptic currents increase and inhibitory currents decrease when cultures are grown in an activity blocker (Murthy *et al.*, 2001; Burrone *et al.*, 2002; Kilman *et al.*, 2002).

It is estimated that over 90% of gerbil IC neurons are synaptically excited to spike threshold by contralateral sound stimulation, whereas only approximately 25% are so activated by the ipsilateral ear (Semple & Kitzes, 1985; Brückner & Rübsamen, 1995). These studies also describe the ipsilateral excitatory input as weaker in that it is recruited at higher sound levels, and it elicits a lower maximal discharge rate. Although the sequelae of unilateral cochlear ablation are numerous, these *in vivo* recordings suggest that one basic difference exists between the two lobes of the IC; the IC lobe that is contralateral to the ablation should be more deprived of excitatory input. If decreased postsynaptic activity leads to a down-regulation of inhibitory synaptic function as suggested by the *in vitro* experiments cited above, then we would expect to observe a greater decrease of inhibitory strength contralateral to the deafened ear.

Gerbil IC neurons display spontaneous activity prior to the onset of hearing (Kotak & Sanes, 1995). Maintained discharge at the level of the eighth nerve and ventral cochlear nucleus (VCN) has been shown to originate within the cochlea of adult and juvenile mammals (Koerber *et al.*, 1966; Bock & Webster, 1974; Tucci *et al.*, 1999; Cook *et al.*, 2002), and prehatch and posthatch birds (Born & Rubel, 1988; Born *et al.*, 1991; Lippe, 1994). Spontaneous activity in the dorsal cochlear nucleus is relatively unaffected following cochlear damage in adult animals (Koerber *et al.*, 1966), and can even increase after intense noise exposure (Kaltenbach *et al.*, 2000; Brozoski *et al.*, 2002). An alteration in VCN spontaneous activity may have some impact on IC electrical activity. In the gerbil, 2-deoxyglucose studies demonstrate that the metabolic activity is decreased in the contralateral lobe of the IC following either conductive hearing loss or sensorineural hearing loss, even in relative silence (Tucci *et al.*, 1999, 2001). However, *in vivo* recordings from adult IC neurons, following unilateral neonatal hearing loss, show an increase in spontaneous and stimulus-evoked discharge rate contralateral to the deafened ear (McAlpine *et al.*, 1997). Taken together, these findings suggest that unilateral cochlear ablation, either in infancy or adulthood, results in a large, but not an exclusive, loss of peripheral excitatory transmission and action potentials during the period immediately after ablation. The discharge of IC neurons, by contrast, may be transiently reduced following neonatal ablation, but is increased by ablation in adulthood and following long recovery after neonatal ablation.

Two of the altered synaptic properties, inhibitory equilibrium potential and paired-pulse facilitation, are independent of afferent number. The positive shift in IPSC reversal potential is clearly a physiological alteration of postsynaptic IC neurons. As compared to control neurons, unilateral cochlear ablation caused an E_{IPSC} depolar-

ization of 23 mV in the contralateral IC, and a 10-mV depolarization in the ipsilateral IC. The effect on the contralateral IC was similar in magnitude to that reported after bilateral deafferentation of P7 or P9 animals (Vale & Sanes, 2000, 2002). The E_{IPSC} depolarization indicates the intracellular chloride homeostasis is disrupted by deafening. Our previous report on bilaterally deafened P9 animals indicates that E_{IPSC} depolarization is independent of cell death in the anteroventral cochlear nucleus (Vale & Sanes, 2002). Therefore, the current results suggest that the larger depolarization of E_{IPSC} in the contralateral IC is due to the relatively greater loss of excitatory drive to that lobe.

Unilateral ablation also had a distinct effect on inhibitory neurotransmitter release in the ipsilateral and contralateral IC. Paired-pulse facilitation of inhibitory synapses ipsilateral to the deaf ear was eliminated, but the effect was not as pervasive in the contralateral IC. We have previously reported a similar decrease in facilitation in both ICs of bilaterally deafened animals (Vale & Sanes, 2000, 2002). It is thought that short-term facilitation is due to a rise in residual calcium within the presynaptic terminal and an associated increase in release probability (see Regehr & Stevens, 2001). Our findings suggest that calcium homeostasis within inhibitory terminals is adversely affected by cochlear ablation. As the effect is greater for LL afferents ipsilateral to the deafened ear, we propose that these inhibitory afferents experience greater deafferentation and inactivation, as compared to those ascending through the contralateral LL. Interestingly, an alteration in calcium homeostasis and short-term plasticity has recently been demonstrated in the cochlear nucleus of the deafness mutant mouse (Oleskevich & Walmsley, 2002).

As the LL-evoked total inhibitory conductance declines (both ipsi and contra to the ablation), but GABAergic conductance is not reduced significantly, we conclude that glycinergic transmission is reduced. As the GABAergic conductance values are lowest in CCA neurons, the results suggest that GABAergic transmission may be reduced slightly. The decrease of total inhibitory conductance could be due to the amount of neurotransmitter released per afferent, the number of functional postsynaptic receptors, and/or a decrease in the number of stimulated afferents. There is evidence from previous experiments in support of each of these possibilities. A decrease in the number of inhibitory afferents via the LL to the IC would be consistent with studies showing that neonatal unilateral cochlear ablation increases cell death in the cochlear nucleus (Parks, 1979; Hashisaki & Rubel, 1989; Tierney *et al.*, 1997). For example, it is possible that cochlear damage leads to a loss of inhibitory projection neurons in nuclei of the superior olivary complex or the lateral lemniscus. Cochlear ablation also leads to a complex rearrangement of projections throughout the ascending auditory brain stem, and these changes could certainly influence the relative density of inhibitory afferents that were activated with LL stimulation (Nordeen *et al.*, 1983b; Moore & Kitzes, 1985; Sanes & Takács, 1993; Kitzes *et al.*, 1995; Russell & Moore, 1995; Gabriele *et al.*, 2000). A decrease in inhibitory neurotransmitter release has been described in the IC and nuclei projecting to the IC after complete (Bledsoe *et al.*, 1995) or partial sensorineural hearing loss in adults (Suneja *et al.*, 1998a). Similarly, a decrease in GABA synthesizing enzyme is observed in the adult mammalian IC after unilateral cochlear ablation (Mossop *et al.*, 2000). Changes in the distribution of GABA and glycine receptors have been described in several nuclei projecting to the IC after unilateral cochlear ablation (Koch & Sanes, 1998; Suneja *et al.*, 1998a, 1998b; Potashner *et al.*, 2000). It is likely that each factor contributes to our current observations. It should be noted that the present study did not take into account the electrophysiological diversity in the IC (Li *et al.*, 1998; N'Gouemo & Rittenhouse, 2000; Sivaramakrishnan & Oliver, 2001;

Bal *et al.*, 2002), and it remains possible that the results can be attributed to a subset of IC neurons.

Our present results are consistent with, and may help to clarify, the observations from *in vivo* electrophysiological studies of deafened neonatal animals. The increase in sound-evoked discharges that is consistently observed in the IC on the side of the intact ear to stimulation of that ear (Kitzes, 1984; Kitzes & Semple, 1985; Szczepaniak & Moller, 1995; McAlpine *et al.*, 1997; Mossop *et al.*, 2000) could be due primarily to a decrease of inhibitory synaptic function. While that hypothesis has many elements in common with the results reported here, the *in vivo* work has shown that much, but not all of the change resulting from deafness occurs very rapidly (within minutes) and also occurs in adult-deafened animals (McAlpine *et al.*, 1997; Mossop *et al.*, 2000). Further *in vitro* research will be required to establish how quickly the synaptic changes described here occur after deafferentation and to what extent they occur in mammals that have passed through the very early developmental period of extreme susceptibility to cochlear removal (Tierney *et al.*, 1997). These experiments may benefit from continuing electrical stimulation of the severed cochlear nerve endings after slice removal to simulate normal afferent function (Hyson & Rubel, 1989).

Acknowledgements

Abbreviations

ACSF, artificial cerebrospinal fluid; CCA, contralateral cochlear ablation; CIC, commissure of the inferior colliculus; CN, cochlear nucleus; E_{IPSC}, IPSC reversal potential; IC, inferior colliculus; ICA, ipsilateral cochlear ablation; IPSC, inhibitory postsynaptic current; IPSG, inhibitory synaptic conductance; KYN, kynurenic acid; LL, lateral lemniscus.

References

Adams, J.C. (1979) Ascending projections to the inferior colliculus. *J. Comp. Neurol.*, **183**, 519–538.

Bal, R., Green, G.G., Rees, A. & Sanders, D.J. (2002) Firing patterns of inferior colliculus neurons-histology and mechanism to change firing patterns in rat brain slices. *Neurosci. Lett.*, **317**, 42–46.

Bledsoe, S.C. Jr, Nagase, S., Miller, J.M. & Altschuler, R.A. (1995) Deafness-induced plasticity in the mature central auditory system. *Neuroreport*, **7**, 225–229.

Bock, G.R. & Webster, W.R. (1974) Spontaneous activity of single units in the inferior colliculus of anesthetized and unanesthetized cats. *Brain Res.*, **76**, 150–154.

Born, D.E., Durham, D. & Rubel, E.W. (1991) Afferent influences on brainstem auditory nuclei of the chick: nucleus magnocellularis neuronal activity following cochlea removal. *Brain Res.*, **557**, 37–47.

Born, D.E. & Rubel, E.W. (1988) Afferent influences on brain stem auditory nuclei of the chicken: presynaptic action potentials regulate protein synthesis in nucleus magnocellularis neurons. *J. Neurosci.*, **8**, 901–919.

Brozoski, T.J., Bauer, C.A. & Caspary, D.M. (2002) Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J. Neurosci.*, **22**, 2383–2390.

Brückner, S. & Rübsamen, R. (1995) Binaural response characteristics in isofrequency sheets of the gerbil inferior colliculus. *Hear. Res.*, **86**, 1–14.

Burrone, J., O'Byrne. M. & Murthy, V.N. (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature*, **420**, 414–418.

Coleman, J.R. & Clerici, W.J. (1987) Sources of projections to subdivisions of the inferior colliculus in the rat. *J. Comp. Neurol.*, **262**, 215–226.

Cook, R.D., Hung, T.Y., Miller, R.L., Smith, D.W. & Tucci, D.L. (2002) Effects of conductive hearing loss on auditory nerve activity in gerbil. *Hear. Res.*, **164**, 127–137.

Desai, N.S., Rutherford, L.C. & Turrigiano, G.G. (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nature Neurosci.*, **2**, 515–520.

Dobrunz, L.E. & Stevens, C.F. (1997) Heterogeinity of release probability, facilitation, and depletion at central synapses. *Neuron*, **18**, 995–1008.

Francis, H.W. & Manis, P.B. (2000) Effects of deafferentation on the electrophysiology of ventral cochlear nucleus neurons. *Hear. Res.*, **149**, 91–105.

Gabriele, M.L., Brunso-Bechtold, J.K. & Henkel, C.K. (2000) Plasticity in the development of afferent patterns in the inferior colliculus of the rat after unilateral cochlear ablation. *J. Neurosci.*, **20**, 6939–6949.

Goda, Y. & Stevens, C.F. (1998) Readily releasable pool size changes associated with long term depression. *Proc. Natl Acad. Sci. USA*, **95**, 1283–1288.

Hardie, N., Martsi-McClintock, A., Aitkin, L. & Shepherd, R. (1998) Neonatal sensorineural hearing loss affects synaptic density in the auditory midbrain. *Neuroreport*, **9**, 2019–2022.

Hashisaki, G.T. & Rubel, E.W. (1989) Effects of unilateral cochlea removal on anteroventral cochlear nucleus neurons in developing gerbils. *J. Comp. Neurol.*, **283**, 465–473.

Hyson, R.L. & Rubel, E.W. (1989) Transneuronal regulation of protein synthesis in the brain-stem auditory system of the chick requires synaptic activation. *J. Neurosci.*, **9**, 2835–2845.

Kaltenbach, J.A., Zhang, J. & Afman, C.E. (2000) Plasticity of spontaneous neural activity in the dorsal cochlear nucleus after intense sound exposure. *Hear. Res.*, **147**, 282–292.

Kilman, V., van Rossum, M.C. & Turrigiano, G.G. (2002) Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses. *J. Neurosci.*, **22**, 1328–1337.

Kitzes, L.M. (1984) Some physiological consequences of neonatal cochlear destruction in the inferior colliculus of the gerbil, *Meriones unguiculatus*. *Brain Res.*, **306**, 171–178.

Kitzes, L.M., Kageyama, G.H., Semple, M.N. & Kil, J. (1995) Development of ectopic projections from the ventral cochlear nucleus to the superior olivary complex induced by neonatal ablation of the contralateral cochlea. *J. Comp. Neurol.*, **353**, 341–363.

Kitzes, L.M. & Semple, M.N. (1985) Single-unit responses in the inferior colliculus: effects of neonatal unilateral cochlear ablation. *J. Neurophysiol.*, **53**, 1483–1500.

Koch, U. & Sanes, D.H. (1998) Afferent regulation of glycine receptor distribution in the gerbil LSO. *Microsc. Res. Techn.*, **41**, 263–269.

Koerber, K.C., Pfeiffer, R.R., Warr, W.B. & Kiang, N.Y. (1966) Spontaneous spike discharges from single units in the cochlear nucleus after destruction of the cochlea. *Exp. Neurol.*, **16**, 119–130.

Kotak, V.C., DiMattina, C. & Sanes, D.H. (2001) GABA(B) and Trk receptor signaling mediates long-lasting inhibitory synaptic depression. *J. Neurophysiol.*, **86**, 536–540.

Kotak, V.C. & Sanes, D.H. (1995) Synaptically evoked prolonged depolarizations in the developing auditory system. *J. Neurophysiol.*, **74**, 1611–1620.

Kotak, V.C. & Sanes, D.H. (1996) Developmental influence of glycinergic transmission: regulation of NMDA receptor-mediated EPSPs. *J. Neurosci.*, **16**, 1836–1843.

Kotak, V.C. & Sanes, D.H. (1997) Deafferentation weakens excitatory synapses in the developing central auditory system. *Eur. J. Neurosci.*, **11**, 2340–2347.

Kuwada, S., Batra, R., Yin, T.C.T., Oliver, D.L., Haberly, L.B. & Stanford, T.R. (1997) Intracellular recordings in response to monaural and binaural stimulation of neurons in the inferior colliculus of the cat. *J. Neurosci.*, **17**, 7565–7581.

Li, Y., Evans, M.S. & Faingold, C.L. (1998) *In vitro* electrophysiology of neurons in subnuclei of rat inferior colliculus. *Hear. Res.*, **121**, 1–10.

Lippe, W.R. (1994) Rhythmic spontaneous activity in the developing avian auditory system. *J. Neurosci.*, **14**, 1486–1495.

Lo, Y.-J., Rao, S.C. & Sanes, D.H. (1998) Modulation of calcium by inhibitory systems in the developing auditory midbrain. *Neuroscience*, **83**, 1075–1084.

Manabe, T., Wyllie, D.J., Perkel, D.J. & Nicoll, R.A. (1993) Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *J. Neurophysiol.*, **70**, 1451–1459.

Marder, E. & Prinz, A.A. (2002) Modeling stability in neuron and network function: the role of activity in homeostasis. *Bioessays*, **24**, 1145–1154.

McAlpine, D., Martin, R.L., Mossop, J.E. & Moore, D.R. (1997) Response properties of neurons in the inferior colliculus of the monaurally deafened ferret to acoustic stimulation of the intact ear. *J. Neurophysiol.*, **78**, 767–779.

Moore, D.R. (1992) Developmental plasticty of the brainstem and midbrain auditory nuclei. In Romand, R. (Ed), *Development of Auditory and Vestibular Systems 2*. Elsevier, Amsterdam, pp. 297–320.

Moore, D.R. (1994) Auditory brainstem of the ferret: long survival following cochlear removal progressively changes projections from the cochlear nucleus to the inferior colliculus. *J. Comp. Neurol.*, **339**, 301–310.

Moore, D.R. & Kitzes, L.M. (1985) Projections from the cochlear nucleus to the inferior colliculus in normal and neonatally cochlea-ablated gerbils. *J. Comp. Neurol.*, **240**, 180–195.

Moore, D.R., Kotak, V.C. & Sanes, D.H. (1998) Commissural and lemniscal synaptic input to the gerbil inferior colliculus. *J. Neurophysiol.*, **80**, 2229–2236.

Moore, D.R. & Kowalchuck, N.E. (1988) Auditory brainstem of the ferret: effects of unilateral cochlear lesions on cochlear nucleus volumen and projections to the inferior colliculus. *J. Comp. Neurol.*, **272**, 503–515.

Mossop, J.E., Wilson, M.J., Caspary, D.M. & Moore, D.R. (2000) Down-regulation of inhibition following unilateral deafening. *Hear. Res.*, **147**, 183–187.

Murthy, V.N., Schikorski, T., Stevens, C.F. & Zhu, Y. (2001) Inactivity produces increases in neurotransmitter release and synapse size. *Neuron*, **32**, 673–682.

N'Gouemo, P. & Rittenhouse, A.R. (2000) Biophysical and pharmacological characterization of voltage-sensitive calcium currents in neonatal rat inferior colliculus neurons. *Neurosci.*, **96**, 753–765.

Nordeen, K.W., Killackey, H.P. & Kitzes, L.M. (1983a) Ascending auditory projections to the inferior colliculus in the adult gerbil, *Meriones unguiculatus*. *J. Comp. Neurol.*, **214**, 131–143.

Nordeen, K.W., Killackey, H.P. & Kitzes, L.M. (1983b) Ascending projections to the inferior colliculus following unilateral cochlear ablation in the neonatal gerbil, *Meriones unguiculatus*. *J. Comp. Neurol.*, **214**, 144–153.

Oleskevich, S. & Walmsley, B. (2002) Synaptic transmission in the auditory brainstem of normal and congenitally deaf mice. *J. Physiol. (Lond.)*, **540**, 447–455.

Oliver, D.L., Winer, J.A., Beckius, G.E. & Saint Marie, R.L. (1994) Morphology of GABAergic neurons in the inferior colliculus of the cat. *J. Comp. Neurol.*, **340**, 27–42.

Parks, T.N. (1979) Afferent influences on the development of the brain stem auditory nuclei of the chicken: otocyst ablation. *J. Comp. Neurol.*, **183**, 665–678.

Parks, T.N. (1999) Cochlear influences on development of the brainstem auditory system. In Hyson, R.L. & Johnson, F. (Eds), *The Biology of Early Influences*. New York Academic Press, New York, pp. 15–34.

Potashner, S.J., Suneja, S.K. & Benson, C.G. (2000) Altered glycinergic synaptic activities in guinea pig brain stem auditory nuclei after unilateral cochlear ablation. *Hear. Res.*, **147**, 125–136.

Reetz, G. & Ehret, G. (1999) Inputs from three brainstem sources to identified neurons of the mouse inferior colliculus slice. *Brain Res.*, **816**, 527–543.

Regehr, W.G. & Stevens, C.F. (2001) Physiology of synaptic transmission and short-term plasticity. In Cowan, W.M., Südhof, T.C. & Stevens, C.F. (Eds), *Synapses*. John Hopkins University Press, Baltimore, pp. 135–175.

Rhee, J.S., Ebihara, S. & Akaike, N. (1994) Gramicidin perforated patch-clamp technique reveals glycine-gated outward chloride current in dissociated nucleus solitarii neurons of the rat. *J. Neurophysiol.*, **72**, 1103–1108.

Rubel, E.W. (1978) Ontogeny of structure and function in the vertebrate auditory system. In Jacobson, M., (Ed), *Handbook of Sensory Physiology*, **Vol. IX** Development of Sensory Systems. Springer, New York, pp. 135–237.

Russell, F.A. & Moore, D.R. (1995) Afferent reorganization within the superior olivary complex of the gerbil: development and induction by neonatal, unilateral cochlear removal. *J. Comp. Neurol.*, **352**, 207–225.

Sanes, D.H., Markowitz, S., Bernstein, J. & Wardlow, J. (1992) The influence of inhibitory afferents on the development of postsynaptic dendritic arbors. *J. Comp. Neurol.*, **321**, 637–644.

Sanes, D.H. & Takács, C. (1993) Activity-dependent refinement of inhibitory connections. *Eur. J. Neurosci.*, **5**, 570–574.

Semple, M.N. & Kitzes, L.M. (1985) Single-unit responses in the inferior colliculus: different consequences of contralateral and ipsilateral auditory stimulation. *J. Neurophysiol.*, **53**, 1467–1482.

Sivaramakrishnan, S. & Oliver, D.L. (2001) Distinct K currents result in physiologically distinct cell types in the inferior colliculus of the rat. *J. Neurosci.*, **21**, 2861–2877.

Smith, P.H. (1992) Anatomy and physiology of multipolar-cells in the rat inferior collicular cortex using the *in vitro* brain slice techniques. *J. Neurosci.*, **12**, 3700–3715.

Suneja, S.K., Benson, C.G. & Potashner, S.J. (1998b) Glycine receptors in adult guinea pig brain stem auditory nuclei: regulation after unilateral cochlear ablation. *Exp. Neurol.*, **154**, 473–488.

Suneja, S.K., Potashner, S.J. & Benson, C.G. (1998a) Plastic changes in glycine and GABA release and uptake in adult brain stem auditory nuclei after unilateral middle ear ossicle removal and cochlear ablation. *Exp. Neurol.*, **151**, 273–288.

Syka, J. (2002) Plastic changes in the central auditory system after hearing loss, restoration of function, and during learning. *Physiol. Rev.*, **82**, 601–636.

Szczepaniak, W.S. & Moller, A.R. (1995) Evidence of decreased GABAergic influence on temporal integration in the inferior colliculus following acute noise exposure: a study of evoked potentials in the rat. *Neurosci. Lett.*, **196**, 77–80.

Tierney, T.S., Russell, F.A. & Moore, D.R. (1997) Susceptibility of developing cochlear nucleus neurons to deafferentation-induced death abruptly ends just before the onset of hearing. *J. Comp. Neurol.*, **378**, 295–306.

Tucci, D.L., Cant, N.B. & Durham, D. (1999) Conductive hearing loss results in a decrease in central auditory system activity in the young gerbil. *Laryngoscope*, **109**, 1359–1371.

Tucci, D.L., Cant, N.B. & Durham, D. (2001) Effects of conductive hearing loss on gerbil central auditory system activity in silence. *Hear. Res.*, **155**, 124–132.

Vale, C. & Sanes, D.H. (2000) Afferent regulation of inhibitory synaptic transmission in the developing auditory midbrain. *J. Neurosci.*, **20**, 1912–1921.

Vale, C. & Sanes, D.H. (2002) The effect of bilateral deafness on inhibitory and excitatory synaptic strength in the inferior colliculus. *Eur. J. Neurosci.*, **16**, 2394–2404.

Vale, C., Schoorlemmer, J. & Sanes, D.H. (2003) Deafness disrupts chloride transporter function and inhibitory synaptic transmission. *J. Neurosci.*, **23**, 7516–7524.

Wagner, T. (1996) Lemniscal input to identified neurons of the central nucleus of mouse inferior colliculus: an intracellular brain slice study. *Eur. J. Neurosci.*, **8**, 1231–1239.

Wu, L. & Borst, J.G. (1999) The reduced release probability of releasable vesicles during recovery from short-term synaptic depression. *Neuron*, **23**, 821–832.

Wu, S.H. & Kelly, J.B. (1996) *In vitro* brain slice studies of the rat's dorsal nucleus of the lateral lemniscus. III. synaptic pharmacology. *J. Neurophysiol.*, **75**, 1271–1282.

Zucker, R.S. & Regehr, W.C. (2002) Short-term plasticity. *Ann. Rev. Physiol.*, **64**, 355–405.