Age-dependent effect of hearing loss on cortical inhibitory synapse function

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Takesian AE, Kotak VC, Sanes DH. Age-dependent effect of hearing loss on cortical inhibitory synapse function. J Neurophysiol 107: 937–947, 2012. First published November 16, 2011; doi:10.1152/jn.00515.2011.—The developmental plasticity of excitatory synapses is well established, particularly as a function of age. If similar principles apply to inhibitory synapses, then we would expect manipulations during juvenile development to produce a greater effect and experience-dependent changes to persist into adulthood. In this study, we first characterized the maturation of cortical inhibitory synapse function from just before the onset of hearing through adulthood. We then examined the long-term effects of developmental conductive hearing loss (CHL). Whole cell recordings from gerbil thalamocortical brain slices revealed a significant decrease in the decay time of inhibitory currents during the first 3 mo of normal development. When assessed in adults, developmental CHL led to an enduring decrease of inhibitory synaptic strength, whereas the maturation of synaptic decay time was only delayed. Early CHL also depressed the maximum discharge rate of fast-spiking, but not low-threshold-spiking, inhibitory interneurons. We then asked whether adult onset CHL had a similar effect, but neither inhibitory current amplitude nor decay time was altered. Thus inhibitory synapse function displays a protracted development during which deficits can be induced by juvenile, but not adult, hearing loss. These long-lasting changes to inhibitory function may contribute to the auditory processing deficits associated with early hearing loss.

auditory cortex; conductive hearing loss; development; fast-spiking interneuron; γ-aminobutyric acid A receptor

A GENERAL THEORY of neural development holds that early sensory experience can permanently alter the functional properties of synapses. Consistent with this idea, a broad range of auditory cortical inhibitory synaptic properties are disrupted immediately following developmental hearing loss (Kotak et al. 2005, 2008; Takesian et al. 2010; Xu et al. 2010). Inhibitory synapse maturation plays a significant role in regulating the plasticity of cortical excitatory circuits (Fagioliini and Hensch 2000; Fagioliini et al. 2004; Hensch 2005; Hensch and Stryker 2004; Hensch et al. 1998; Huang et al. 1999; Iwai et al. 2003; Katagiri et al. 2007; Southwell et al. 2010; Sugiyama et al. 2008; Yazaki-Sugiyama et al. 2009), yet information on the age dependence of inhibitory plasticity is limited. If inhibitory synapse function does display age-dependent plasticity, then it should fail to mature properly following developmental conductive hearing loss (CHL) but remain relatively unaffected by adult hearing loss. Furthermore, if inhibitory dysfunction is causally related to adult perceptual deficits, then aberrant properties should persist into adulthood. Therefore, we asked whether the identical hearing loss manipulation produced the same outcome when carried out in juvenile and adult animals, and whether the developmental effect was transient or permanent.

Immediately after the onset of hearing, both subcortical and cortical circuits are vulnerable to perturbations of the sensory environment, such as chronic deprivation or continuous stimulation (Sanes and Bao 2009). At the cellular level, deprivation leads to a net increase in the excitability of both midbrain and cortical neurons that is due, in part, to a decline in the magnitude of inhibitory synaptic events (Kotak et al. 2005, 2008; Takesian et al. 2010; Vale and Sanes 2000). Although adult hearing loss is thought to affect inhibitory synapse function, this has only been measured indirectly (Bledsoe et al. 1995; Burianova et al. 2009; Caspary et al. 1990, 1995, 1999; Ling et al. 2005; Rajan 1998, 2001; Raza et al. 1994). Here, we were particularly interested in the effects of a less severe form of auditory deprivation, one in which animals hear, albeit with higher thresholds, and the relative impact of this deprivation in juvenile and adult animals.

The effects of sensory deprivation on synaptic properties are extensive, yet the assessment age is generally restricted to a narrow time window during juvenile development. At least one study in the visual cortex has demonstrated that inhibitory synapse strength remains diminished in adult animals raised with visual deprivation (Morales et al. 2002). In the auditory system, recordings from the cochlear nucleus of mice with a congenital form of progressive hearing loss show that changes to excitatory synapses and membrane properties are present in young adults (Wang and Manis 2005, 2006). However, some cellular effects, such as changes in expression of glutamic acid decarboxylase (GAD) or Fos, occur only transiently following deprivation (Mossop et al. 2000; Sun et al. 2009). Direct measures of inhibitory synapse function in adults following a developmental manipulation have not been made.

In this report, we describe the long-term alterations to inhibitory synapses in auditory cortex that are associated with moderate developmental CHL. First, we show that CHL induced during early life profoundly affects inhibitory synapses recorded in juvenile animals and that these effects persist into adulthood. We then show that CHL induced in adulthood does not alter inhibitory synapses, suggesting that at least one form of inhibitory plasticity in auditory cortex is restricted to a developmental period.

MATERIALS AND METHODS

Experimental animals. Gerbils (Meriones unguiculatus) aged postnatal day (P) 8–210 born from breeding pairs (Charles River Laboratories) were used. Animal care, maintenance, and surgeries were in accordance with the guidelines and rules of the Institutional Animal Care and Use Committee, New York University (NYU), approved by the Office of Laboratory Animal Welfare, Office of Extramural Research, U.S. National Institutes of Health (NIH; Bethesda, MD). The data are drawn from voltage-clamp recordings of 173 pyramidal
cells from 55 animals and from current-clamp recordings of 50 interneurons from 14 animals.

Conductive hearing loss. CHL was induced by tympanic membrane puncture and malleus extirpation using procedures similar to those described previously (Tucci et al. 1999; Xu et al. 2007). This form of CHL elevates auditory thresholds by about 35–45 dB and is thought to be a reasonable model for childhood hearing loss associated with certain cases of otitis media (Tucci et al. 1999; Whitton and Polley 2011). Gerbil pups were anesthetized with halogenated ethyl methyl ether methoxyflurane (Metofane), and anesthetic induction occurred within 10 min, which was confirmed by a complete elimination of responses to nociceptive stimuli. A postauricular incision was made, the tympanic membrane was punctured, and the malleus was removed with forceps. The postauricular wound was closed with cyanoacrylate glue, and the procedure was repeated on the other side. The success of each surgery was confirmed anatomically after the brain slice was prepared to ensure that the stapes was preserved during malleus removal.

Thalamocortical brain slice preparation. Thalamocortical brain slices (500 μm) were generated from gerbils as described previously (Kotak et al. 2005). The brain was sectioned perihorizontally to preserve the ventral medial geniculate (MGv) and its ascending (Kotak et al. 2005). The brain was sectioned perihorizontally to account for developmental changes in the relative location of the auditory pathways to the auditory cortex (Cruikshank et al. 2002). To preserve the ventral medial geniculate (MGv) and its ascending pathways to the auditory cortex, thalamocortical slices were cut at a modified 25° angle in adult rats. In some experiments using P180–P210 animals, thinner slices (200 μm) were used to allow for the visualization of inhibitory interneurons under infrared differential interface contrast (IR-DIC).

The slices were incubated in artificial cerebral spinal fluid (ACSF) at 32°C for 30 min, then at room temperature for 60 min, and were transferred to a recording chamber continuously superfused (3 ml/min) with ACSF at 32°C. The ACSF contained (in mM) 125 NaCl, 4 KCl, 1.2 KH₂PO₄, 1.3 MgSO₄, 24 NaHCO₃, 15 glucose, 2.4 CaCl₂, and 0.4 l-ascorbic acid (pH 7.3 when bubbled with 95%O₂-5%CO₂). Before each recording, the auditory cortex was identified in all 500-μm-thalamicortical slices by extracellular field responses to MGv stimulation.

Whole cell recordings. To assess synaptic inhibition, whole cell recordings (PC-501A; Warner Instruments, Hamden, CT) were obtained from pyramidal neurons in cortical layers (L) 2/3. Recording electrodes were fabricated from borosilicate glass microcapillaries (outer diameter, 1.5 mm) with a micropipette puller (model P-97; Sutter Instruments, Novato, CA). The internal solution contained (in mM) 125 NaCl, 4 KCl, 1.2 KH₂PO₄, 1.3 MgSO₄, 24 NaHCO₃, 15 glucose, 2.4 CaCl₂, and 0.4 l-ascorbic acid (pH 7.3 when bubbled with 95%O₂-5%CO₂). Before each recording, the auditory cortex was identified in all 500-μm-thalamicortical slices by extracellular field responses to MGv stimulation.

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curves, only FS cells with firing rates > 100 Hz were used. One FS cell from each control and CHL animals were excluded that fired < 100 Hz. Their exclusion did not affect the significance of the difference in firing rates between FS cells from control and CHL animals. Spike adaptation was calculated as the average of the last two interspike intervals (ISIs) divided by the average of the first two ISIs in response to a depolarizing step that evoked firing rates of ~50 Hz.

All data are means ± SE. Statistical tests were performed using statistical software (JMP; SAS Institute, Cary, NC). These include Levene’s test for equal variance, ANOVA or Student’s t-test for data distributed normally (t), and Kruskal-Wallis or Wilcoxon’s nonparametric tests for data not distributed normally (χ²). Traces shown are individual traces from single neurons. Stimulus artifacts were decreased.

RESULTS

Development of cortical inhibitory synapses. To facilitate the interpretation of experience-dependent changes, the normal development of inhibitory synaptic properties was first examined. In principle, deprivation can prevent or delay normal maturation, induce a relapse to an earlier state, or initiate a degenerative response. Therefore, inhibitory synaptic properties in auditory cortex were first evaluated at several developmental periods: before hearing onset (P8–P11), following hearing onset (P17–P22), before sexual maturity, which occurs at ~P70 in gerbils (P30–P60), and at two adult ages (P90–P110, P180–P210). sIPSCs were recorded in L2/3 pyramidal cells in the presence of ionotopic glutamate receptor antagonists (Fig. 1A).

As shown in Fig. 1C, there was a modest change in amplitude across the age groups examined [sIPSC amplitude (pA): P8–P11: 30.4 ± 3.0, n = 13; P17–P22: 29.0 ± 4.0, n = 14; P30–P60: 25.4 ± 2.8, n = 11; P90–P110: 27.2 ± 2.7, n = 29; P180–P210: 34.5 ± 2.5, n = 26; control: 11.6 ± 1.1 ms, n = 24; χ² = 11.5, degrees of freedom (df) = 4, P = 0.02] that occurred after P90–P110 (P17–P22 vs. P180–P210, χ² = 4.8, df = 1, P = 0.03; P30–P60 vs. P180–P210, χ² = 7.1, df = 1, P = 0.008; P90–P110 vs. P180–P210, χ² = 8.0, df = 1, P = 0.005). Furthermore, the sIPSC decay kinetics, measured from single exponential fits (Fig. 1B), displayed a significant reduction throughout the examined age range [Fig. 1C; sIPSC decay constant (ms): P8–P11: 25.3 ± 1.4, n = 13; P17–P22: 19.0 ± 1.6, n = 13; P30–P60: 17.3 ± 1.3, n = 11; P90–P110: 11.7 ± 1.1, n = 27; P180–P210: 11.6 ± 0.6, n = 24; χ² = 42.5, df = 4, P < 0.0001]. However, there was no significant change in sIPSC frequency across development [sIPSC frequency (Hz): P8–P11: 1.9 ± 0.3, n = 13; P17–P22: 3.5 ± 0.8, n = 14; P30–P60: 2.6 ± 0.8, n = 11; P90–P110: 4.3 ± 0.8, n = 29; P180–P210: 3.6 ± 0.4, n = 26; χ² = 5.7, df = 4, P = 0.22]. Finally, the mean sIPSC charge transfer also showed a significant decrease from the juvenile to adult age range [sIPSC charge transfer (IC): P17–P22: 993 ± 101, n = 14; P180–P210: 665 ± 51, n = 14; t = 2.9, P = 0.007]. Thus, for the parameters examined, several properties of cortical inhibitory synapses were found to display a protracted period of maturation, into early adulthood.

CHL disrupts inhibitory synapse function. To assess the effects of moderate CHL, the malleus was removed bilaterally in gerbils aged P10, elevating auditory thresholds by about 35–45 dB (Tucci et al. 1999; Xu et al. 2007). Animals were then reared for about a week after hearing onset (P17–P22) and compared with age-matched controls reared with normal auditory experience. As shown in Fig. 2A, neurons recorded from animals with CHL displayed significantly smaller sIPSCs compared with age-matched controls [sIPSC amplitude (pA): control: 29.0 ± 4.0, n = 14; CHL: 18.7 ± 2.4, n = 10; χ² = −4.4, P = 0.04]. However, there was no significant change in the mean sIPSC frequency [sIPSC frequency (Hz): control: 3.5 ± 0.8 Hz, n = 14; CHL: 4.2 ± 0.6 Hz, n = 10; χ² = 1.7, P = 0.20]. To determine whether CHL alters the kinetics of sIPSCs, ~50 events from each neuron were fitted with a single exponential (see MATERIALS AND METHODS). Figure 2B shows that the mean decay time constant was longer in CHL neurons compared with controls [sIPSC decay constant (ms): control: 19.0 ± 1.6, n = 13; CHL: 26.1 ± 3.5, n = 9; χ² = 3.6; P = 0.05].

Finally, to determine whether CHL affects the strength of putative unitary inhibitory connections to L2/3 pyramidal neurons, IPSCs were elicited by minimum stimulation to L4. Figure 2C shows that me-IPSCs recorded in slices from CHL animals were significantly smaller than those recorded in controls [me-IPSC amplitude (pA): control: 12.1 ± 1.3, n = 9; CHL: 5.3 ± 0.5, n = 8; χ² = 8.9, P = 0.003]. However, there was no significant change in the me-IPSC decay time constant [me-IPSC decay constant (ms): control: 32.1 ± 3.5, n = 9; CHL: 38.0 ± 10.1, n = 8; t = 0.6, P = 0.57].

CHL-induced changes in inhibition persist into adulthood. The effect of sensory deprivation on synaptic function is typically examined at 1–2 wk after the manipulation, but it has yet to be determined whether early hearing loss induces long-term changes in synaptic inhibition. If inhibitory synaptic deficits persist past the juvenile stage and into adulthood, these effects would be potential candidates to explain the adult perceptual deficits associated with early hearing loss (Whitton and Polley 2011). Therefore, we sought to determine whether CHL-induced changes at inhibitory synapses persist into adulthood.

CHL was induced at P10, and animals were reared past sexual maturation. Pyramidal neurons were recorded at either of two ages (P90–P110 and P180–P210) in CHL and age-matched control animals. Figure 3A shows that sIPSC amplitude remained significantly smaller in CHL neurons compared with controls when recorded at P90–P110 (control: 27.2 ± 2.7 pA, n = 29; CHL: 16.5 ± 2.1 pA, n = 11; χ² = 8.2, P = 0.004) or at P180–P210 (control: 34.5 ± 2.5 pA, n = 26; CHL: 24.1 ± 1.9 pA, n = 39; χ² = 14.8, P = 0.0001). A significant increase in mean sIPSC frequency emerged in CHL neurons recorded at P180–P210 (P90–P110: control: 4.3 ± 0.8 Hz, n = 29; CHL: 3.1 ± 1.4 Hz, n = 11; χ² = 0.55, P = 0.46; P180–P210: control: 3.6 ± 0.4 Hz, n = 26; CHL: 6.0 ± 0.5 Hz, n = 39; χ² = 9.5, P = 0.002). Finally, the mean sIPSC decay time constant remained longer in CHL neurons recorded at P90–P110 compared with age-matched controls (control: 11.7 ± 1.1 ms, n = 27; CHL: 14.4 ± 0.7 ms, n = 9; t = 2.1; P = 0.04) but did not show a significant difference in CHL neurons recorded at P180–P210 (control: 11.6 ± 0.6 ms, n = 24; CHL: 10.2 ± 0.7 ms, n = 37; t = −1.4; P = 0.16).

Comparison of the adult evoked IPSCs also confirmed a long-term reduction in IPSC strength. Figure 3B shows that putative unitary inhibitory connections to pyramidal cells were significantly reduced in CHL neurons recorded at P90–P110 [me-IPSC amplitude (pA): control: 13.0 ± 1.7, n = 13; CHL: 6.4 ± 0.7, n = 6; χ² = 4.8, P = 0.028]. Furthermore, me-IPSCs...
decay time constants were longer in CHL neurons [me-IPSC decay constant (ms): control: 14.6 ± 1.7, n = 13; CHL: 29.7 ± 5.7, n = 6; \( \chi^2 = 6.9, P = 0.009 \)]. Similarly, max-IPSCs were significantly smaller in CHL animals compared with controls [Fig. 3, C–E; max-IPSC amplitude (pA): control: 315 ± 83, n = 12; CHL: 73 ± 22, n = 6; \( \chi^2 = 4.2, P = 0.04 \)] and showed prolonged decay time constants [max-IPSC decay constant (ms); control: 24.0 ± 4.7, n = 12; CHL: 51.3 ± 12.5, n = 6; \( \chi^2 = 5.1, P = 0.02 \)].

Since inhibitory synapses display long-lasting effects of early CHL, we asked whether the cellular intrinsic properties of inhibitory interneurons that formed these synapses were also altered. To examine the long-term effects of developmental CHL on adult interneuron intrinsic properties, current-clamp recordings were obtained from two major interneuron subtypes, FS and LTS cells, from adult control animals (P180–P210) and age-matched animals with CHL induced at P10. Intrinsic firing properties were evaluated on the basis of suprathreshold responses to current injection (1,500 ms). The results showed that CHL had a significant impact on the firing properties of FS, but not LTS, interneurons. As shown in Fig. 4, the average firing rates in response to increasing current injections were significantly reduced in FS, but not LTS, cells from animals with long-term CHL. CHL resulted in a significant decrease in the maximum spike rate evoked by up to 800-pA current injection in FS cells (control: 235 ± 24 Hz,
Adult CHL does not affect inhibition. To determine whether inhibitory synapses are equivalently deprived in adulthood, CHL was induced after sexual maturation (P83). As with the younger experimental group, animals were reared for about a week (P90–P110) and compared with age-matched controls. Following adult CHL, there was not a significant change in sIPSC amplitude, frequency, or decay constant compared with controls [Fig. 5A; sIPSC amplitude (pA): control: 27.2 ± 2.7, n = 29; CHL83: 24.0 ± 2.1, n = 20; \( \chi^2 = 0.17, P = 0.68 \); sIPSC frequency (Hz): control: 4.3 ± 0.8, n = 29; CHL83: 4.0 ± 0.9, n = 20; \( \chi^2 = 0.002, P = 0.97 \); sIPSC decay constant (ms): control: 11.7 ± 1.1, n = 27; CHL83: 11.5 ± 0.6, n = 16; \( \chi^2 = 1.2, P = 0.27 \)]. Furthermore, adult CHL did not induce a significant change in me-IPSC amplitude or decay time constant [Fig. 5B; me-IPSC amplitude (pA): control: 13.0 ± 1.7, n = 13; CHL83: 11.7 ± 1.5, n = 16; \( t = -0.6, P = 0.56 \); me-IPSC decay constant (ms): control: 14.6 ± 1.7, n = 13; CHL83: 18.7 ± 1.3, n = 16; \( t = 1.9, P = 0.07 \)].

**DISCUSSION**

When animals are raised in a degraded environment, central nervous system (CNS) function can become disrupted. Depending on the manipulation, these effects are less prominent or absent when adult animals are subjected to the same environmental impact (Harris and Rubel, 2006; Hensch 2005; Hooks and Chen 2007; Lewis and Maurer 2005; Knudsen 2004; Sanes and Polley 2011). In this report, we present evidence that moderate hearing loss induced near the onset of hearing does result in enduring changes to cortical GABAergic inhibition. However, when induced in adulthood, inhibitory function was unaffected.

**Inhibitory functional deficits persist to adulthood.** Conductive hearing loss, which produces a moderate increase in the threshold for hearing, was shown to impact cortical inhibitory synapse function when induced early in development (Fig. 2) The 35% decrease in sIPSC amplitude following CHL is consistent with the 30% decrease in the amplitude of miniature (m) IPSCs following developmental deprivation in the adult cortex (Maffei et al. 2010). Furthermore, the decrease in sIPSC and me-IPSC amplitudes following CHL is comparable in magnitude to that elicited by the most severe manipulation: sensorineural hearing loss (SNHL) (Kotak et al. 2008). This is consistent with recent findings that CHL and SNHL produce comparable effects on synaptic and intrinsic temporal features of L2/3 pyramidal cells (Takekian et al. 2010; Xu et al. 2007) as well as a similar reduction of long-term inhibitory potentiation (Xu et al. 2010).

The early CHL-induced reduction in inhibitory synaptic strength was as prominent in adult animals as it was within a week of the manipulation (Fig. 3). This was not entirely expected, because some previous reports have shown that hearing loss can result in transient adjustments in neural function (Mossop et al. 2000; Sun et al. 2009). In contrast, our results indicate that the CHL-induced decrease in inhibitory current amplitude did not recover in adults after more than 6 mo. This is consistent with the long-term change in 2-deoxy-PiSC amplitude.
glucose uptake in the auditory brain stem and midbrain following unilateral CHL (Tucci et al. 1999) and the persistent enhancement of ipsilaterally evoked responses in the inferior colliculus following unilateral neonatal cochlear ablation (Kitzes 1984; Kitzes and Semple 1985).

The long-term effect of early hearing loss is not uniform across all inhibitory synaptic properties. The kinetic properties of inhibitory synapses eventually recover to control levels over 6 mo. By comparing the inhibitory properties of control neurons from before hearing onset to adulthood (Fig. 1) with neurons from CHL animals (Fig. 3), we could establish that CHL delays the maturation of IPSC kinetics. In normal animals, IPSC kinetics show a protracted development during postnatal life: the IPSC decay time constant continues to decline for several months, corresponding to a significant decrease in IPSC charge transfer across development. This is consistent with longer duration inhibitory postsynaptic potentials (IPSPs) in kittens compared with adult cats (Purpura et al. 1965, 1968) and with a prolonged maturation of IPSP decay time in the primate prefrontal cortex (Hashimoto et al. 2009).

Both pre- and postsynaptic changes may underlie the developmental and CHL-induced effects on inhibitory function. For example, the maturation of sIPSCs in the primate prefrontal cortex corresponds with the emergence of the adult GABA_A receptor subunit composition (Hashimoto et al. 2009). Similarly, in rat, the expression of GABA_A receptor subunits undergoes dramatic changes during postnatal development (Laurie et al. 1992), and hippocampal inhibitory currents dis-
play age-dependent changes in responses to GABA<sub>A</sub>ergic modulatory agents, extending into adulthood (Cohen et al. 1995). Finally, both pre- and postsynaptic markers of human GABA<sub>A</sub>ergic synaptic function show prolonged developmental trajectories that extend into adulthood (Pinto et al. 2010). Therefore, it is plausible that developmental CHL may produce a long delay in the expression of the mature GABA<sub>A</sub> receptor subunits that confer the fast activation and deactivation kinetics (Bosman et al. 2005; Ducić et al. 1995; Gingrich et al. 1995; Tia et al. 1996). This is consistent with previous observations from SNHL animals in which slower IPSC kinetics are associated with a failure or delay in the emergence of functional α1- and β2/3-subunits (Kotak et al. 2008; Sarro et al. 2008). Similar observations have been made in the visual cortex following developmental monocular deprivation (Maffei et al. 2010). In addition to postsynaptic alterations in GABA<sub>A</sub> receptor subunit composition, CHL may induce parallel presynaptic changes. This is supported by our present finding that long-term CHL leads to an increase in sIPSC frequency. Furthermore, our previous study showed that early CHL prevents a developmental shift from short-term depression to facilitation of IPSCs, suggesting that presynaptic release properties are affected (Takesian et al. 2010).

The effect of developmental CHL on IPSC amplitude and kinetics is most likely to occur at the inhibitory synapses formed by FS interneurons. We have previously reported that FS-evoked IPSCs display a threefold decrease in amplitude and slower kinetics after developmental SNHL; in contrast, this does not occur at LTS synapses (Takesian et al. 2010). Our present results further reveal that early hearing loss influences FS interneuron discharge properties into adulthood, but not those of LTS cells (Fig. 4). CHL permanently diminishes the maximum discharge rate of FS cells, presumably by disrupting developmental mechanisms that have been characterized previously (Itami et al. 2007; Okaty et al. 2009). For example, CHL may prevent the developmental upregulation of potas-

Table 1. Effects of early CHL on adult FS and LTS cell intrinsic properties

<table>
<thead>
<tr>
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<th>FS control</th>
<th>FS CHL</th>
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<td>–19 ± 1</td>
<td>–14 ± 1</td>
<td>–14 ± 1</td>
</tr>
<tr>
<td>AP amp, mV</td>
<td>87 ± 5</td>
<td>98 ± 2</td>
<td>104 ± 2</td>
<td>107 ± 2</td>
</tr>
<tr>
<td>Adaptation ratio</td>
<td>1.4 ± 0.2</td>
<td>3.6 ± 0.71</td>
<td>5.3 ± 1.1</td>
<td>8.1 ± 2.2</td>
</tr>
<tr>
<td>Max spike rate, Hz</td>
<td>235 ± 23</td>
<td>141 ± 20*</td>
<td>49 ± 10</td>
<td>47 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = no. of cells sampled) of intrinsic properties of fast-spiking (FS) and low-threshold-spiking (LTS) interneurons from adult (postnatal day (P)180–P210) control and conductive hearing loss (CHL) animals. *P < 0.05; †P < 0.01. RMP, resting membrane potential; R<sub>in</sub>, input resistance; τ, membrane time constant; I thresh, current threshold required to elicit a spike; V thresh, voltage threshold to spike; AP width, action potential half-width; AP amp, action potential amplitude; adaptation ratio, average of last 2 interspike-intervals (ISI) divided by average of first 2 ISIs; Max spike rate, maximum spike rate evoked by ±800-pA current injection.

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sium channels Kv3.1b/Kv3.2 (Grabert and Wahle 2008) that endow normal adult FS cells with the capability to fire at high frequencies with little spike frequency adaptation (Du et al. 1996; Erisir et al. 1999; Okaty et al. 2009; Tansey et al. 2002). This is consistent with disrupted maturation of parvalbumin-positive inhibitory cells in noise-exposed regions of the auditory cortex (de Villers et al. 2008). Our results also agree with previous reports from auditory, visual, and somatosensory cortices, each of which demonstrates that FS and non-FS interneurons display a differential effect of developmental manipulations (Bartley et al. 2008; Maffei et al. 2004; Sun 2009; Takesian et al. 2010). The cumulative effects of decreased FS cell firing and diminished inhibitory synaptic strength are expected to result in less FS cell stimulus-evoked inhibition following CHL. Such a deficit may affect auditory tuning and temporal response properties that are thought to rely on strong FS cell feed-forward inhibition (Oswald et al. 2006).

**Age-dependent influence of CHL on inhibitory synapse function.** Although the decline of cortical plasticity with age has been explored at the cellular level, previous studies generally focused on the developmental properties of excitatory afferents, particularly in the visual pathway (Hensch 2005; Hooks and Chen 2007). Since developmental hearing loss has a profound impact on inhibitory synapse development, we have proposed that this effect may explain some of the associated perceptual deficits (Sanes et al. 2009; Takesian et al. 2009). However, if similar changes occur following adult hearing loss, then this idea would be less compelling. In the present study, we found that CHL had a significant effect on inhibitory synapse function when induced during early life, whereas the same manipulation in adulthood had no effect. This suggests that there exists a developmental period during which CHL is maximally effective. Future studies that systematically vary the manipulation age across development will be instrumental in identifying the period of maximum sensitivity (i.e., critical or sensitive period) to this particular manipulation. Our results are consistent with several studies in visual cortex showing that the effects of deprivation on inhibitory function are age dependent (He et al. 2006; Maffei et al. 2010; Morales et al. 2002; Yazaki-Sugiyama et al. 2009).

The extent and type of inhibitory plasticity likely depend on several parameters, including the type of hearing loss, age of onset, magnitude, and duration (Sanes et al. 2009; Takesian et al. 2009). Inhibitory plasticity clearly occurs after early life. For example, it has been shown that adult cortical GABAergic neurons continue to undergo dendritic remodeling, suggesting that they remain plastic (Lee et al. 2006). Similarly, aging is associated with a decrease in GABAergic transmission, and this has been proposed to reflect a loss of hearing (presbycusis) analogous to changes induced by developmental hearing loss (Caspar et al. 2008). However, age-related hearing loss is likely to involve a prolonged degeneration of hair cell function, in contrast to CHL, and this could induce changes to the adult CNS. Changes in GABAergic inhibition may also lead to auditory cortex suppression in adults following passive exposure to band-limited tone pip ensembles (Noreña et al. 2006; Pienkowski et al. 2011). Our study suggests that, at a minimum, cortical inhibitory plasticity occurs over a slower time scale in the adult compared with the developing animal.

**Implications for auditory dysfunction after early CHL.** Many previous reports have demonstrated that the early acoustic environment influences central coding properties and maps (Chang and Merzenich 2003; Chang et al. 2005; Kandler et al. 2009; Knudsen et al. 1984a, 1984b; Poon et al. 1990; Razak et
al. 2008; Sanes and Constantine-Paton 1985; Zhang et al. 2002; Zhou et al. 2008). When the identical manipulation is performed at different times during development, age-dependent effects are often observed, indicating a critical period (de Villers-Sidani et al. 2007; Insanally et al. 2009). This principle appears to extend to hearing loss (Kral et al. 2002). For example, ear-plugging mice at P17–P21 leads to a dramatic increase in sensitivity to sound, but the same manipulation at P42–P46 does not induce this sensitivity (McGinn and Henry 1975). Similarly, monaural CHL shifts cortical tonotopic maps and augments responses from the unaffected ear, but only when it is induced at an early age (Popescu and Polley 2010).

CHL-induced alterations in central response properties in developing animals may underlie the impairments in sound localization (Clements and Kelly 1978) and binaural masking (Moore et al. 2003) that persist even after CHL is reversed. In humans, children with a history of severe CHL resulting from ear infections are at risk for receptive language deficits that can persist even after normal audiometric hearing is restored (Whitton and Polley 2011). We suggest that a persistent change in inhibitory synaptic strength may be involved in such long-term deficits associated with early CHL. Understanding the precise synaptic mechanisms underlying these changes will be instrumental in identifying targets to recover inhibitory function in adulthood.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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