Synaptic Transmission at the Cochlear Nucleus Endbulb Synapse During Age-Related Hearing Loss in Mice

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Wang, Yong and Paul B. Manis. Synaptic transmission at the cochlear nucleus endbulb synapse during age-related hearing loss in mice. J Neurophysiol 94: 1814–1824, 2005. First published May 18, 2005; doi:10.1152/jn.00374.2005. Age-related hearing loss (AHL) typically starts from high-frequency regions of the cochlea and over time invades lower-frequency regions. During this progressive hearing loss, sound-evoked activity in spiral ganglion cells is reduced. DBA mice have an early onset of AHL. In this study, we examined synaptic transmission at the endbulb of Held synapse between auditory nerve fibers and bushy cells in the anterior ventral cochlear nucleus (AVCN). Synaptic transmission in hearing-impaired high-frequency areas of the AVCN was altered in old DBA mice. The spontaneous miniature excitatory postsynaptic current (mEPSC) frequency was substantially reduced (about 60%), and mEPSCs were significantly slower (about 115%) and smaller (about 70%) in high-frequency regions of old (average age 45 days) DBA mice compared to normal hearing young and old DBA mice. Moreover, synaptic release probability was about 30% higher in high-frequency regions of young DBA mice than that in old DBA mice. Auditory nerve-evoked EPSCs showed less rectification in old DBA mice, suggesting recruitment of GluR2 subunits into the AMPA receptor complex. No similar age-related changes in synaptic release or EPSCs were found in age-matched, normal hearing young and old CBA mice. Taken together, our results suggest that auditory nerve activity plays a critical role in maintaining normal synaptic function at the endbulb of Held synapse after the onset of hearing. Auditory nerve activity regulates both presynaptic (release probability) and postsynaptic (receptor composition and kinetics) function at the endbulb synapse after the onset of hearing.

INTRODUCTION

The anterior ventral cochlear nucleus (AVCN) principal neurons, the stellate and bushy cells, receive obligatory synaptic input from the central projections of spiral ganglion cells. There is a well-organized tonotopic innervation pattern in the AVCN such that high-frequency auditory nerve fibers project to the dorsal region, whereas low-frequency fibers innervate the ventral regions of the AVCN (Berglund and Brown 1994; Rose et al. 1960; Rouiller and Ryugo 1984). To faithfully convey fine acoustic temporal features, a specialized synapse is formed between multiple release sites, called the endbulb of Held, connects the auditory nerve fibers to the bushy cells. AVCN bushy neurons also have a prominent low-threshold K+ conductance that is partially activated at rest, endowing the cells with a low input resistance and short membrane time constant (Manis and Marx 1991; Oertel 1983; Rothman and Manis 2003). α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated synaptic currents in AVCN neurons are large and among the briefest in the nervous system (Brenowitz and Trussell 2001; Gardner et al. 1999, 2001; Issaean and Walmsley 1996; Raman and Trussell 1992). The postsynaptic receptors lack GluR2 subunits and consist primarily of flop subunit splice variants (Brenowitz and Trussell 2001; Gardner et al. 1999, 2001; Lawrence and Trussell 2000; Parks 2000). These features are in part responsible for the rapid desensitization of the receptors in auditory neurons (Geiger et al. 1995), thereby achieving exquisite temporal coding by minimizing postsynaptic temporal summation.

Because the principal neurons of the AVCN receive direct afferent innervation from the cochlea and provide essential timing information to other auditory centers for both sound localization and pitch detection, the effect of age-related hearing loss on synaptic transmission in these cells could have a profound impact on the residual auditory function (Syka 2002). A number of morphological, biochemical, and physiological changes occur in cochlear nucleus (CN) neurons after hearing loss produced by cochlear ablation. These effects include cell shrinkage (Pasic and Rubel 1989; Willott et al. 1987), abnormal expression of transcription factors and synaptic proteins (Ilting et al. 1997; Luo et al. 1999; Sie and Rubel 1992; Sunega et al. 1998), altered neuronal excitability (Francis and Manis 2000; Lu et al. 2004), and, depending on ages, cell death (Mostafapour et al. 2000; Tierney et al. 1997; Zirpel et al. 2000). The shape of the endbulbs is also modified by deafness in cats (Ryugo et al. 1998) and mice (Limb and Ryugo 2000). Recent studies in young (P7–10) congenitally deaf mice have shown that the auditory nerve-evoked excitatory postsynaptic current (EPSC) amplitude is larger and transmitter release probability is higher than comparable normal-hearing animals (Oleskevich and Walmsley 2002; Oleskevich et al. 2004). Many other functional changes in both excitatory and inhibitory synaptic transmission have been shown along the central auditory brain stem pathways in both congenitally deaf and cochlear ablated animals (Kotak and Sanes 1997; Leao et al. 2004; Oleskevich et al. 2004; Vale and Sanes 2002).

To investigate the consequences of peripheral hearing loss [age-related hearing loss (AHL)] on synaptic transmission in the first central auditory relay nucleus, AVCN, we chose to use the inbred DBA strain of mouse. DBA mice possess multiple major recessive genes on chromosome 10 that are responsible for AHL (Johnson et al. 1997; Noben-Trauth et al. 2003). At the onset of hearing, which occurs between 2 and 3 wk of age, DBA mice have normal-hearing thresholds (Willott and Erway 1991; Oertel 1983; Rothman and Manis 2003).

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Slice preparation

Slices of cochlear nucleus were prepared as previously described (Manis 1989, 1990). Briefly, mice were anesthetized with sodium pentobarbital [50 mg/kg, administered intraperitoneally (ip)] or ketamine (100 mg/kg) and xylazine (10 mg/kg, ip), and then decapitated. The brain stem including the cochlear nucleus was immediately dissected out and immersed in preswallowed (34°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 122 NaCl, 3 KCl, 1.25 KH2PO4, 20 glucose, 25 NaHCO3, 2 Na-pyruvate, 3 myo-inositol, 0.4 ascorbic acid, 0.1 CaCl2, 3.7 MgSO4, and bubbled with 95% O2-5% CO2 to a pH of 7.4. Brain stems were trimmed and mounted on a cutting block and 200–250 μm parasagittal sections of the cochlear nucleus were sliced on a vibratome. After incubation for ≥30 min at 34°C, each slice was secured in the recording chamber and superfused with recording ACSF (same as dissection ACSF except 2 mM CaCl2, 2 mM MgSO4) at a rate of 3–5 ml/min.

Electrophysiological recordings

AVCN neurons were visualized with a water-immersion objective (40×) using Nomarski differential interference contrast optics on a Zeiss FS Axioskop (Zeiss, Oberkochen, Germany). To enhance image contrast in slices from older animals, the field diaphragm was closed nearly all the way, no infrared filter was used, and the condenser was aligned slightly eccentrically (Gardner et al. 2001; Kachar 1985). Patch electrode pipettes (3–8 MΩ) were pulled from borosilicate glass (KG-33, Garner Glass, Claremont, CA) with a Sutter P2000 puller (Sutter Instruments, San Francisco, CA), coated with Sylgard 184 (Dow Corning, Midland, MI) before use. The standard electrode solution contained (in mM) 110 K gluconate, 4 NaCl, 20 KCl, 10 HEPES, 0.2 EGTA, 4 Mg2+ -ATP, 10 creatine phosphate, and 0.3 GTP. The pH was adjusted to 7.2 with KOH, and the final osmolarity was about 295 mOsm. For evoked EPSC recordings, a Cs+-based electrode solution containing 2–5 mM QX-314 (Tocris Cookson, Bristol, UK) was used to minimize contamination from potassium and sodium conductances. The solution contained (in mM) 125 CsMeSO4, 15 CsCl, 5 EGTA, 10 HEPES, 4 MgATP, 10 creatine phosphate, 0.3 GTP, and was adjusted to pH 7.2 with CsOH. N-(4-Hydroxyphenyl)-propionyl)-spermine trihydrocholoride (NHPP-spermine; Tocris Cookson), 50 μM, was added to the electrode solution when studying EPSC rectification. For most recordings, the fluorescent dye Alexa Fluor488 (Molecular Probes, Eugene, OR) was added to aid cell visualization and identification. For recordings made with potassium gluconate electrodes, the membrane potential of each cell was adjusted by −12 mV to account for the liquid junction potential of the gluconate solution. Recordings using cesium solutions were not corrected. Recordings pertaining to spontaneous miniature excitatory postsynaptic current (mEPSC) data were performed at 34°C using an Axopatch 200B (Axon Instruments, Foster City, CA) under the
control of in-house software written in Matlab (The MathWorks, Natick, MA). Whole cell access resistance was <15 MΩ and compensated to >70% on-line with a 20-µs lag time. Spontaneous synaptic responses were low-pass filtered at 5 kHz and sampled at 10 kHz. Some experiments were also repeated with 10-kHz low-pass filter and 50-kHz sampling rate.

For evoked EPSCs, recordings were made at room temperature (about 23°C) to reduce the peak-evoked EPSC amplitude and slow the time course for better voltage-clamp control. A concentric bipolar stimulating electrode was placed on the auditory nerve root (Fig. 1A). A stimulus–response function was collected to determine the EPSC threshold. The stimulus strength was then adjusted to be 1.5 to 2 × the current required to elicit reliable EPSCs. Only cells with large all-or-none EPSCs to graded stimulation intensities were selected for analysis because these responses are typical of bushy cells in AVCN (Isaacson and Walmsley 1995). When cells showed more than one EPSC level, the stimulus was adjusted to reliably produce EPSCs at the first (lowest) amplitude, while not triggering larger EPSCs.

Stationary mean–variance analysis was used to estimate the initial release probability (P) of the synapse (Oleskevich et al. 2000; Reid and Clements 1999). P was manipulated by varying the external calcium concentration (1–3 mM), while adjusting extracellular magnesium concentration to maintain a constant total divalent cation concentration in the ACSF. The mean EPSC amplitude and the variance were obtained over a stable epoch of 60–80 trials (<20% drift). A parabola was fit against the mean–variance data by minimizing least mean square error and forcing the function through the origin. The equation for the parabola is

\[ y = Ax + Bx^2 \]

where y is EPSC variance and x is EPSC mean amplitude. Release probability can then be calculated as

\[ P_r = x(-B/A) \]

where x corresponds to the mean amplitude of the EPSC at the standard calcium concentration (2 mM). A lower limit of the number of release sites can be estimated as

\[ N_{min} = -1/B \]

Data analysis

Each cell recorded in current clamp was characterized by its responses to current pulses. The input resistance of the cell was measured as the maximum slope of the current–voltage relationship in the range 0–40 mV negative to the resting membrane potential (RMP). The shape of the action potential was quantified by measuring spike height, spike width at half-height, and the maximum rising and falling rates (Francis and Manis 2000). Action potential current threshold was measured as the smallest current step that elicited an action potential.

mEPSCs were analyzed in two steps. First, large events were identified using simple amplitude detection with the threshold set to 4SD of the background noise level. A template mEPSC was created using the rising and decay τ of the averaged mEPSC acquired in the first analysis run. In the second step, this template was used to detect mEPSCs with a scaled-template method (Clements and Bekkers 1997) implemented in Matlab, with the detection criterion set at three to four times the SD of the recording noise. mEPSC amplitude, 20–80% rise time, decay τ, and interevent interval were measured.

Statistical significance (P < 0.05) was determined using parametric unpaired Student’s t-test using GraphPad software (Prism, San Diego, CA).

RESULTS

A total of 104 DBA mice and 43 CBA mice were used in this series of experiments. To minimize contributions of develop-

mental maturation, no animals younger than 17 days old were used. The hearing loss in old DBA mice was tested behaviorally with Preyer’s reflex before brain stem slice preparation. Old DBA mice uniformly lacked a Preyer’s reflex, whereas young DBA mice and CBA mice had a normal Preyer’s reflex. We later confirmed (in a separate study; Wang and Manis, unpublished observations) with auditory brain stem–evoked responses that click thresholds were elevated by about 20 dB in old DBA mice.

The majority of VCN neurons recorded belonged to two classes, type I and type II, as previously described by Oertel (1983). Type II neurons have been previously identified as spherical or globular bushy cells (Brawer et al. 1974; Oertel 1983; Osen 1969; Wu and Oertel 1984). In this study, we focused on type II cells. As reported previously, the electrical properties of the type II cells appeared to be scarcely affected by the status of the auditory periphery (Francis and Manis 2000; Lu et al. 2004). The resting membrane potential, input resistance, action potential (AP) height, AP width (at half-height), and AP afterhyperpolarization (AHP) were not statistically different between cells in high-frequency regions of normal-hearing young and impaired-hearing old DBA mice. However, the amount of current necessary to evoke an action potential was found to be significantly elevated in the old DBA mice (Table 1).

Spontaneous mEPSC event frequency is reduced in older hearing-impaired DBA mice

Spontaneous mEPSCs were recorded for ≥40 s while holding the cell membrane at −60 mV. Figure 2A shows representative traces from bushy cells in high-frequency regions of old and young DBA mice, as well as young CBA mice. Spontaneous mEPSC event frequency from high-frequency regions in young DBA mice was 124% higher than that in old DBA (young DBA: 11.50 ± 2.27 Hz, n = 12; old DBA: 5.13 ± 1.34 Hz, n = 13; P = 0.022) (Fig. 2B). Spontaneous mEPSC event frequency was 14.52 ± 4.90 Hz (n = 3) for low-frequency cells in old DBA mice. The event frequencies were not statistically different between the young HF cells and the old LF cells in DBA mice. In addition, mEPSC event frequency was not different between young and old CBA HF bushy cells (6.43 ± 1.54 Hz, n = 6; 6.93 ± 2.49 Hz, n = 8; P = 0.88), although event frequencies were lower in CBA mice than in young DBA mice. Pairwise comparisons between the normal-hearing young DBA HF and young CBA HF or between young DBA HF and old CBA HF also did not reveal differences (young DBA HF vs. old CBA HF, P = 0.16; young DBA HF vs. young CBA HF, P = 0.20) (Fig. 2B). Thus the regions of the AVCN subject to hearing loss selectively exhibit a lower spontaneous mEPSC frequency, whereas this is not seen in age-matched CBA mice or in the low-frequency regions of old DBA mice.

Release probability is lower in high-frequency bushy cells of hearing-impaired DBA

Lower spontaneous EPSC event frequency in the hearing-impaired animals may be attributable either to lower synaptic release probability at the endbulb of Held or to a reduction in the number of active release sites. To test the hypothesis that
release probability changed, we measured evoked EPSC from auditory nerve stimulation using a paired-pulse protocol. As typical of many synapses (reviewed by Thomson 2000), endbulb synapses exhibit an inverse linear relationship between the initial release probability and the paired-pulse ratio (Oleskevich and Walmsley 2002; Oleskevich et al. 2000). High release probability tends to result in paired-pulse depression, whereas low release probability tends to produce paired-pulse facilitation. At 23°C paired-pulse depression was observed in HF bushy cells from young DBA mice, whereas paired-pulse facilitation was seen in HF bushy cells from old DBA mice (Fig. 3A). In the HF bushy cells of young DBA mice, the P2/P1 ratio was 0.87 ± 0.06 (n = 19), whereas in the HF bushy cells of old DBA mice the ratio was 1.09 ± 0.06 (n = 9, P = 0.04, Fig. 3B). In Fig. 3C, paired-pulse ratios from all individual cells were plotted against the spontaneous mEPSC event frequency. Cells with strong paired-pulse depression had higher mEPSC event frequency (P = 0.039, event frequency was 5.95 ± 0.71 for young, and 4.06 ± 0.50 for old DBA at 23°C). The evoked EPSC amplitude in old and young DBA mice was not significantly different (2.66 ± 0.52 nA, n = 8; 4.03 ± 0.61 nA, n = 18, P = 0.10), although there was a tendency for old animals to have smaller AMPA concentrations (Fig. 3D).

To further assess release probability (P), we used stationary EPSC mean–variance analysis (see METHODS), and manipulated the release probability by varying external Ca2+ concentration (Fig. 4). These experiments also demonstrated a difference in release probability of HF bushy cells between young and old DBA mice. The estimated release probability at physiological Ca2+ concentration (2 mM) was 0.65 ± 0.03 (n = 9) for young DBA mice, whereas it was 0.47 ± 0.08 (n = 5) for old DBA mouse (P = 0.039). Although the mean–variance technique does not permit a direct measure of the number of release sites, a lower limit can be estimated (Oleskevich et al. 2000). The minimum number of release sites onto HF bushy cells was not significantly different between young (Nrel.sites = 59 ± 17, n = 9) and old DBA mice (Nrel.sites = 86 ± 28, n = 5; P = 0.39). The difference in release probability (about 30%) could readily account for the nearly 30% reduction of evoked EPSCs we observed in older DBA mice (Fig. 3C).

**Spontaneous mEPSCs have a slower decay time constant in high-frequency bushy cells of hearing-impaired DBA mice**

In AVCN, mEPSCs in mature mice are mediated predominantly by AMPA receptors (Bellingham et al. 1998; Isaacson and Walmsley 1996), which mostly contain GluR3 and GluR4 subunits (reviewed by Parks 2000). Consistent with previous reports (Brenowitz and Trussell 2001; Gardner et al. 1999), mEPSCs recorded in DBA and CBA mice were characteristically brief. Figure 5 illustrates the distribution of mEPSCs and decay time constants from a HF bushy cell in a young DBA mouse. Figure 5A shows the normalized average of all the mEPSCs (about 500 events) from this cell. The mEPSC am-

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**TABLE 1. Parameters measured within DBA and CBA mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DBA</th>
<th>CBA</th>
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<tr>
<td></td>
<td>HF Old (n = 21)</td>
<td>HF Young (n = 14)</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>–60.9 ± 1.4</td>
<td>–61.5 ± 1.1</td>
</tr>
<tr>
<td>Rm, Ω</td>
<td>121.0 ± 13.2</td>
<td>140.7 ± 18.9</td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>1.05 ± 0.09</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>AP threshold I, pA</td>
<td>313.1 ± 30.1</td>
<td>189.8 ± 28.0*</td>
</tr>
<tr>
<td>AP height, mV</td>
<td>36.8 ± 3.6</td>
<td>40.1 ± 2.8</td>
</tr>
<tr>
<td>AP half-width, ms</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>AHP, mV</td>
<td>9.2 ± 1.0</td>
<td>7.5 ± 1.7</td>
</tr>
<tr>
<td>Max rising rate of spike, mV/ms</td>
<td>74.9 ± 8.8</td>
<td>88.3 ± 6.7</td>
</tr>
<tr>
<td>Max falling rate of spike, mV/ms</td>
<td>73.9 ± 19.2</td>
<td>60.7 ± 9.2</td>
</tr>
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Values are means ± SE. Except the current required to reach spike threshold, no pairwise significant difference was observed for all parameters measured within DBA or CBA mice. With the exception of Rm between young DBA and old CBA mice, none of the parameters measured was significantly different between DBA and CBA mice. *P < 0.01.
amplitudes (124.4 ± 51.8 pA; CV = 0.42) showed a Gaussian distribution (Fig. 5C). Although there was a broad range of mEPSC amplitudes, the majority of mEPSCs had a similar decay time constant (0.15 ± 0.04 ms, CV = 0.27; Fig. 5B). The intervals between mEPSCs were Poisson distributed with a CV = 1.05 (Fig. 5D).

The mEPSC decay time constant was 115% slower in cells from the HF region of old DBA mice than that in tonotopically
matched young DBA mice (Fig. 6, A and B). Decay time constants were 0.13 ± 0.02 ms (n = 12) for young and 0.28 ± 0.06 ms (n = 13) (P = 0.039) for old DBA bushy cells in the HF region (Fig. 6B). Because the decay time constants were so fast, we repeated this experiment in a separate group of cells, using a faster A/D conversion rate (20 μs per point), and obtained very similar results (0.12 ± 0.01 ms, n = 8 young DBA cells vs. 0.24 ± 0.04 ms, n = 9, old DBA cells, P = 0.033). Decay time constants for all the cells that were not affected by hearing loss were similar to those seen in HF bushy cells of young DBA mice; the decay time constants (in milliseconds) were 0.16 ± 0.06 (n = 3, old DBA LF), 0.19 ± 0.02 (n = 8, young CBA HF), and 0.15 ± 0.02 (n = 6, old CBA HF). In spite of the significant difference in mEPSC decay time constants of HF bushy cells between young and old DBA mice, there was no difference in the decay time constants of the evoked EPSC at 23°C (0.51 ± 0.04, n = 18, young vs. 0.54 ± 0.06, n = 8, old). Presumably, the effects of asynchronous release at the endbulb terminal (Isaacson and Walmsley 1995; Oleskevich and Walmsley 2002) obscured any difference in the decay rate. Spontaneous mEPSC amplitude was about 30% smaller in old, hearing-impaired DBA mice than that in young DBA mice (80.9 ± 10.8 vs. 113.8 ± 8.9 pA; P = 0.029; Fig. 6C). In contrast, mEPSC amplitudes were not significantly different between the LF cells of old DBA and HF cells of young DBA mice (102.0 ± 15.3 vs. 113.8 ± 8.9 pA, P = 0.555). When comparing amplitudes of HF cells in old and young CBA mice, the opposite relationship appeared, although it was not significant (119.7 ± 19.7 pA, n = 6 young CBA cells vs. 83.1 ± 7.7 pA, n = 8 old CBA cells; P = 0.13).

mEPSCs in cells not affected by hearing loss tended to be larger and more frequent with a wide range of variability (Fig. 7A). The amplitude and event frequency for the two normal-hearing groups (young DBA HF, old DBA LF) were significantly different from those of impaired-hearing old DBA high-frequency group. Larger mEPSCs tended to have faster decay time constant (Pearson’s correlation coefficient r² = 0.72; Fig. 7B). A similar systematic relationship between the mEPSC amplitude and decay time constant was also observed at the endbulb synapse in avian nucleus magnocellularis during development (Brenowitz and Trussell 2001). mEPSCs with a slow decay τ did not have a slow rise time (Fig. 7C). A linear regression to the data in Fig. 7C yielded a slope of 0.05 (r² = 0.03). There were no apparent differences between either the amplitude-decay time constant nor the rise-decay time constant relationships between the different groups.

Synaptically activated AMPA currents show different rectification in high-frequency bushy cells of young and old DBA mice

The increase in mEPSC decay time constant in the HF cells of old DBA mice suggested that there may be a change in postsynaptic receptor subunit assembly because the kinetics of the AMPA receptor–mediated EPSC depends on the subunit composition (Geiger et al. 1995; Seeburg 1996). Four AMPA receptor subunits (GluR1–GluR4) have been identified (Boulter et al. 1990; Hollmann et al. 1994; Keinanen et al. 1990), and they can form homomeric or heteromeric receptor complexes. GluR2 plays a critical role in determining AMPA receptor properties. Receptors with GluR2 show slower decay kinetics and are impermeable to Ca²⁺ (Geiger et al. 1995; Jonas and Burnashev 1995; Seeburg 1996). AMPA receptors lacking the GluR2 subunits show greater Ca²⁺ permeability and an inward rectification in I–V relationship at positive

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voltages, arising from a voltage-dependent block by intracellular polyamines (Bowie and Mayer 1995; Kamboj et al. 1995; Washburn and Dingledine 1996). To test whether changes in AMPA receptor composition accompany hearing loss, we recorded auditory nerve–evoked EPSCs at various holding voltages with recording electrodes containing 50 \mu M NHPP-spermine, a polyamine that enhances rectification when applied intracellularly (Washburn and Dingledine 1996). The rectification index was computed by taking the ratio of the synaptic conductance at \(-40\) mV and \(-60\) mV [similar results were obtained when the \(I-V\) relationship was fitted with the Woodhull equation (Woodhull 1973), which models the voltage-dependent block of the receptors by a charged compound]. Representative evoked EPSC current–voltage plots of individual bushy cells are shown in Fig. 8A. High-frequency cells from young DBA mice showed more rectification than those from old DBA mice (Fig. 8B). Overall, the rectification index in young DBA HF bushy cells was \(0.30 \pm 0.07\) \((n = 5)\), whereas the rectification index in HF cells from old DBA mice was \(0.57 \pm 0.03\) \((n = 8, P = 0.001)\). To determine whether this shift reflected continued development, we also measured rectification in HF bushy cells from age-matched groups of CBA mice. No age-dependent change in EPSC rectification was observed in CBA mice \((0.43 \pm 0.04, n = 11; 0.34 \pm 0.06, n = 7\) for old and young CBA, respectively; \(P = 0.22)\). These changes in the polyamine-dependent rectification of the EPSCs are consistent with a change in subunit composition of the receptor.

**DISCUSSION**

We have shown that there are changes in synaptic transmission between the remaining auditory nerve fibers and their postsynaptic targets in the hearing-impaired high-frequency region of the AVCN in \(\geq 6\)-wk-old DBA mice. The principal changes in synaptic transmission in the HF regions include a reduction of mEPSC frequency, a slowing of mEPSC decay time course, an increase in paired-pulse potentiation and a corresponding decrease in the measured release probability, and a decrease in the voltage-dependent rectification of the EPSC. These results suggest that the deafness in these mice results in a cluster of changes in synaptic function that involve both presynaptic and postsynaptic mechanisms.

It is difficult to identify the proximal cause of the changes that we see because the exact cause of the early onset of hearing loss in DBA mice is not clear. A mutation of an important protein in the stereocilia of hair cells, cadherin-23, has been implicated in hearing loss in DBA mice (Di Palma et al. 2001; Noben-Trauth et al. 2003), and is consistent with the ultrastructurally documented stereociliary disarray reported in this strain (Hultcrantz and Spangberg 1997). The disruption of the hair cell stereocilia has also been shown to result in a significant reduction of spontaneous activity in the auditory nerve fibers (Liberman and Dodds 1984). However, the hearing loss in DBA mice does not lead to immediate death of spiral ganglion cells (Willott and Erway 1998). Moreover, stimulation of the eighth nerve stump in brain slices of DBA mice (this study) produces EPSCs in high-frequency regions of the
AVCN that are comparable to those previously reported in normal-hearing animals (Isaacson and Walmsley 1996; Oertel 1983). Thus auditory nerve fibers appear to be intact in our DBA mice and synaptic transmission remains functional. However, the hearing loss is accompanied by a reduction in sound-evoked activity, and the structural changes in the stereocilia bundle seem likely to result in a decrease or total loss of spontaneous activity in the auditory nerve. Data from a recent study of single auditory nerve fiber recordings in another AHL strain of mice, C57, suggest a decreased maximal spontaneous rate among fibers from the high-frequency hearing-impaired region (Taberner and Liberman 2005; MC Liberman, personal communication). Although we suggest that the changes we see result from a decrease in spike frequency over time at the endbulb synapses, it is unclear whether there is a complete or only a partial loss of activity.

The changes in synaptic transmission at the endbulbs are largely confined to those synapses from the HF regions of the old DBA AVCN, suggesting that the synaptic plasticity is...
caused by the hearing loss. Alternatively, although hearing threshold becomes adultlike around P20 (Ehret 1976; Mikaelian et al. 1965), the changes could also reflect continued postnatal development in DBA mice because endbulbs show continued morphological maturation up to 60 days of age in C57 mice (Limb and Ryugo 2000). Studies in rat AVCN (Bellingham et al. 1998), chick nucleus magnocellularis (Brenowitz and Trussell 2001), and mouse MNTB (Joshi and Wang 2002) have all shown that both miniature and evoked EPSCs become progressively larger and faster at the endbulb/calyx of Held synapses during the early stages of postnatal development. In contrast, the physiological changes seen in the old DBA mice are in the opposite direction of those reported during normal development. We observed smaller, slower, and less-frequent mEPSCs in the hearing loss–affected HF bushy cells of old DBA mice (Figs. 2, 6, and 7). It is interesting to note that despite the changes observed in the mEPSC, we did not observe a significant difference in evoked EPSC time course and amplitude. Asynchronous release at the endbulb terminal (Isaacson and Walmsley 1995; Oleskevich and Walmsley 2002) contributes to the time course of the EPSC, and may obscure subtle changes that depend on receptor kinetics. In addition, compensatory mechanisms that change the number of functional release sites may regulate the EPSC amplitude (see following text).

Two other lines of evidence also suggest that the hearing loss itself, rather than continued development, may be responsible for altering synaptic transmission at these synapses. First, the differences in mEPSC frequency, amplitude, and decay 

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\text{were seen only between HF regions of old DBA and young DBA mice, but not between HF cells from age-matched groups in CBA mice. Second, there was no difference in mEPSC frequency and kinetics between cells in the LF regions of old DBA mice and the HF regions of young DBA mice. Both of these observations suggest that, after P20, maturation of mechanisms affecting mEPSCs does not occur in regions of the AVCN that retain normal hearing.}
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Two of the striking effects we observed in synapses from regions of the AVCN with hearing loss were decreased spontaneous mEPSC frequency and release probability for evoked EPSCs. Because an association clearly exists between endbulb morphology and the auditory nerve activity (Ryugo et al. 1996), a reduction in mEPSC frequency could result from fewer active release sites, perhaps as a consequence of reduced endbulb complexity, or from lower vesicle release probability. Our mean–variance analysis data showed that the overall number of functional release sites on bushy cells did not change significantly at this stage of hearing loss in DBA mice, although interestingly the estimate of the number of sites in older animals was greater than that in younger animals. The number of release sites estimated in our study is somewhat smaller than that reported in “normal-hearing” P11–P16 CBA mice (N_rel. sites = 91), as well as congenitally deaf dn/dn mice (N_rel. sites = 97) (Oleskevich and Walmsley 2002), in part because we did not correct for the asynchronous release. In contrast, the vesicle release probability appears reduced at affected synapses. Both paired-pulse and mean–variance analyses demonstrated that the release probability is significantly lower in HF endbulb synapses of hearing-impaired mice (Fig. 4). Although we cannot unequivocally rule out a contribution of continued development, this decreased release probability is in striking contrast to a twofold increase in release probability at the same synapse in congenitally deaf dn/dn mice (Oleskevich and Walmsley 2002). Because the dn/dn mice never attain hearing status, the increased release probability in those mice may reflect the consequences of abnormal development, as opposed to the onset of deafness that occurs after a period of normal development.

The changes in mEPSC time course and voltage-dependent rectification of the EPSC in the HF regions of old DBA, but not in CBA mice, suggest a concurrent change in postsynaptic AMPA receptor subunit composition with hearing loss in DBA mice. AVCN principal neurons express different levels of glutamate receptor variants (Hunter et al. 1993; Parks 2000; Wang et al. 1998). During development, AMPA receptors in AVCN neurons gradually transit to flop isoforms, and lose GluR2 (Gardner et al. 2001 1999; Isaacson and Walmsley 1996; Lawrence and Trussell 2000). Activity plays a critical role in specifying and determining the composition of AMPA receptors. In rat cerebellar stellate cells, evoked and spontaneous synaptic activity can recruit GluR2 subunits to the receptor complex, altering both voltage-dependent rectification and Ca\(^{2+}\) permeability (Gardner et al. 2005; Liu SJ and Cull-Candy 2002; Liu SQ and Cull-Candy 2000). In addition, GluR2 expression can be regulated by trophic factors such as BDNF (Narisawa-Saito et al. 2002), which may promote the N-ethylmaleimide–sensitive factor-dependent translocation of GluR2-containing receptors to the cell surface (Nishimune et al. 1998; Song et al. 1998). Alternatively, activity-dependent trafficking and recycling of other GluR subunits can affect the amount of GluR2 in the receptor complex (Ju et al. 2004; Zhu et al. 2000). Thus there is an ample precedent for the idea that prolonged decreases in synaptic activity associated with hearing loss in AVCN bushy cells could alter the receptor subunit composition. The changes we observed in evoked EPSC rectification (Fig. 8) and mEPSC kinetics are consistent with the notion that GluR2 subunits are being reintroduced into the receptor complex, either through up-regulation of GluR2 subunits, or indirectly as a result of down-regulation of other subunits.

In conclusion, we have shown functional changes in both presynaptic release of transmitter and in postsynaptic responses at the endbulb synapse in DBA mice with hearing loss. Although the immediate cause of these changes is not known, the case can be made that driven activity is decreased, and it seems likely that spontaneous activity is also reduced, in the high-frequency hearing-impaired auditory nerve fibers of DBA mice. Such changes are similar to those expected in cases of acquired deafness in humans. The hearing loss in our study produces a different pattern of changes in the function of endbulb synapses from those reported previously for congenital hearing loss. These results also indicate that activity-dependent changes in the central auditory pathway accompany peripheral hearing loss, and highlight the importance of early intervention with cochlear implants (Sharma et al. 2002; Zwolan et al. 2004).

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**References**

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