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The effect of bilateral deafness on excitatory and inhibitory synaptic strength in the inferior colliculus

Carmen Vale[1,3] and Dan H. Sanes[1,2]
[1]Center for Neural Science and [2]Department of Biology, 4 Washington Place, New York University, New York, NY 10003, USA
[3]School of Medicine and Centro Regional de Investigaciones Biomedicas (CRIB), University of Castilla–La Mancha, Campus Universitario s/n, Albacete, 02071, Spain

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Abstract

The consequences of deafness on the central auditory nervous system have been examined at many levels, from molecular to functional. However, there has never been a direct and selective measurement of excitatory synaptic function following total hearing loss. In the present study, gerbils were deafened at postnatal day 9, an age at which there is no deafferentation-induced cell death of ventral cochlear nucleus neurons. One to five days after bilateral cochlear ablation, the amplitude of evoked excitatory postsynaptic currents (EPSC) was measured with whole-cell voltage-clamp recordings in an inferior colliculus (IC) brain slice preparation in response to electrical stimulation of the ipsilateral lateral lemniscus (LL) or the commissure of the inferior colliculus (CIC). Deafness resulted in larger LL- and CIC-evoked EPSC amplitudes and durations. This result was observed at a depolarized holding potential. In addition, deafness caused a decrease in excitatory neurotransmitter release at the LL pathway, as assessed with a paired-pulse stimulation protocol. In contrast to its effect on excitatory synapses, bilateral cochlear ablation reduced inhibitory synaptic strength in IC neurons. The effects included a postsynaptic decrease in IPSC conductance, a 25-mV depolarization in the IPSC equilibrium potential and a decrease of neurotransmitter release. Thus normal innervation differentially affects excitatory and inhibitory synaptic strength in IC neurons, and these changes may contribute to alterations in auditory coding properties following sensory deprivation.

Introduction

A decrease in electrical activity or synaptic transmission can lead to degenerative changes in the developing nervous system (Linden, 1994) and interfere with the maturation or maintenance of single neuron coding properties (Sherman & Spear, 1982; Katz & Shatz, 1996; Gold & Knudsen, 1999; Kelly *et al*., 1999; Huang & Pallas, 2001). While degenerative anatomical alterations account for some alterations in neuronal processing, little is known about changes in synaptic physiology that accompany deafferentation or sensory deprivation.

In the central auditory system, partial sensory deafferentation results in reduced sound-evoked inhibitory responses, in both the inferior colliculus (Kitzes & Semple, 1985; Bledsoe *et al*., 1995; McAlpine *et al*., 1997) and the auditory cortex (Rajan, 1998). There are many possible reasons for this effect, including loss of afferents due to deafferentation-induced cell death in the cochlear nucleus (Parks, 1979; Hashisaki & Rubel, 1989; Tierney *et al*., 1997; Mostafapour *et al*., 2000), compensatory sprouting of afferents (Moore & Kitzes, 1985; Kitzes *et al*., 1995; Russell & Moore, 1995), a direct alteration of membrane properties (Francis & Manis, 2000) or a change in synaptic strength (Kotak & Sanes, 1996).

We reported previously that unilateral cochlear damage produces large changes in the strength of both inhibitory and excitatory connections within the gerbil lateral superior olive (Kotak & Sanes, 1996, 1997). In the gerbil inferior colliculus (IC), bilateral cochlear ablation at postnatal day (P)7 causes a rapid loss of inhibitory synaptic strength through both pre- and postsynaptic mechanisms, including a 24-mV depolarizing shift of the inhibitory equilibrium potential (Vale & Sanes, 2000). Because the IC receives many excitatory projections, it is critical to know whether the strength of these afferents are also influenced by deafness.

In the present study, gerbils were used to examine the effect of deafness on excitatory synaptic connections after the period when cochlear nucleus neurons are susceptible to cochlea removal. Cochlear ablation at P7 leads to a 50% cell death as well as soma shrinkage in the ventral cochlear nucleus within 2 weeks (Hashisaki & Rubel, 1989). However, cochlear ablation at P9 does not produce neuronal cell death (Tierney *et al*., 1997). The strength of excitatory synaptic connections was assessed independently of inhibition in brain slices through the gerbil inferior colliculus. Our results indicated that hearing loss induces large changes in the strength of both inhibitory and excitatory synapses. This creates an imbalance of synaptic drive that can account for observed alterations in auditory coding properties following partial hearing loss.

Materials and methods

Cochlear ablation

All protocols were reviewed and approved by the New York University Institutional Animal Care and Use Committee. Cochlear ablations were performed using procedures similar to those described

Correspondence: Dr Dan H. Sanes, [1]Center for Neural Science, as above.
E-mail: sanes@cns.nyu.edu

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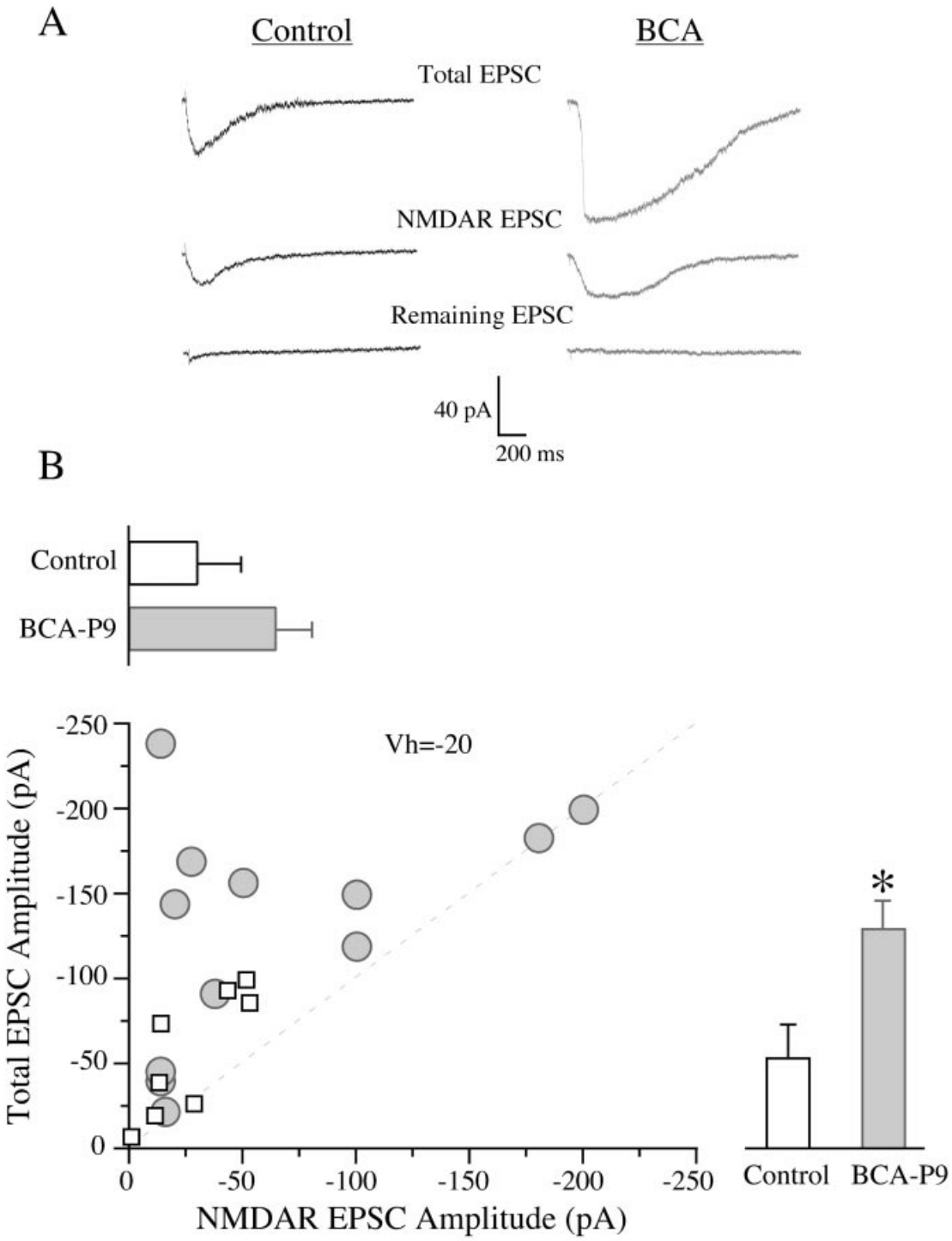


FIG. 1. Evoked EPSCs were larger in BCA neurons than in controls. (A) LL-evoked EPSCs are shown for a control (left) and a BCA neuron (right) under different pharmacological conditions. Total EPSCs were obtained in the presence of SN and BIC. The addition of CNQX (NMDA-mediated EPSC) led to a decrease in the EPSC amplitude for both neurons. The further addition of AP-5 eliminated the remaining EPSC in both neurons. Both control and BCA recordings were obtained from P12 animals at a V_{HOLD} of –20 mV. (B) The graph plots the size of total and NMDA-mediated EPSCs for each control and BCA neuron tested at a V_{HOLD} of –20 mV. The average total and NMDA-mediated EPSC size is plotted on bar graphs. Values are expressed as mean ± SEM. The average total EPSC amplitude was significantly greater in BCA neurons than in controls (see text for statistics). In the presence of CNQX, some BCA neurons exhibited a large reduction in amplitude while others displayed almost no reduction (i.e. those lying near to the dashed line). This result suggests that some BCA neurons acquire a greater number of functional NMDA receptors whereas other acquire a greater number of non-NMDA receptors.

previously (Sanes *et al*., 1992). Gerbil (*Meriones unguiculatus*) pups at P9 were anaesthetized with the halogenated ethyl methyl ether, methoxyflurane (Metofane). Anaesthetic induction occurred within 3 min, and produced complete elimination of responses to nociceptive stimuli. A small hole was made in the cochlear wall, and the contents were rapidly removed with forceps. A piece of gelfoam was

then placed in the cavity and the wound was closed. Ablations were performed bilaterally. Following surgery, animals were warmed on a heating pad and returned to the litter when respiration and motor activity had recovered. Prior to each brain slice experiment, successful ablations were confirmed 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. The age of surgery was chosen based upon the work of Tierney *et al*. (1997) showing that anteroventral cochlear nucleus cell number is unaffected by cochlear ablation at P9.

Brain slice preparation

Control and bilaterally ablated P10–14 gerbils were anaesthetized with chloral hydrate (400 mg/kg). Following decapitation, 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). The tissue was cut in cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 123; KCl, 4; KH_2PO_4, 1.2; $MgSO_4$, 1.3; $NaHCO_3$, 28; glucose, 15; $CaCl_2$, 2.4; and L-ascorbic acid, 0.4 (pH = 7.3 when oxygenated with 95% O_2 : 5% CO_2). Coronal slices of 300 μm were obtained in cold ACSF and maintained in an incubation chamber at room temperature for 2 h. Slices containing the rostral IC were transferred to a recording chamber and superfused with oxygenated ACSF (7 mL/min) at room temperature (22–24 °C).

Electrophysiology

Whole-cell and gramicidin perforated-patch recordings (Warner Instruments PC-501A) were obtained as described previously (Vale & Sanes 2000). For whole-cell recordings, the internal pipette solution contained (in mM): caesium gluconate, 127.5; EGTA, 0.6; HEPES, 10; $MgCl_2$, 2; KCl, 5; ATP, 2; GTP, 0.3; and QX-314, 5 (pH = 7.2). The tip resistance was 5–10 MΩ. Access resistance was balanced throughout the recordings and generally ranged between 10 and 30 MΩ. For perforated-patch recordings (Reichling *et al*., 1994; Ebihara *et al*., 1995), the internal pipette solution contained 2–5 μg/mL gramicidin (Sigma), and caesium gluconate was replaced by KCl to avoid alterations in the potassium–chloride cotransporter mechanism (Kazaku *et al*., 1999). The presence of depolarization-evoked breakaway action potentials was taken as indication of the integrity of the patch (i.e. QX-314 did not enter the neuron). 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.

Extracellular stimuli (200-μs pulses, ranging from 5 to 100 mV) were delivered through Teflon-insulated paired platinum electrodes driven by isolated biphasic stimulators (Intronics Inc, Norwood, MA, USA). Stimulating electrodes were placed in the afferent pathways from the commissure of the inferior colliculus (CIC) and the lateral lemniscus (LL).

Data were collected using a Macintosh PPC running a custom-designed IGOR (WaveMetrics, v3.14; WaveMetrics Inc, Lake Oswego, OR, USA) macro called *SLICE* (Kotak *et al*., 2001). Stimuli were delivered and data sampled via an ITC-18 Computer Interface (Instrutech Corporation, Port Washington, NY, USA). Analyses of peak postsynaptic current (PSC) amplitude, duration and reversal potential were performed off-line using a second IGOR macro called *SLICE ANALYSIS*. Peak PSC amplitude was measured by determining the maximum value after the stimulus artifact and subtracting the mean value obtained during the prestimulus period. PSC duration was measured by determining the latency at which the trace returned to baseline and subtracting the latency at which the trace first deviated from the baseline values. The software and a description of the algorithms are available at http://www.cns.nyu.edu/~sanes/slice_software/index.html.

Measurements of excitatory postsynaptic currents (EPSC) were performed in whole-cell voltage-clamp mode. Glycine and $GABA_A$ receptors were blocked by adding 4 μM strychnine (SN; Sigma-Aldrich Corp, St. Louis, MO, USA) and 50 μM bicuculline methobromide (BIC; RBI-Sigma, Natick, MA, USA), respectively. The total excitatory postsynaptic current was blocked by the sequential addition of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris Cookson Inc, Ellisville, MO, USA), followed by 20 μM DL-2-amino-5-phosphonopentanoic-acid (AP-5; Tocris Cookson Inc). The effect of CNQX and AP-5 were reversible after removal. The maximum amplitude of evoked EPSCs was obtained at membrane holding potentials (V_{HOLD}) of –20 and –80 mV. Measurements of inhibitory postsynaptic current (IPSC) equilibrium potential (E_{IPSC}) were performed with gramicidin perforated-patch electrodes in voltage-clamp mode in the presence of 5 mM kynurenic acid (KYN). The E_{IPSC} was calculated from linear fits of the current-voltage curves plotting IPSC amplitude vs. membrane holding potential. Inhibitory and excitatory neurotransmitter release was measured using a paired-pulse protocol. For excitatory currents, short stimulus intervals occasionally elicited long-lasting currents, particularly at a V_{HOLD} of –20 and –80 mV, possibly mediated by voltage-gated calcium channels. Therefore, excitatory paired-pulse responses were analysed at a V_{HOLD} of 0 mV, a potential at which voltage-gated calcium channels were inactivated.

For each parametric measure, a one-way analysis of variance (ANOVA) followed by pairwise comparisons (Student's *t*-test) was used to assess whether significant differences existed between neurons from control and bilaterally ablated animals, except as noted in Results. All values are expressed as mean ± SEM, with the number of observations in parentheses.

Results

To study the influence of cochlear damage without ventral cochlear nucleus cell death, bilateral cochlear ablations (BCA) were performed at P9 when neuron survival no longer depends on eighth-nerve afferents (Tierney *et al*., 1997). In perforated-patch recordings, the resting membrane potential of control and BCA neurons was not significantly different (control –53 ± 1 mV, $n = 38$; BCA –55 ± 1 mV, $n = 14$). The data presented here were obtained from a total of 46 control animals and 20 BCA animals.

Excitatory postsynaptic currents in control IC neurons

Following pharmacological isolation of excitatory synaptic current, the LL- and CIC-evoked EPSCs were analysed at a V_{HOLD} of –20 and –80 mV. At –20 mV, the EPSCs are mediated by both NMDA and non-NMDA receptors, whereas at hyperpolarizing membrane voltages the response is predominantly mediated by the non-NMDA component in Mg^{2+}-containing ACSF (Wu & Kelly, 1996).

Figure 1A shows examples of LL-evoked EPSCs in a control and a BCA-P9 neuron under each pharmacological condition. A summary of LL- and CIC-evoked maximum EPSC amplitudes are shown in Tables 1 and 2. In control animals, the average LL-evoked total EPSCs were –57 ± 9 pA ($n = 17$) at a V_{HOLD} of –20 mV. This was significantly larger than the EPSC amplitude elicited with CIC stimulation, –29 ± 4 pA ($n = 15$) (d.f. = 30, $t = 2.668$, $P = 0.012$).

Bath application of 20 μM CNQX reduced the LL-evoked total inward EPSC amplitude by 63% (d.f. = 24, t = –2.156, P = 0.041) at a V_{HOLD} of –20 mV. The further addition of 20 μM AP-5 eliminated the remaining inward current (Fig. 1 and Table 1). At a V_{HOLD} of –80 mV, the LL-evoked total EPSC was reduced by 85% in the presence of CNQX (d.f. = 20, t = –2.997, P = 0.007). CIC-evoked total EPSCs exhibited a similar sensitivity to holding potential and glutamate receptor antagonists (Table 2). For example, at a V_{HOLD} of –80 mV, CIC-evoked EPSCs were reduced by 86% in the presence of CNQX (d.f. = 17, t = –3.293, P = 0.004).

Excitatory postsynaptic currents increased following bilateral cochlear ablation

Bilateral cochlear ablation produced a significant increase in the total EPSC amplitude, and this effect was more prominent at a V_{HOLD} of –20 mV. The total EPSC amplitudes were 70% greater in neurons from BCA animals than in control neurons (control –57 ± 9 pA, n = 17; BCA –102 ± 16 pA, n = 17; d.f. = 32, t = –2.393, P = 0.023). The duration of LL-evoked total EPSCs was also larger in BCA neurons (control 499 ± 73 ms, n = 11; BCA 723 ± 66 ms; d.f. = 24, t = 2.251, P = 0.034). A similar observation was made in the presence of CNQX, but the difference did not reach significance due to the high variability of the NMDA-mediated currents (d.f. = 20, t = –1.579, P = 0.130 for EPSC amplitude and d.f. = 15, t = 1.386, P = 0.186 for EPSC duration). Table 1 shows that the LL-evoked total EPSCs were also larger in BCA neurons at a V_{HOLD} of –80 mV, but this was not significant (d.f. = 26, t = –0.789, P = 0.437 for EPSC amplitude and d.f. = 17, t = 1.498, P = 0.152 for EPSC duration). Multiple ANOVA of data in Table 1 showed a significant effect of experimental condition (F = 9.11, P = 0.0054) and age (F = 6.60; P = 0.016) on LL-evoked total EPSC amplitude at a V_{HOLD} of –20 mV. At a V_{HOLD} of –80 mV, no effect of age (F = 0.47, P = 0.50) or experimental condition (F = 1.68, P = 0.21) was observed.

Figure 1B plots the LL-evoked total and NMDA-mediated EPSC amplitudes. Both the control and BCA neurons displayed variability in the amount of current blocked by CNQX. Most EPSCs were composed of both NMDA and non-NMDA components, but a few cells in each group were composed entirely of an NMDA component (points on the dashed line in Fig. 1B). However, the ratio of total to NMDA EPSC amplitude did not differ significantly between control and BCA neurons (control 2.1 ± 0.4, n = 8; BCA 2.8 ± 0.6, n = 11; d.f. = 17, t = 0.903, P = 0.38). The graph illustrates that larger EPSCs in deafened animals occurred through two different mechanisms. One group of BCA neurons displayed larger total EPSCs but similar NMDA-mediated EPSCs, suggesting that AMPA receptors had increased. A second group of BCA neurons displayed larger total and NMDA-mediated EPSCs, suggesting that NMDA receptors had increased.

A similar set of observations were made on EPSCs which were elicited by stimulation of the CIC pathway (Table 2). Maximum total EPSC amplitude was significantly larger in BCA neurons at a V_{HOLD} of –20 mV (control –29 ± 4, n = 15; BCA –77 ± 17, n = 13; d.f. = 26, t = 2.874, P = 0.008). Total CIC-evoked EPSC duration was also larger at a V_{HOLD} of –20 mV (control 339 ± 45 ms, n = 9; BCA 693 ± 107 ms, n = 12; d.f. = 19, t = 2.706, P = 0.014). At a V_{HOLD} of –80 mV there was not a significant difference between total EPSC amplitude (d.f. = 21, t = –0.603, P = 0.553) or total EPSC duration (d.f. = 13, t = 1.292, P = 0.219). In the presence of CNQX, at a V_{HOLD} of –20 mV, the NMDA-mediated EPSCs were larger in BCA neurons but, as with lemniscal stimulation, the result was not significant. No significant differences were found between control and BCA neurons at a V_{HOLD} of –80 mV. Multiple ANOVA of data in Table 2 showed a significant effect of experimental condition (F = 5.04, P = 0.034), but no effect of age (F = 0.19, P = 0.668) on CIC-evoked total EPSC amplitude at a V_{HOLD} of –20 mV. At a V_{HOLD} of –80 mV, no effect of age (F = 1.31, P = 0.26) or experimental condition (F = 1.60, P = 0.22) was observed.

Paired-pulse facilitation at excitatory synapses

To determine whether the increased excitatory responses in IC neurons was due, in part, to alterations in presynaptic function, paired-pulse responses were recorded as an indirect measure of 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/depression observed after paired-pulse stimulation (Manabe *et al.*, 1993; Dobrunz & Stevens, 1997). Two pulses of equal strength were delivered to the LL or CIC pathways at intervals of 33–1000 ms in the presence of inhibitory blockers (BIC and SN), and with the subsequent addition of an AMPA receptor antagonist (CNQX). The amplitude of the second EPSC was measured and expressed as a percentage of the first EPSC amplitude. This analysis was performed using stimuli which evoked a minimum EPSC amplitude (presumed to reflect the response of one or a few terminals), and measurements were obtained at a V_{HOLD} of 0 mV. As illustrated in Fig. 2, control neurons displayed an increase in the amplitude of the second LL-evoked EPSC at interpulse intervals of 33, 50 and 100 ms. In contrast, neurons from BCA animals either showed less facilitation (50 ms intervals) or exhibited paired-pulse depression (at intervals of

TABLE 1. LL-evoked excitatory postsynaptic currents

V_{HOLD}	Total EPSC (pA)	n	NMDA EPSC (pA)	n	Difference	n
–20 mV						
Control	–57 ± 9	17	–27 ± 7	8	–4 ± 2	6
BCA	–102 ± 16	17*	–63 ± 18	12	–1 ± 1	11
–80 mV						
Control	–91 ± 19	14	–14 ± 6	8	0	3
BCA	–122 ± 33	14	–7 ± 4	7	0	7

Values are given as mean ± SEM. Total EPSC recorded in 50 μM BIC + 4 μM SN; NMDA EPSC recorded in 50 μM BIC + 4 μM SN + 20 μM CNQX; Remaining recorded in 50 μM BIC + 4 μM SN + 20 μM CNQX + 20 μM AP-5; n, number of observations. Data were obtained from eight control and 10 BCA animals. *P < 0.05.

TABLE 2. CIC-evoked excitatory postsynaptic currents

V_{HOLD}	Total EPSC (pA)	n	NMDA EPSC (pA)	n	Difference	n
–20 mV						
Control	–29 ± 4	15	–22 ± 6	8	–1 ± 2	4
BCA	–77 ± 17	13*	–39 ± 14	10	0	4
–80 mV						
Control	–57 ± 11	12	–8 ± 5	7	0	4
BCA	–70 ± 20	11	–8 ± 3	8	0	7

Values are given as mean ± SEM. Total EPSC recorded in 50 μM BIC + 4 μM SN; NMDA EPSC recorded in 50 μM BIC + 4 μM SN + 20 μM CNQX; Remaining recorded in 50 μM BIC + 4 μM SN + 20 μM CNQX + 20 μM AP-5; n, number of observations. Data were obtained from eight control and 10 BCA animals. *P < 0.01.

100–500 ms). A similar trend was observed when CNQX was added. These data are summarized in Table 3 for LL stimulation. When EPSCs were elicited with paired-pulse stimuli delivered to the CIC pathway, no significant differences were found between control and BCA neurons (Table 4).

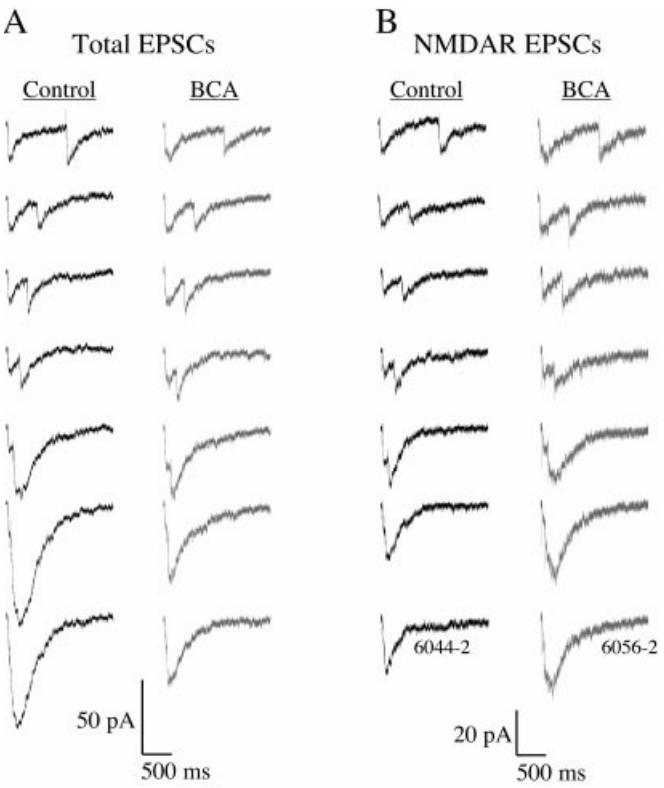


FIG. 2. Analysis of excitatory neurotransmitter release using a paired-pulse stimulus protocol. (A) Stimuli that evoked the minimum EPSC amplitude were delivered to the LL pathway at interpulse intervals of 1000, 500, 333, 200, 100, 50 and 33 ms (from top to bottom; arrows indicate stimulus time). For the control neuron (left), the amplitude of the second EPSC was larger than the first EPSC at stimulus intervals of 100, 50 and 33 Hz. For the BCA neuron (right), the second EPSC was no larger than the first for any interval tested. Recordings were obtained in the presence of SN and BIC. (B) The paired-pulse protocol was repeated in the presence of CNQX. For the control neuron (left), paired-pulse facilitation was observed at stimulus intervals of 100, 50 and 33 ms and there was no increase for any interval tested in the BCA neuron (right).

Depolarization of E_{IPSC} in neurons from BCA animals

In our previous study on the effects of deafness on inhibitory connections, the ablations were performed at P7 (Vale & Sanes, 2000), an age at which significant cell death occurs (Tierney *et al.*, 1997). Therefore, we reexamined IPSC reversal potential and paired-pulse facilitation of IPSCs for ablations at P9, an age at which cell death does not occur in the cochlear nucleus (Tierney *et al.*, 1997). For this analysis, 38 control, 14 BCA-P7 and eight BCA-P9 animals were used.

Figure 3A shows LL-evoked IPSCs obtained at a series of holding potentials for a control and a BCA-P9 neuron. The value for E_{IPSC} was 25 mV more depolarized in deafened animals compared to controls (control –75 ± 2 mV, $n = 38$; BCA-P9 –51 ± 3 mV, $n = 14$; d.f. = 50, $t = 6.923$, $P < 0.0001$). When the age of recording was matched, a comparison of the effects of ablation at P7 and at P9 showed that the E_{IPSC} depolarization was nearly identical for both ages of surgery (Fig. 3B). The E_{IPSC} values from control and BCA-P9 neurons diverged within 1–2 days of deafferentation (Fig. 3C). A similar depolarization was observed for CIC-evoked inhibitory current reversal potentials (control –73 ± 2 mV, $n = 23$; BCA –50 ± 4 mV, $n = 7$; d.f. = 28, $t = 5.370$, $P < 0.0001$). Again, the age of deafening did not influence the result.

To determine whether there was a smaller postsynaptic response independent of driving force ($E_{HOLD} - E_{IPSC}$), we calculated the inhibitory conductance from the slope of the current–voltage curves. Bilateral ablation at P9 decreased LL-evoked IPSC conductance by ≈50% (control 0.9 ± 0.1 nS, $n = 34$; BCA 0.45 ± 0.12 nS, $n = 13$; d.f. = 45, $t = 2.192$, $P < 0.03$). A similar observation was made when conductance was calculated for CIC-evoked IPSCs (control 1.6 ± 0.4 nS, $n = 17$; BCA 0.39 ± 0.1 nS, $n = 8$; d.f. = 23, $t = 2.203$, $P < 0.04$).

Decrement of paired-pulse facilitation at inhibitory synapses

The neurotransmitter release characteristics of inhibitory terminals was evaluated by delivering paired stimulus pulses to the afferent pathway and measuring the relative amplitude of the second IPSC. Pulses of equal strength were delivered at several intervals, and the stimulus strength was set to evoke a minimum IPSC. As shown in Fig. 4A, control neurons displayed an increase in the amplitude of the second LL-evoked IPSC, compared to the first, at all interpulse intervals tested. This facilitation was absent in BCA neurons. Figure 4B shows the mean values for control and BCA neurons and a comparison with previous data from animals deafferented at P7. Significant differences between control and BCA-P9 neurons were observed at stimulus intervals of 100 ms (d.f. = 23, $t = -2.85$, $P < 0.009$), 50 ms (d.f. = 23, $t = -2.52$, $P < 0.019$) and 33 ms (d.f. = 23, $t = -2.60$, $P < 0.016$). For pulses delivered to the CIC

TABLE 3. Excitatory responses to paired-pulse LL stimulation

	Excitatory responses (%) at inter-pulse intervals of													
	1000 ms	(*n*)	500 ms	(*n*)	333 ms	(*n*)	200 ms	(*n*)	100 ms	(*n*)	50 ms	(*n*)	33 ms	(*n*)
BIC + SN														
Control	100 ± 18	(9)	100 ± 11	(11)	93 ± 7	(11)	92 ± 9	(12)	155 ± 20	(11)	214 ± 38	(12)	216 ± 46	(13)
BCA	83 ± 10	(19)	65 ± 10*	(19)	59 ± 9*	(19)	60 ± 9*	(17)	78 ± 17**	(17)	118 ± 20*	(18)	174 ± 25*	(19)
CNQX														
Control	151 ± 25	(8)	121 ± 8	(8)	102 ± 8	(8)	106 ± 12	(9)	145 ± 18	(8)	127 ± 20	(8)	150 ± 19	(8)
BCA	101 ± 13*	(14)	76 ± 7**	(14)	70 ± 7*	(14)	75 ± 10	(13)	115 ± 20	(12)	119 ± 17	(13)	130 ± 21	(13)

Data were analysed by multiple ANOVA. Values are given as mean ± SEM with *n* the number of observations. *$P < 0.05$ vs. control; **$P < 0.01$ vs. control, *t*-test. Data were obtained from eight control and 10 BCA animals.

pathway, significant differences were also found at 100 ms (d.f. = 13, $t = -3.20$, $P < 0.007$), 50 ms (d.f. = 12, $t = -3.00$, $P < 0.011$) and 33 ms (d.f. = 12, $t = -3.59$, $P < 0.004$).

Discussion

The major findings of this study were that deafening had distinct postsynaptic effects on inhibitory and excitatory synapses on IC neurons, and that these effects were independent of cochlear nucleus cell death. The amplitude and duration of evoked EPSCs increased significantly for excitatory afferents travelling through both the LL and CIC pathways. In contrast, evoked IPSCs were significantly smaller following deafening, confirming our previous observations in P7 deafened animals (Vale & Sanes, 2000). An important distinction of the present findings is that animals were deafened at P9, and the observed changes were not attributable to deafferentation-induced neuron cell death (Tierney *et al*., 1997). The presence of a narrow temporal window for deafferentation-induced cell death of cochlear nucleus neurons has also been demonstrated in mice (Mostafapour *et al*., 2000) and rats (Moore *et al*., 1998). Neurotransmitter release characteristics of both excitatory and inhibitory afferents were also influenced by disuse, exhibiting a smaller response to the second of two stimulus pulses. Together, these findings demonstrate that multiple changes to synaptic physiology occur within 24 h of deafness, leading to a dramatic imbalance of excitation and inhibition.

Many anatomical studies have shown that reduced neural activity or deafferentation lead to postsynaptic cell death, atrophy and altered metabolism in the central auditory nervous system (Levi-Montalcini, 1949; Coleman & O'Connor, 1979; Parks, 1979, 1981; Webster & Webster, 1979; Feng & Rogowski, 1980; Smith *et al*., 1983; Deitch & Rubel, 1984, 1989; Durham & Rubel, 1985; Steward & Rubel, 1985; Born & Rubel, 1988; McMullen *et al*., 1988; Hashisaki & Rubel, 1989; Hyson & Rubel, 1989). *In vivo* electrophysiology studies also confirm large changes. For example, excitatory responses are enhanced in the IC after partial (Kitzes, 1984; McAlpine *et al*., 1997; Mossop *et al*., 2000) or total (Bledsoe *et al*., 1995; Shepherd *et al*., 1999) damage to the cochlea. It is not known whether there is a compensatory sprouting or loss of afferents after bilateral ablation, as occurs after unilateral ablation (Moore & Kitzes, 1985; Kitzes *et al*., 1995; Russell & Moore, 1995), or whether cochlear ablation at P9 alters neuron survival in central structures other than the cochlear nucleus.

In contrast to the well established influence of disuse on central anatomy and processing, there are few direct measurements of physiology, particularly synaptic transduction (Kotak & Sanes, 1996, 1997; Vale & Sanes, 2000; Futai *et al*., 2001). The IC is an excellent model for these experiments because it receives multiple inhibitory and excitatory projections through the LL and CIC pathways (Adams, 1979; Nordeen *et al*., 1983; 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).

Postsynaptic changes following deafness

The present results suggest that two postsynaptic changes underlie altered sound-evoked responses in the IC of deafened juvenile animals. First, the total evoked EPSC amplitudes were enhanced following deafening at P9, and this was apparently due to an increase in either AMPA or non-NMDA receptor function (Fig. 1B). The enhancement of excitation was observed for two different pathways, the LL and the CIC (Tables 1 and 2). Second, there is a decrease in both IPSC conductance and a depolarization of IPSC reversal potential (Fig. 3). This dramatic weakening of inhibition was also observed in both pathways that were assessed.

Although excitatory synaptic currents were larger in BCA neurons, we found that the ratio of total : NMDA receptor-dependent currents was similar to that in the control group, suggesting that the amount of glutamate release may have increased. However, a significant difference between evoked EPSCs in control and BCA neurons was obtained when neurons were voltage-clamped to –20 mV, but not at a holding potential of –80 mV (Table 1). If glutamate release alone was responsible for the enhanced excitatory response, then a difference should have been observed at a holding potential of –80 mV. Several BCA neurons displayed large EPSCs that were blocked primarily with CNQX (Fig. 1B), suggesting that some neurons may selectively increase functional non-NMDA receptors. A clear answer to this question must ultimately include a molecular analysis of glutamate receptor expression (Parks, 2000; Sato *et al*., 2000).

An activity-dependent regulation of excitatory transmission has been reported in several systems. In the visual system, NMDA receptor expression and the maturation of EPSCs is regulated by visual experience (Carmignoto & Vicini, 1992; Fox *et al*., 1992; Czepita *et al*., 1994; Binns *et al*., 1999; Quinlan *et al*., 1999; Xue & Cooper 2001). In the auditory brainstem, bilateral cochlear ablation at P7 resulted in persistent increase of NMDA receptor expression (Futai *et al*., 2001). Furthermore, the amplitudes of evoked EPSCs are larger in the cochlear nucleus of deaf mutant mice, and this appears to be purely presynaptic (Oleskevich & Walmsley, 2002). Even the selective blockade of inhibitory inputs to lateral superior olive neurons leads to an increase in NMDA-mediated EPSPs (Kotak & Sanes, 1996). However, deafferentation in adult animals often leads to a transient or persistent decrease in glutamate receptors (Sato *et al*.,

TABLE 4. Paired-pulse excitatory response to CIC stimulation

	Excitatory responses (%) at inter-pulse intervals of													
	1000 ms	(*n*)	500 ms	(*n*)	333 ms	(*n*)	200 ms	(*n*)	100 ms	(*n*)	50 ms	(*n*)	33 ms	(*n*)
BIC + SN														
Control	99 ± 12	(11)	86 ± 7	(11)	95 ± 9	(11)	102 ± 14	(11)	141 ± 15	(12)	151 ± 14	(11)	171 ± 15	(12)
BCA	97 ± 21	(13)	88 ± 9	(14)	69 ± 11	(14)	89 ± 19	(13)	145 ± 21	(12)	250 ± 51	(13)	152 ± 17	(12)
CNQX														
Control	106 ± 7	(9)	114 ± 8	(9)	106 ± 8	(9)	125 ± 15	(9)	162 ± 24	(9)	189 ± 26	(9)	184 ± 23	(9)
BCA	109 ± 21	(9)	107 ± 22	(9)	75 ± 10*	(9)	93 ± 10	(10)	163 ± 28	(10)	151 ± 23	(9)	155 ± 23	(10)

Data were analysed by multiple ANOVA. Values are given as mean ± SEM with *n* the number of observations. *$P < 0.05$ vs. control (*t*-test). Data were obtained from eight control and 10 BCA animals.

2000; Suneja *et al*., 2000). Therefore, the effects of deafness during early development may have a qualitatively different effect on synaptic excitation.

Deafness also leads to a reduction of inhibitory strength. Using gramicidin perforated-patch recordings, with physiological levels of K^+ in the internal pipette solution, we found that cochlear ablation induced a 25-mV depolarization of E_{IPSC} (Fig. 3) and decreased the inhibitory conductance. These effects were identical to those reported previously for BCA at P7 (Vale & Sanes, 2000). Because the present results are largely independent of cell death or loss of afferents to the IC, we conclude that the absence of auditory activity and synaptic transmission causes the observed changes in synaptic function. These results are consistent with a loss of surround inhibition within the auditory cortex following partial cochlear damage (Rajan, 1998).

The E_{IPSC} depolarization indicates that intracellular chloride concentration has increased, resulting in a decrease in the driving force for chloride (Kotak & Sanes, 1996; Vale & Sanes, 2000). In immature neurons, intracellular chloride concentration is regulated by the NKCC and the KCC cotransporters (Payne *et al*., 1996; Plotkin *et al*., 1997; Kazaku *et al*., 1999; Rivera *et al*., 1999; Williams *et al*., 1999). Therefore, a perturbation of chloride homeostasis following hearing loss may also be due to an alteration of chloride transport mechanisms. It is not yet clear what signals regulate chloride transport, although there are several candidates (Sun & Murali, 1998; Ganguly *et al*., 2001; Schomberg *et al*., 2001).

The effect of juvenile deafness on inhibitory synaptic transmission bears some relationship to studies of age-related hearing loss. These studies generally show that GABA and glycine synthesis and release decrease with age, although there appear to be compensatory changes in receptor expression (Caspary *et al*., 1995; Willott *et al*., 1997; Caspary *et al*., 1999).

Presynaptic changes following deafness

Bilateral deafness led to a depression of excitatory neurotransmitter release, as assessed with a paired-stimulus protocol. An important factor determining whether depression or facilitation is produced after paired-pulse stimulation is the probability of transmitter release after the first (conditioning) pulse (Manabe *et al*., 1993; Thomson *et al*., 1993; Debanne *et al*., 1996). As shown in Table 3, LL excitatory afferents displayed paired-pulse facilitation over a range of interpulse intervals from 33 to 100 ms. For all intervals tested, BCA neurons exhibited a smaller average response to the second pulse than did controls, and significance was attained for most intervals. This deafness-induced change in neurotransmitter release properties was not observed for excitatory afferents coursing through the CIC pathway (Table 4). Partial sensory deafferentation of adult animals does not modify excitatory neurotransmitter release within the ipsilateral IC and LL, although aspartate release is depressed within subdivisions of nuclei projecting to the IC after 5 days (Potashner *et al*., 1997). As with postsynaptic changes induced by deafness, it is quite possible that hearing loss during development may produce qualitatively different results.

Bilateral deafness had a more profound effect on inhibitory neurotransmitter release properties than on excitatory ones. Control neurons displayed inhibitory paired-pulse facilitation for pulse intervals of 10–30 ms, and this was observed for both the LL and

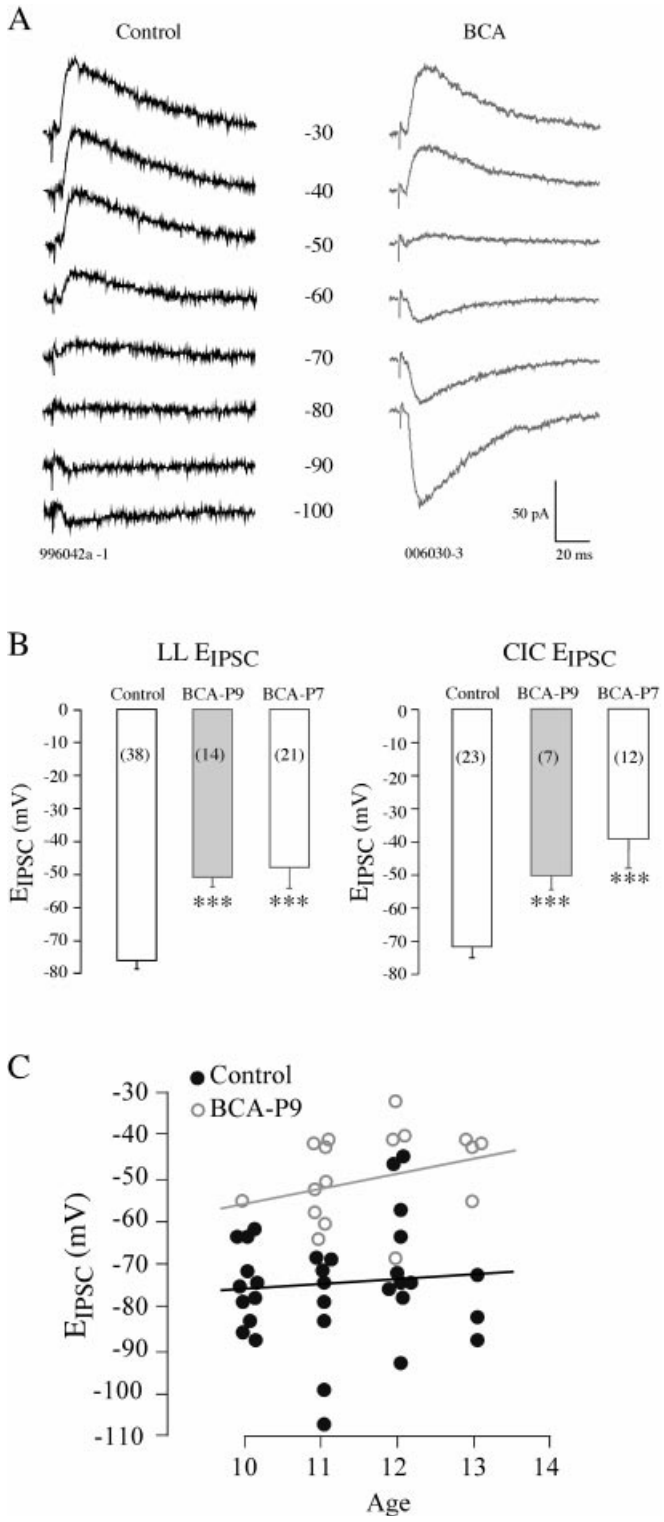
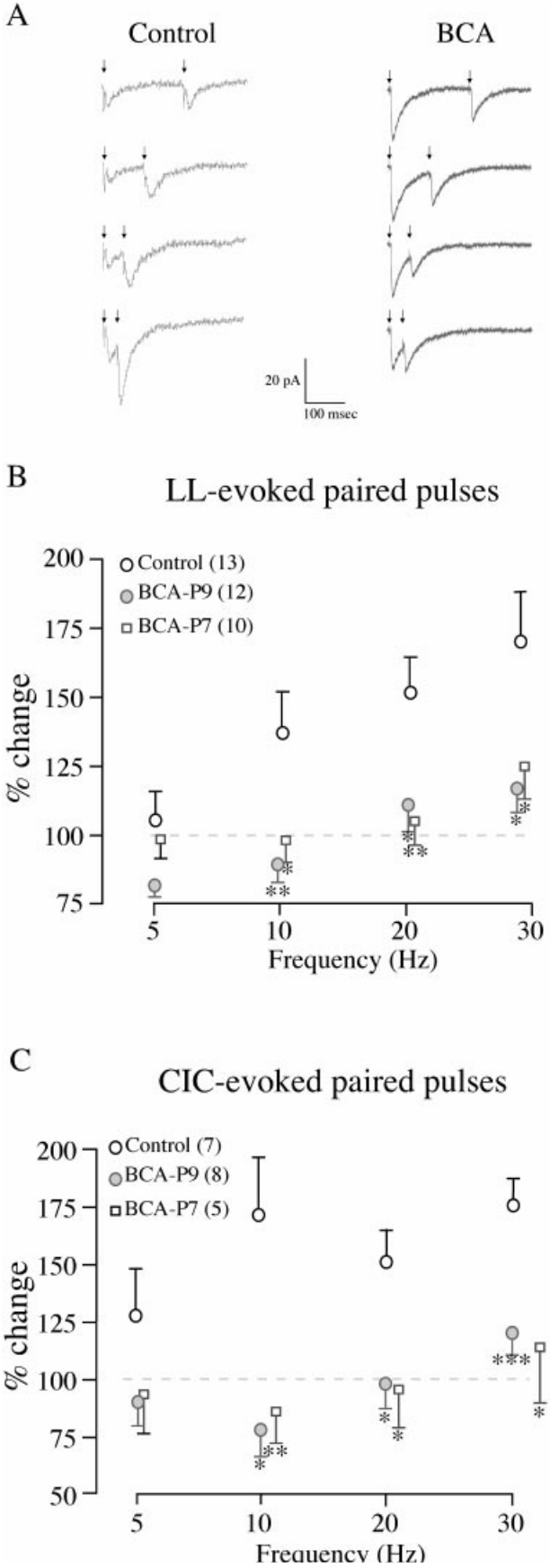


FIG. 3. BCA lead to depolarization of the IPSC reversal potential. (A) LL-evoked IPSCs are shown for a P10 control neuron (left) and a P11 BCA neuron (right) at different holding potentials. The E_{IPSC} was ≈–80 mV for the control neuron and –50 mV for the BCA neuron. (B) Mean E_{IPSC} is plotted for both LL (left) and CIC (right) evoked IPSCs from control, P9 BCA and P7 BCA neurons (all data were restricted to recording ages of P10–14). Bilateral ablation at either P7 or P9 caused an ≈25 mV depolarization of the mean E_{IPSC}. Data from P7 BCA neurons were recomputed from Vale & Sanes (2000) after restricting the recording age to match the present study. Values are means ± SEM with the number of observations shown in each bar (***$P < 0.0001$ vs. control). See text for statistics. (C) The distribution of E_{IPSC} is plotted for neurons from control (black circles) and BCA-P9 animals (open circles) during the age range analysed. E_{IPSC} depolarization in BCA-P9 neurons was observed within 1–2 days of deafferentation.



CIC pathways (Fig. 4). In BCA neurons, inhibitory paired-pulse facilitation was eliminated. Furthermore, this depression of transmitter release was similar in animals deafferented at P7 (Vale & Sanes, 2000) or P9, indicating that the decrease in inhibitory neurotransmitter release was not due to afferent loss produced by cell death. A decrease in inhibitory neurotransmitter release has been described in the IC and nuclei projecting to the IC after complete (Bledsoe *et al*., 1995) and partial (Suneja *et al*., 1998) sensory deprivation. A decrease in GABA synthesis in the mammalian IC and the chick nucleus magnocelluris has also been reported after unilateral cochlea ablation (Code *et al*., 1990; Mossop *et al*., 2000).

Conclusions

The present results are also in agreement with recent findings obtained from cortical neurons grown in culture. For example, decreasing all activity with tetrodotoxin results in an increase in the size of spontaneous EPSCs and a decrease in the size of spontaneous IPSCs (Turrigiano *et al*., 1998; Kilman *et al*., 2002). Likewise, deafness increased evoked excitation and decreased evoked inhibition. However, it is unlikely that each area of the brain responds in an identical fashion to disuse. Neurons in the visual cortex lose their response to an eye that is deprived of vision during early life (Wiesel & Hubel, 1963a), whereas the visually evoked responses of lateral geniculate nucleus neurons remain largely unchanged, even after months of monocular deprivation (Wiesel & Hubel, 1963b; Levitt *et al*., 2001). In the inferior colliculus of deafened adult rats, suprathreshold electrical stimulation results in a greater number of Fos-positive neurons, compared to controls, suggesting a more potent excitatory drive (Nagase *et al*., 2000). In the auditory cortex of congenitally deaf cats, cortical activity and synaptic efficacy are depressed, as assessed with a middle latency response to electrical stimulation of the cochlea (Klinke *et al*., 1999).

In the present experiments, bilateral deafening occurred prior to the onset of hearing, and synaptic physiology was assessed both before (P10) and after (P14) the age at which animals would first respond to airborne sound. Because changes in synaptic strength occurred within 24 h of deafening, these results suggest that spontaneous neural activity is necessary for the normal maturation of inhibitory and excitatory synaptic properties. In fact, spontaneous activity has been recorded in the gerbil cochlear nucleus and IC at P9, the age of deafferentation (Woolf & Ryan, 1985; Kotak & Sanes, 1995). Furthermore, cochlear damage has been suggested to decrease spontaneous activity in the IC of acutely deafened animals (Shepherd *et al*., 1999).

FIG. 4. Analysis of inhibitory neurotransmitter release using the paired-pulse stimulus protocol. (A) Minimum IPSCs were evoked by electrical stimulation of the LL pathway at stimulus intervals of 200, 100, 50 and 33 ms (from top to bottom; arrows indicate stimulus time) in a control (left) and a BCA (right) neuron. The amplitude of the second IPSC was larger in the control neuron at stimulus intervals of 100, 50 and 33 ms, but was unchanged in the BCA neuron. The V_{HOLD} was –80 mV. (B) Summary of paired-pulse responses from LL stimulation in age-matched control (open circles), P9 BCA (grey circles) and P7 BCA neurons (grey rectangles). Inhibitory paired-pulse facilitation was observed in control neurons, but not in either set of BCA neurons. (C) Summary of paired-pulse facilitation for CIC stimulation. Again, control neurons displayed inhibitory paired-pulse facilitation, but not in either group of BCA neurons. Data were obtained in the presence of kynurenic acid. Values are Means ± SEM with the number of observations shown next to the legend (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ vs. control). Data from P7 BCA neurons were recomputed from Vale & Sanes (2000) after restricting the recording age to match the present study.

In vivo manipulations of auditory activity clearly produce several pre- and postsynaptic alterations, each of which may influence synaptic integration. The novel sound-evoked coding properties observed after sensory deprivation (Kitzes & Semple, 1985; Bledsoe *et al*., 1995; Rajan, 1998; Sheperd *et al*., 1999) may be attributed, in part, to the physiological changes at both inhibitory and excitatory synapses.

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Abbreviations

ACSF, artificial cerebrospinal fluid; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP-5, DL-2-amino-5-phosphonopentanoic acid; BCA, bilateral cochlear ablation; BIC, bicuculline methobromide; CIC, commissure of the inferior colliculus; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; E_{IPSC}, IPSC equilibrium potential; EPSC, excitatory postsynaptic currents; IC, inferior colliculus; IPSC, inhibitory postsynaptic currents; KYN, kynurenic acid; LL, lateral lemniscus; NMDA, N-methyl-D-aspartate; P, postnatal day; PSC, postsynaptic current; SN, strychnine; V_{HOLD}, holding potential.

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