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Aged-Related Loss of Temporal Processing: Altered Responses to Amplitude Modulated Tones in Rat Dorsal Cochlear Nucleus

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Abstract

Loss of temporal processing is characteristic of age-related loss of speech understanding observed in the elderly. Inhibitory glycinergic circuits provide input onto dorsal cochlear nucleus (DCN) projection neurons which likely serve to modulate excitatory responses to time-varying complex acoustic signals. The present study sought to test the hypothesis that age-related loss of inhibition would compromise the ability of output neurons to encode sinusoidally amplitude modulated (SAM) tones. Extracellular recordings were obtained from young and aged FBN rat DCN putative fusiform cells. Stimuli were SAM tones at 3 modulation depths (100, 50, and 20%) at 30 dB HL with the carrier frequency set to the unit's characteristic frequency. Discharge rate and synchrony were calculated to describe SAM responses. There were significant age-related changes in the shape and peak vector strength [best modulation frequency (BMF)] of temporal modulation transfer functions (tMTFs), with no significant age-related changes in rate modulation transfer functions (rMTFs) at BMF. Young neurons exhibited band-pass tMTFs for most SAM conditions while aged fusiform cells exhibited significantly more low-pass or double-peaked tMTFs. There were significant differences in tMTFs between buildup, pauser- buildup, and wide-chopper temporal response types. Young and aged wide-choppers displayed significantly lower vector strength values than the other two temporal DCN response types. Age-related decreases in the number of pauser-buildup response types and increases in wide-chopper types with age reported previously, could account, in part, for the observed loss of temporal coding of the aged fusiform cell. Age-related changes in SAM coding were similar to changes observed with receptor blockade of glycinergic inhibition onto fusiform cells and consistent with previously observed age-related loss of endogenous glycine levels and changes in normal adult glycine receptor function. DCN changes in SAM coding could, in part, underpin temporal processing deficits observed in the elderly.

Keywords

Dorsal Cochlear Nucleus; Aging; Auditory; Glycine; Amplitude Modulation; Temporal Processing

Presbycusis, age-related hearing loss, is a complex disorder that may result in a slow deterioration of peripheral auditory input to auditory regions of the brain (see Willott, 1991;

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Syka, 2002). One common complaint of age-related hearing loss is the difficulty understanding speech under acoustically-challenging listening conditions (see CHABA, 1988; Willott, 1991, Chapter 6, for review). Manifestations of this include age-related temporal processing deficits (Welford, 1984; Gordon-Salant and Fitzgibbons, 1993; Schneider et al., 1994; Fitzgibbons and Gordon-Salant, 1995; Turner et al., 1995; Snell, 1997; Fitzgibbons et al., 2006; Gifford et al., 2007; Pichora-Fuller et al., 2007). Amplitude-modulation is an important temporal feature of sounds found in speech and other species-specific vocalizations. The envelope of sinusoidally amplitude modulated (SAM) sound is coded at all levels of the central auditory system (Joris et al., 2004; Ter-Mikaelian et al., 2007), where inhibitory circuits are thought to enhance temporal coding (Koch and Grothe 1998; Backoff et al., 1999; Krishna and Semple, 2000; Caspary et al., 2002; Ter-Mikaelian et al., 2007). Humans show age-related deficits in amplitude modulation depth discrimination (Takahashi and Bacon, 1992; Leigh-Paffenroth and Fowler, 2006) and numerous other temporally-challenging discrimination tasks including speech discrimination, gap detection and localization of sounds in space (Warren et al., 1978; Brown, 1984; Barsz et al., 2002).

In rodents, age-related changes in neural processing of AM signals have been found in inferior colliculus (IC) (Palombi et al., 2001; Walton et al., 2002; Simon et al., 2004). Functional and neurochemical studies in animal models suggest that sensory aging may begin as a slow progressive peripheral deafferentation, which may trigger an activity-dependent compensatory down-regulation of central inhibitory neurotransmission (Caspary et al., 1990, 2002, 2005; Schmolesky et al., 2000; Mendelson and Ricketts, 2001; Leventhal et al., 2003; Yu et al., 2006). Thus, clinically observed age-related central sensory processing deficits may be attributable at least in part, to decrements in inhibitory neurotransmission (Betts et al., 2007). A better understanding of the underlying inhibitory changes could enable identification of unique receptor targets for selective pharmacotherapy to reinstate inhibitory processing deficits.

The dorsal cochlear nucleus (DCN) receives primary excitatory auditory inputs from acoustic nerve fibers as well as intrinsic and extrinsic/descending inputs from auditory and non-auditory sources (Hackney et al. 1990; Cant and Benson, 2003; Young and Oertel, 2004; Zhou and Shore, 2004; Arnott et al., 2004). Neuro-anatomic, pharmacologic and physiological studies have contributed to a model of DCN neuronal circuitry in which multiple inhibitory inputs shape the response properties of DCN output neurons (fusiform and giant cells) (Voigt and Young, 1980; Caspary et al., 1987; Davis and Young, 2000; Young and Oertel, 2004; Rubio and Juiz, 2004; Kanold and Manis, 1999, 2005; Street and Manis, 2007). DCN output neurons, especially fusiform cells, receive focused glycinergic input from tonotopically-aligned vertical cells (Rhode, 1999) and less frequency-focused inputs from glycinergic D-multipolar cells in the ventral cochlear nucleus (VCN) (Saint-Marie et al., 1991; Kolston et al., 1992; Doucet et al., 1999). Vertical cells, termed narrow-band inhibitors, project strong glycinergic inhibition onto fusiform cells when stimulated at frequencies near characteristic frequency (CF) (Osen, 1969; Caspary et al., 1987; Osen et al., 1990; Rhode, 1999; Davis and Young, 2000). Fusiform cells are identifiable by their temporal responses to CF tone-burst stimuli resulting in, buildup, pauser-buildup and wide-chopper patterns (Rhode and Smith, 1986). The temporal response patterns are shaped, in part, by the magnitude and timing of inhibitory activation of transient potassium currents prior to excitation by an acoustic stimulus (Rhode and Smith, 1986; Hewitt and Meddis, 1995; Kanold and Manis, 1999, 2005; Street and Manis, 2007). Fusiform cells from young adult animals exhibit strong phase-locking to the SAM envelope and likely play a role in processing temporal information (Kim et al., 1990; Frisina et al., 1994; Rhode and Greenberg, 1994; Joris and Smith, 1998; Joris et al., 2004). These cells appear to enhance coding of SAM tones with respect to auditory nerve fibers by preferentially locking to a restricted range of envelope frequencies (band-pass tMTFs) with maximal sensitivity at a particular (best) modulation frequency (BMF) (Moller, 1974, 1976; Kim et al., 1990; Langner,

1992; Frisina et al., 1994; Rhode and Greenberg, 1994; Zhao and Liang, 1995, 1997; Joris et al., 2004). Application of the glycine receptor antagonist strychnine, onto DCN neurons increased discharge rate and reduced synchronization to the SAM envelope at low and near BMF frequencies, which in turn, resulted in a flatter less band-pass like tMTF (Backoff et al., 1999).

In light of these neurophysiological and neurochemical studies (also see discussion) suggestive of an age-related decrease in glycinergic inhibition in DCN as well as studies suggesting a role for inhibition in shaping temporal response properties, the present study examined age-related functional changes in the response properties of putative DCN output/fusiform cells to SAM stimuli. Response properties of these putative fusiform cells to simple stimuli have been previously reported (Caspary et al., 2005). We hypothesize that DCN output neurons will show age-related response changes consistent with a functional loss of inhibition, which will resemble changes observed with glycine-receptor blockade. These changes will result in a degraded temporal output of the DCN and input to the IC in aged animals.

Experimental Procedures

Surgical Protocol

Surgical methods were similar to those described in Caspary et al., (2005) and are summarized below. Young-adult (4–5 month) and aged (31–33 month) Fischer Brown Norway (FBN) rats (Harlan Sprague-Dawley, Inc.) were housed for less than a month at Southern Illinois University (SIU) and used under protocols approved by the SIU Laboratory Animal Care Committee. A full discussion of the anesthesia protocol used in aging studies is described in Palombi and Caspary (1996). Briefly, young rats were anesthetized with a mixture of ketamine (75 mg/kg, Aveco, Fort Dodge, Iowa) and xylazine (5 mg/kg, Lloyd Laboratories, Shennandoah, Iowa) along with 0.10 ml sodium pentobarbital (independent of weight, Abbott Laboratories, Illinois). Anesthesia was maintained by alternating hourly boosters of ketamine alone (~33 mg/kg) or ketamine/xylazine mixture (approximately one-third the original dose). Aged rats received lower doses of anesthesia (~85% of the young animal doses) to compensate for reduced liver function (Finlayson and Caspary, 1993; Palombi and Caspary, 1996; Turner et al., 2005). Booster times were similar for young and aged FBN rats suggesting that the dose adjustment for aged animals was appropriate. Body temperature was maintained at 37°C using a thermostatically-regulated heating pad.

Rats were placed in an IAC sound-attenuating booth. The right pinna was removed and the rat was placed in a stereotaxic frame using a small metal plate attached to the dorsal surface of the skull with bone screws and dental acrylic. The occipital bone was partially removed to access the posterior fossa. Glass micropipettes (8–16 M Ω) filled with either 2M potassium acetate or horseradish peroxidase (HRP) in 0.5M KCl Tris buffer (Sigma, St. Louis, Missouri) were advanced through the cerebellum into the right DCN.

Acoustic and Recording Protocol

Acoustic signals were generated by Tucker Davis Technologies (TDT) System II hardware (Alachua, Florida), amplified (Phase 3, HA-2B), transduced (Beyerdynamic DT931) and juxtaposed to the right ear canal using polypropylene tubing. The system was calibrated using a one-quarter inch Bruel & Kjaer model 4938 microphone (Naerum, Denmark) and a simulated rat ear (Palombi and Caspary, 1996). Calibration software performed a Fourier analysis on the signal generation system in response to a 1 Vrms click to generate calibration tables in dB sound pressure level (SPL re: 20 μ pa) for use by programmable attenuators (Windows-based software provided by Ken Hancock, Boston University; Hancock and Voigt, 2002a,b). Pure

tone intensities in dB SPL were accurate within 2 dB for frequencies up to 50 kHz. Signal generation and data acquisition were controlled by the above software.

Monaural auditory brainstem responses (ABRs) to broadband noise and 4, 8, 16 and 31.5 kHz tones were obtained from all animals at the beginning of each experiment with hearing thresholds determined visually (Casparly et al., 2005).

Upon isolation of a single unit, response maps were obtained to determine CF and threshold. Temporal response classification was as described in Casparly et al., (2005). Post-stimulus time histograms (PSTHs) were generated using 50 ms CF tone bursts (5 ms rise-fall), 30 dB above CF threshold. Data presented herein were collected from units judged to be fusiform cells based on previously published criteria from anesthetized preparations (Casparly, 1972; Godfrey et al., 1975; Rhode et al., 1983; Casparly et al., 1994; Backoff et al., 1997; Brozoski et al., 2002; Casparly et al., 2005). These criteria include: (1) Putative fusiform-cell recording depths were measured from the cerebellar surface and, when possible, from point of entry into the DCN. A mean depth of 3.52 mm in young animals and 3.36 mm in aged animals as measured from the cerebellar surface was equivalent to a depth of approximately 300 μ m into the DCN, as determined by the depth from the point at which sound is produced by the electrode puncturing the overlying pia mater until the appearance of a sound-evoked slow-wave; (2) units displaying buildup, pauser-buildup or wide-chopper responses and (3) units with large broad triphasic spikes. In most cases, histological verification revealed an electrode track coursing through the fusiform-cell layer with termination of the track shortly beyond the granule cell layer.

Histology

Following acquisition of electrophysiologic data, animals were given an overdose of anesthetic and perfused transcardially with 0.9% normal saline followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer. The brain removed and 40 μ frozen serially sections obtained and stained with thionin. HRP sections were stained for 5 min at 23°C in 0.06% diaminobenzidine HCl (Sigma), and mounted on glass microscope slides. HRP marks and electrode tracks were examined to verify recording sites within DCN (Palombi and Casparly, 1996). HRP was injected once in every experiment following acquisition of a complete data set from a putative fusiform cell. Recovery of HRP marks was near 50% with remaining tracks reconstructed onto DCN templates.

Acoustic Stimuli and Data Analysis

Spike data were imported into an Excel spreadsheet. Responses to SAM tones (*250 ms duration, 5 ms ramp, 2/s rate, carrier frequency set to CF*) were gathered with modulation frequencies stepping up from 16.8 Hz to 20% of CF in 0.75 octave steps (*10 presentations at each modulation frequency step*). SAM signals were set at 30 dB above CF threshold and varied by depth of modulation (100, 50, and 20%). Temporal responses of neurons to SAM signals (tMTFs) were analyzed for temporal synchronicity to the stimulus envelope. Vector strength was evaluated as a function of envelope frequency and depth of modulation. Average discharge rate was plotted against modulation frequency to generate a rMTF. Graphs of sync/vector strength vs. modulation frequency, referred to as tMTFs, were compared with respect to tMTF type, vector strength and the modulation frequency at the peak vector strength value (BMF). tMTFs were classified into the following categories:

Low-pass (LP) –vector strength is high at low modulation frequencies, decreases by > 3 dB and remains below 3 dB of the BMF as modulation frequency is increased,

Band-pass (BP) –vector strength progressively increases at low modulation frequencies until it peaks at a middle frequency, then vector strength decreases at higher modulation

frequencies, vector strengths at both low and high frequency extremes deviate from maximal vector strength by > 3 dB,

Low-pass notched (LPN) – similar to low-pass tMTF but contains a notch (drop of > 3 dB) within the high vector strength area, following the notch, high vector strength returns then decreases and remains low as modulation frequency is increased,

Double-peaked (DP) – similar to band-pass tMTF but contains a notch of > 3 dB within the high vector strength portion of the tMTF,

Complex (C) – vector strength fluctuates several times over the range of modulation frequencies.

Frequency of occurrence data were analyzed using the Chi-square test. Analysis of variance (ANOVA) and follow-up comparisons using Bonferroni corrections were used to analyze continuous data. Significance of vector strength was evaluated using the Rayleigh test.

Results

Age-related comparisons were made between 93 DCN neurons from 15 young-adult FBN rats and 88 DCN neurons from 16 aged FBN rats. DCN neurons included in the present study showed buildup, pauser-buildup or wide-chopper temporal responses, large broad triphasic spikes and were localized to the fusiform cell layer (see Methods and Caspary et al., 2005).

Age-related changes in response properties to simple tones

Young rat DCN neurons had a CF range of 1.3 kHz to 37.9 kHz whereas aged rat units had a CF range of 1.3 kHz to 39.1 kHz. Although frequency range was similar between young and aged units, fewer aged units were encountered at the frequency extremes. Single unit CF thresholds were significantly higher ($F=289.06$, $df=1,177$, $p<0.001$) in aged rats (mean 35 dB SPL) while young putative fusiform cells had a mean of 7.05 dB SPL. Likely due to a loss of near CF glycinergic inhibition, putative fusiform cells showed a significant age-related increase in maximum evoked discharge rate to CF tones (90 vs. 106 spikes/sec., $F=8.76$, $df=1,177$, $p=0.004$) and an increase in CF dynamic range (27dB vs. 34dB, $F=5.41$, $df=1,177$, $p=0.021$) (see Caspary et al., 2005). Caspary et al. (2005) reported a significant age-related change in the distribution of temporal (PSTH) response types for the same population of putative fusiform cells used in the present study. DCN fusiform cells in response to CF tonal stimuli at 30 dB above threshold primarily showed three classic PSTH categories: buildup, pauser-buildup and wide-chopper (Caspary et al., 2005). There was a 23% age-related loss of buildup and pauser-buildup response types (71 young, 55 in old) with a 65% increase in wide-chopper response types (17 young, 28 old) ($\chi^2=4.578$, $df=1$, $p=.032$).

Age-related changes in temporal response to SAM tones: Combined Data

SAM tones were presented at 30 dB above CF threshold and had modulation depths of 100%, 50%, or 20%. The carrier frequency was set to each unit's CF. Responses to all SAM conditions were obtained from 91 young and 84 aged putative fusiform cells. An overall analysis of each of the dependent variables (sync and rate) was conducted with age (young, aged), modulation frequency (16.8–1810.2 Hz), and modulation depth (100, 50, 20%) as independent variables. In brief, the present findings include a significant age-related loss of temporal coding of SAM stimuli in the absence of any significant age-related changes in rate coding. The shapes of tMTFs from young and aged neurons were classified as described above (Figure 1). The majority of young and aged neurons responded strongly to SAM tones with a band-pass tMTF (Figure 1). Aged neurons showed fewer band-pass tMTFs, especially for the 100% depth condition (Figure 1). At 100% modulation depth, aged neurons exhibited significantly more low-pass and low-pass notched units than corresponding young neurons ($\chi^2=17.1$, $df=1$,

$p < 0.001$). At 20% depth, aged units exhibited more double-peaked tMTFs than their young counterparts ($\chi^2 = 10.24$, $df = 1$, $p = 0.001$).

The mean and standard deviation of sync values for all young and aged neurons at each modulation frequency and depth were used to create composite tMTFs (Figure 2). When tMTFs from young fusiform cells were compared to tMTFs from aged fusiform cells there were significant differences at all three modulation depths (Figure 2). The largest age-related changes were observed at or below BMF and at the shallower modulation depths (Figure 2). For all three modulation depths, there was a significant interaction of modulation frequency with age (100% $F = 2.777$, $df = 9, 1491$, $p = 0.003$; 50% $F = 2.998$, $df = 9, 1481$, $p = 0.002$; 20% $F = 3.729$, $df = 9, 1436$, $p < 0.001$). The aged tMTF peak, BMF, was significantly lower for all modulation depths tested (100% $F = 6.266$, $df = 1, 157$, $p = 0.013$; 50% $F = 10.074$, $df = 1, 156$, $p = 0.002$; 20% $F = 10.861$, $df = 1, 152$, $p = 0.001$). The age-related increases in variability in the modulation frequency at which peak sync was reached contributed to a broadening of the aged tMTF function at 50% and 20% modulation depth (50% $F = 1.779$, $df = 73, 85$, $p = 0.005$; 20% $F = 33.959$, $df = 71, 83$, $p < 0.001$). Significant levels of synchronization and high vector strength values were only obtained for modulation frequencies at or below BMF, since vector strength drops off rapidly above BMF (Figure 2). Significant vector strength values were never obtained for modulation frequencies above 640 Hz (Rayleigh $p > 0.05$), and only under certain conditions at 380.5 Hz.

In contrast to the age-related changes in temporal coding, there were no significant age-related changes in discharge rate at or near BMF as a function of modulation frequency (Figure 3). Young and aged rMTFs were relatively flat and similar at 50% and 20% modulation depth. The only significant rMTF finding was an increasing rate as modulation frequency increased at 100% modulation depth for both young and aged groups ($F = 13.176$, $df = 9, 1709$, $p > 0.001$).

Age-related changes in temporal response to SAM tones: PSTH Temporal Response Types

A more detailed analysis in response to SAM tones was conducted on sync and rate data at BMF (134.4 Hz). Fusiform cells were broken down by their CF temporal response type; buildup, pauser-buildup, or wide-chopper and were analyzed separately by depth of modulation (Figure 4A). Significant differences between response types were found at modulation depths of 100% and 50% ($F = 6.503$, $df = 2, 143$, $p = 0.002$; $F = 4.898$, $df = 2, 142$, $p = 0.009$) respectively. While there were no significant differences between buildup and pauser-buildup response types, post hoc comparisons at 100% and 50% modulation depths revealed that wide-chopper response types had significantly (Bonferroni corrected) poorer vector strength than either buildup or pauser-buildup units (100%: B vs WC, $p = .013$; B-P vs WC, $p = .003$; 50%: B vs WC, $p = .014$; B-P vs WC, $p = .029$). Additionally, there was a significant age-related decrease for all three response types at 50% and 20% modulation depth (50%: $F = 6.757$, $df = 1, 142$, $p = 0.01$; 20%: $F = 8.704$, $df = 1, 138$, $p = 0.004$). Aging effects at BMF were independent of response type.

In contrast to the vector strength results at BMF, discharge rate at BMF (Figure 4B) failed to show any significant age-related changes. Subsequent comparisons of PSTH response type at each modulation depth confirmed no differences between buildup and pauser-buildup units, with wide-chopper units showing significantly (Bonferroni corrected) higher discharge rates than either the buildup or pauser-buildup response types (100%: B vs WC, $p = .001$; B-P vs WC, $p = 0.009$; 50%: B vs WC, $p = 0.010$; B-P vs WC, $p = 0.034$; 20%: B vs WC, $p = 0.020$). Additional analyses of vector strength and rate data across all modulation frequencies were conducted for the specific response types and confirmed the forgoing analyses.

Discussion

The present study of DCN fusiform cells finds a significant age-related loss of temporal precision when coding SAM stimuli in the absence of any significant age-related rate changes in SAM coding. Previous studies described age-related changes in processing of SAM stimuli in IC, a structure which primarily employs a rate code for encoding SAM stimuli (Palombi et al., 2001; Walton et al., 2002; Simon et al., 2004). The present results show an age-related loss of temporal precision at or near BMF is not simply the result of an increase in discharge rate, but implies that an age-related reduction of timed IPSCs may be responsible for the loss of temporal coding (Kanold and Manis, 1999; 2005; Street and Manis, 2007). Age-related rate increases at or near BMF could decrease synchrony by increasing temporal jitter but since no such changes were observed for buildup and pauser-buildup units, a simple change of rate cannot reasonably explain the present age-related tMTF findings. Although the present study finds that all the three PSTH response types show age-related reductions in synchrony near BMF, it is also significant that the different PSTH response types differ significantly in their ability to sync to the SAM stimulus (see Figure 4A). Both young and aged buildup and pauser-buildup response types displayed significantly higher peak synchrony vector strength values than did wide-chopper units which also had higher firing rates (Figure 4 A&B). The 65% age-related increase in the number of fusiform cells showing wide-chopper response types combined with the 23% age-related decrease in the number of buildup and pauser-buildup response types could, in part, explain the age-related loss of peak synchrony at or near BMF. In vivo and in vitro studies show that fusiform cell temporal responses are shaped, by well timed IPSCs or tonic inhibitory, inputs (Hancock and Voigt, 2002a; Kanold and Manis, 1999; 2005; Street and Manis, 2007). Inhibitory inputs onto fusiform cells can alter rapidly inactivating K^+ currents which help determine the nature of the PSTH temporal response and can affect temporal precision over tens of milliseconds (Rhode and Smith, 1996; Manis, 1990; Hewitt and Meddis, 1995; Hancock and Voigt, 2002a; Kanold and Manis, 1999; 2005; Street and Manis, 2007). The degree of hyperpolarization existing just prior to excitation by an acoustic stimulus likely determines the relative deinactivation of rapidly inactivating K^+ currents (Manis, 1990; Hancock and Voigt, 2002a; Kanold and Manis, 2005; Street and Manis, 2007). We hypothesize that there is an age-related loss of, or small/mis-timed IPSCs, or loss of tonic inhibition impinging on the aged fusiform cell. This age-related loss of inhibitory input onto fusiform cells results in a shift from pauser-buildup to wide-chopper temporal response types resulting in the loss of temporal precision at or near BMF. As a corollary to the reduced peak synchrony there are increased numbers of low-pass tMTFs in the aged DCN (see Figure 1, Figure 2 and Figure 4). At all temporal modulation depths, and at either age, buildup and pauser-buildup units have higher peak modulation sync functions than units showing wide-chopper temporal responses at all temporal modulation depths, and at either age. Thus the age-related shift toward wide-chopper response types, a temporal response type with higher firing rates and a decreased ability to synchronize to a SAM envelop could explain the present findings and is consistent with a loss of glycinergic inhibition.

Inhibitory circuitry may play a role in maintaining/enhancing synchronization to SAM tones (Frisina et al., 1994; Rhode and Greenberg, 1994; Zhao and Liang, 1995; Young, 1998; Backoff et al., 1999; Krishna and Semple, 2000; Hancock and Voigt, 2002b; Joris et al., 2004; Kanold and Manis, 2005; Street and Manis, 2007). The present findings from aged DCN output neurons resemble what is seen when inhibitory neurotransmission is blocked during temporal processing paradigms in CN and IC (Koch and Grothe, 1998; Backoff et al., 1999; Caspary et al., 2002). Most notably, Backoff and colleagues (1999) found that application of inhibitory neurotransmitter glycine onto CN neurons tended to increase synchrony in response to SAM tones while strychnine blockade of glycine receptors reduced vector strength at the peak and flattened the tMTF (Backoff et al., 1999). The present study found that aged putative DCN output neurons exhibited significantly decreased peak sync values, consistent with a decrease

in glycinergic inhibition. Furthermore, the age-related shift toward more low-pass tMTFs with notches (LPN, DP and complex tMTFs) is expected with a flattening observed with age-related decreases in vector strength near BMF.

Physiologic changes associated with aging observed in the present study and age-related pre- and postsynaptic glycinergic neurochemical changes described above are suggestive of a net loss of glycinergic inhibition in fusiform and cartwheel cell neurotransmission in aged DCN (Caspary et al., 2005, 2006). Rodent aging studies describe decreased glycine immunostaining in aged C57 mouse DCN (Willott et al., 1997), while Banay-Schwartz et al. (1989) found an age-related decrease in glycine levels in aged rat cochlear nucleus. Age-related decrement in glycine receptor binding was described for both mouse and rat CN (Milbrandt and Caspary, 1995; Willott et al., 1997) which likely reflect age-related glycine receptor subunit changes (Krenning et al., 1998; Caspary et al., 2002; Wang et al., 2008).

Recent findings also suggest that the age-related loss of intensity discrimination seen in older adults may be attributable, in part, to an age-related loss of central auditory inhibition (Harris et al., 2007). Collectively, these results support a loss of temporal precision in the coding of the SAM envelope which could help explain, in part, the loss of temporal resolving power observed in speech processing in the older individuals (Gordon-Salant and Fitzgibbons, 1993, 1995; Strouse et al., 1998; Schneider et al., 1998; Souza and Kitch, 2001; Tremblay et al., 2002).

An age-related decrease in inhibition alters the output of the DCN, specifically for selected modulation frequencies. It is thus reasonable to conclude that this loss of inhibition contributes to an age-related loss of temporal processing at higher levels of the central auditory system.

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References

- Arnott RH, Wallace MN, Shackleton TM, Palmer AR. Onset neurones in the anteroventral cochlear nucleus project to the dorsal cochlear nucleus. *J Assoc Res Otolaryngol* 2004;5:153–170. [PubMed: 15357418]
- Backoff PM, Palombi PS, Caspary DM. Glycinergic and GABAergic inputs affect short-term suppression in the cochlear nucleus. *Hear Res* 1997;110:155–163. [PubMed: 9282898]
- Backoff PM, Palombi PS, Caspary DM. GABA- and glycinergic inputs shape coding of AM in chinchilla cochlear nucleus. *Hearing Res* 1999;134:77–88.
- Banay-Schwartz M, Lajtha A, Palkovits M. Changes with aging in the levels of amino acids in rat CNS structural elements. II. Taurine and small neutral amino acids. *Neurochem Res* 1989;14:563–570. [PubMed: 2761675]
- Barsz K, Ison JR, Snell KB, Walton JP. Behavioral and neural measures of auditory temporal acuity in aging humans and mice. *Neurobiol Aging* 2002;23:565–578. [PubMed: 12009506]
- Betts LR, Sekuler AB, Bennett PJ. The effects of aging on orientation discrimination. *Vision Res* 2007;47:1769–1780. [PubMed: 17466355]
- Brown CH. Directional hearing in aging rats. *Exp Aging Res* 1984;10:35–38. [PubMed: 6734685]
- Brozoski TJ, Bauer CA, Caspary DM. Elevated fusiform-cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J Neurosci* 2002;22:2383–2390. [PubMed: 11896177]
- Cant NB, Benson CG. Parallel auditory pathways: projection patterns of the different neuronal populations in the dorsal and ventral cochlear nuclei. *Brain Res Bull* 2003;60:457–474. [PubMed: 12787867]

- Caspary DM. Classification of subpopulations of neurons in the cochlear nuclei of the kangaroo rat. *Exp Neurol* 1972;37:131–151. [PubMed: 5077556]
- Caspary DM, Pazara KE, Kossl M, Faingold CL. Strychnine alters the fusiform cell output from the dorsal cochlear nucleus. *Brain Res* 1987;417:273–282. [PubMed: 3651816]
- Caspary DM, Raza A, Lawhorn Armour BA, Pippin J, Arneric SP. Immunocytochemical and neurochemical evidence for age-related loss of GABA in the inferior colliculus: implications for neural presbycusis. *J Neurosci* 1990;10:2363–2372. [PubMed: 1973948]
- Caspary DM, Backoff PM, Finlayson PG, Palombi PS. Inhibitory inputs modulate discharge rate within frequency receptive fields of anteroventral cochlear nucleus. *J Neurophysiol* 1994;72:2124–2133. [PubMed: 7884448]
- Caspary DM, Palombi PS, Hughes LF. GABAergic inputs shape responses to amplitude modulated stimuli in the inferior colliculus. *Hear Res* 2002;168:163–173. [PubMed: 12117518]
- Caspary DM, Schatterman TA, Hughes LF. Age-related changes in the inhibitory response properties of dorsal cochlear nucleus output neurons: role of inhibitory inputs. *J Neurosci* 2005;25:10952–10959. [PubMed: 16306408]
- Caspary DM, Hughes LF, Schatterman TA, Turner JG. Age-Related Changes in the Response Properties of Cartwheel Cells in Rat Dorsal Cochlear Nucleus. *Hearing Res* 2006;217:207–215.
- CHABA (Committee on Hearing, Bioacoustics and Biomechanics). Speech understanding and aging. *J Acoust Soc of America* 1988;83:859–895.
- Davis KA, Young ED. Pharmacological evidence of inhibitory and disinhibitory neuronal circuits in dorsal cochlear nucleus. *J Neurophysiol* 2000;83:926–940. [PubMed: 10669505]
- Doucet JR, Ross AT, Gillespie MB, Ryugo DK. Glycine immunoreactivity of multipolar neurons in the ventral cochlear nucleus which project to the dorsal cochlear nucleus. *J Comp Neurol* 1999;408:515–531. [PubMed: 10340502]
- Finlayson PG, Caspary DM. Response properties in young and old Fischer 344 rat lateral superior olive neurons: A quantitative approach. *Neurobiol Aging* 1993;14:127–139. [PubMed: 8487915]
- Fitzgibbons PJ, Gordon-Salant S. Age effects on duration discrimination with simple and complex stimuli. *J Acoust Soc Am* 1995;98:3140–3145. [PubMed: 8550939]
- Fitzgibbons PJ, Gordon-Salant S, Friedman SA. Effects of age and sequence presentation rate on temporal order recognition. *J Acoust Soc Am* 2006;120:991–999. [PubMed: 16938986]
- Frisina RD, Walton JP, Karcich KJ. Dorsal cochlear nucleus single neurons can enhance temporal processing capabilities in background noise. *Exp Brain Res* 1994;102(1):160–164. [PubMed: 7895792]
- Gifford RH, Bacon SP, Williams EJ. An examination of speech recognition in a modulated background and of forward masking in younger and older listeners. *J Speech Lang Hear Res* 2007;50:857–864. [PubMed: 17675591]
- Godfrey DA, Kiang NY, Norris BE. Single unit activity in the dorsal cochlear nucleus of the cat. *J Comp Neurol* 1975;162:269–284. [PubMed: 1150922]
- Gordon-Salant S, Fitzgibbons PJ. Temporal factors and speech recognition performance in young and elderly listeners. *J Speech Hear Res* 1993;36:1272–1285.
- Gordon-Salant S, Fitzgibbons PJ. Recognition of multiply degraded speech by young and elderly listeners. *J Speech Hear Res* 1995;38:1150–1156. [PubMed: 8558883]
- Hackney CM, Osen KK, Kolston J. Anatomy of the cochlear nuclear complex of guinea pig. *Anat Embryol (Berl)* 1990;182(2):123–149. [PubMed: 2244686]
- Hancock KE, Voigt HF. Intracellularly labeled fusiform cells in dorsal cochlear nucleus of the gerbil. I. Physiological response properties. *J Neurophysiol* 2002a;87:2505–2519. [PubMed: 11976387]
- Hancock KE, Voigt HF. Intracellularly labeled fusiform cells in dorsal cochlear nucleus of the gerbil. II. Comparison of physiology and anatomy. *J Neurophysiol* 2002b;87:2520–2530. [PubMed: 11976388]
- Harris KC, Mills JH, Dubno JR. Electrophysiologic correlates of intensity discrimination in cortical evoked potentials of younger and older adults. *Hear Res* 2007;228:58–68. [PubMed: 17344001]
- Hewitt MJ, Meddis R. A computer model of dorsal cochlear nucleus pyramidal cells: intrinsic membrane properties. *J Acoust Soc Am* 1995;97:2405–2413. [PubMed: 7714258]

- Joris PX, Smith PH. Temporal and binaural properties in dorsal cochlear nucleus and its output tract. *J Neurosci* 1998;18(23):10157–10170. [PubMed: 9822769]
- Joris PX, Schreiner CE, Rees A. Neural processing of amplitude-modulated sounds. *Physiol Rev* 2004;84(2):541–577. [PubMed: 15044682]Review.
- Kanold PO, Manis PB. Transient potassium currents regulate the discharge patterns of dorsal cochlear nucleus paramidal cells. *J Neurosci* 1999;19(6):2195–2208. [PubMed: 10066273]
- Kanold PO, Manis PB. Encoding the timing of inhibitory inputs. *J Neurophysiol* 2005;93(5):2887–2897. [PubMed: 15625095]
- Kim DO, Sirianni JG, Chang SO. Responses of DCN-PVCN neurons and auditory nerve fibers in unanesthetized decerebrate cats to AM and pure tones: analysis with autocorrelation/power-spectrum. *Hear Res* 1990;45(1–2):95–113. [PubMed: 2345121]
- Koch U, Grothe B. GABAergic and glycinergic inhibition sharpens tuning for frequency modulations in the inferior colliculus of the big brown bat. *J Neurophysiol* 1998;80(1):71–82. [PubMed: 9658029]
- Kolston J, Osen KK, Hackney CM, Ottersen OP, Storm-Mathisen J. An atlas of glycine- and GABA-like immunoreactivity and colocalization in the cochlear nuclear complex of the guinea pig. *Anat Embryol (Berl)* 1992;186:443–465. [PubMed: 1443654]
- Krenning J, Hughes LF, Caspary DM, Helfert RH. Age-related glycine receptor subunit changes in the cochlear nucleus of Fischer-344 rats. *Laryngoscope* 1998;108:26–31. [PubMed: 9432062]
- Krishna BS, Semple MN. Auditory temporal processing: responses to sinusoidally amplitude-modulated tones in the inferior colliculus. *J Neurophysiol* 2000;84:255–273. [PubMed: 10899201]
- Langner G. Periodicity coding in the auditory system. *Hear Res* 1992;60(2):115–142. [PubMed: 1639723] Review.
- Leigh-Paffenroth ED, Fowler CG. Amplitude-modulated auditory steady-state responses in younger and older listeners. *J Am Acad Audiol* 2006;17:582–597. [PubMed: 16999253]
- Leventhal AG, Wang Y, Pu M, Zhou Y, Ma Y. GABA and its agonists improved visual cortical function in senescent monkeys. *Science* 2003;300:812–815. [PubMed: 12730605]
- Manis PB. Membrane properties and discharge characteristics of guinea pig dorsal cochlear nucleus neurons studied in vitro. *J Neurosci* 1990;10(7):2338–2351. [PubMed: 2376777]
- Mendelson JR, Ricketts C. Age-related temporal processing speed deterioration in auditory cortex. *Hear Res* 2001;158:84–94. [PubMed: 11506940]
- Milbrandt JC, Caspary DM. Age-related reduction of [3H]strychnine binding sites in the cochlear nucleus of the Fischer 344 rat. *Neurosci* 1995;67:713–719.
- Moller AR. Responses of units in the cochlear nucleus to sinusoidally amplitude-modulated tones. *Exp Neurol* 1974;45(1):105–117. [PubMed: 4415598]
- Moller AR. Dynamic properties of excitation and two-tone inhibition in the cochlear nucleus studied using amplitude-modulated tones. *Exp Brain Res* 1976;25(3):307–321. [PubMed: 954895]
- Osen KK. Cytoarchitecture of the cochlear nuclei in the cat. *J Comp Neurol* 1969;136:453–484. [PubMed: 5801446]
- Osen, KK.; Ottersen, OP.; Storm-Mathisen, J. Colocalization of Glycine-like and GABA-like Immunoreactivities: A Semiquantitative Study of Individual Neurons in the Dorsal Cochlear Nucleus of Cat. In: Ottersen, OP.; Storm-Mathisen, J., editors. *Glycine Neurotransmission*. West Sussex, England: John Wiley & Sons Ltd; 1990. p. 417–451.
- Palombi PS, Caspary DM. Physiology of the aged Fischer 344 rat inferior colliculus: responses to contralateral monaural stimuli. *J Neurophysiol* 1996;76:3114–3125. [PubMed: 8930259]
- Palombi PS, Backoff PM, Caspary DM. Responses of Young and Aged Fischer 344 Rat Inferior Colliculus Neurons to Sinusoidally Amplitude Modulated Tonal Stimuli. *Hearing Res* 2001;153:174–180.
- Pichora-Fuller MK, Schneider BA, Macdonald E, Pass HE, Brown S. Temporal jitter disrupts speech intelligibility: a simulation of auditory aging. *Hear Res* 2007;223:114–121. [PubMed: 17157462]
- Rhode WS. Vertical cell responses to sound in cat dorsal cochlear nucleus. *J Neurophysiol* 1999;82:1019–1032. [PubMed: 10444694]
- Rhode WS, Smith PH. Physiological studies on neurons in the dorsal cochlear nucleus of cat. *J Neurophysiol* 1986;56(2):287–307. [PubMed: 3760922]

- Rhode WS, Oertel D, Smith PH. Physiological response properties of cells labeled intracellularly with horseradish peroxidase in cat ventral cochlear nucleus. *J Comp Neurol* 1983;213:448–463. [PubMed: 6300200]
- Rhode WS, Greenberg S. Encoding of amplitude modulation in the cochlear nucleus of the cat. *J Neurophysiol* 1994;71:1797–1825. [PubMed: 8064349]
- Rubio ME, Juiz JM. Differential distribution of synaptic endings containing glutamate, glycine, and GABA in the rat dorsal cochlear nucleus. *J Comp Neurol* 2004;477(3):253–272. [PubMed: 15305363]
- Saint-Marie RL, Benson CG, Ostapoff EM, Morest DK. Glycine immunoreactive projections from the dorsal to the anteroventral cochlear nucleus. *Hear Res* 1991;51:11–28. [PubMed: 1672865]
- Schmolesky MT, Wang Y, Pu M, Leventhal AG. Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys. *Nat Neurosci* 2000;3:384–390. [PubMed: 10725929]
- Schneider BA, Pichora-Fuller MK, Kowalchuk D, Lamb M. Gap detection and the precedence effect in young and old adults. *J Acoust Soc Am* 1994;95:980–991. [PubMed: 8132912]
- Schneider BA, Speranza F, Pichora-Fuller MK. Age-related changes in temporal resolution: envelope and intensity effects. *Can J Exp Psychol* 1998;52:184–191. [PubMed: 10095852]
- Simon H, Frisina RD, Walton JP. Age reduces response latency of mouse inferior colliculus neurons to AM sounds. *J Acoust Soc Am* 2004;116(1):469–477. [PubMed: 15296006]
- Snell KB. Age-related changes in temporal gap detection. *J Acoust Soc Am* 1997;101:2214–2220. [PubMed: 9104023]
- Souza PE, Kitch V. The contribution of amplitude envelope cues to sentence identification in young and aged listeners. *Ear Hear* 2001;22:112–119. [PubMed: 11324840]
- Street SE, Manis PB. Action potential timing precision in dorsal cochlear nucleus pyramidal cells. *J Neurophysiol* 2007;97(6):4162–4172. [PubMed: 17442767]
- Strouse A, Ashmead DH, Ohde RN, Grantham DW. Temporal processing in the aging auditory system. *J Acoust Soc Am* 1998;104:2385–2399. [PubMed: 10491702]
- Syka J. Plastic changes in the central auditory system after hearing loss, restoration of function, and during learning. *Physiol Rev* 2002;82:601–636. [PubMed: 12087130]
- Takahashi GA, Bacon SP. Modulation detection, modulation masking, and speech understanding in noise in the elderly. *J Speech Hear Res* 1992;35(6):1410–1421. [PubMed: 1494284]
- Ter-Mikaelian M, Sanes DH, Semple MN. Transformation of temporal properties between auditory midbrain and cortex in the awake Mongolian gerbil. *J Neurosci* 2007;27:6091–6102. [PubMed: 17553982]
- Tremblay KL, Piskosz M, Souza P. Aging alters the neural representation of speech cues. *Neuroreport* 2002;13:1865–1870. [PubMed: 12395081]
- Turner CW, Souza PE, Forget LN. Use of temporal envelope cues in speech recognition by normal and hearing-impaired listeners. *J Acoust Soc Am* 1995;97(4):2568–2576. [PubMed: 7714274]
- Turner JG, Hughes LF, Caspary DM. Divergent response properties of layer-V neurons in rat primary auditory cortex. *Hear Res* 2005;202:129–140. [PubMed: 15811705]
- Voigt HF, Young ED. Evidence of inhibitory interactions between neurons in dorsal cochlear nucleus. *J Neurophysiol* 1980;44:76–96. [PubMed: 7420140]
- Walton JP, Simon H, Frisina RD. Age-related alterations in the neural coding of envelope periodicities. *J Neurophysiol* 2002;88(2):565–578. [PubMed: 12163510]
- Wang HN, Ling LL, Brozoski TJ, Turner JG, Parrish JL, Hughes LF, Caspary DM. Altered Markers of Glycinergic function in the DCN of a Rat Model of Tinnitus. *Assoc Res Otolaryngol* 2008;31:2008.
- Warren LR, Wagener JW, Herman GE. Binaural analysis in the aging auditory system. *J Gerontol* 1978;33:731–736. [PubMed: 299563]
- Welford AT. Between bodily changes and performance: some possible reasons for slowing with age. *Exp Aging Res* 1984;10:73–88. [PubMed: 6389152]
- Willott JF. *Aging and the Auditory System*. San Diego: Singular Publishing Group, Inc; 1991. p. 100-131.
- Willott JF, Milbrandt JC, Bross LS, Caspary DM. Glycine immunoreactivity and receptor binding in the cochlear nucleus of C57BL/6J and CBA/CaJ mice: effects of cochlear impairment and aging. *J Comp Neurol* 1997;385:405–414. [PubMed: 9300767]

- Young, ED. Cochlear nucleus. In: Shepherd, GM., editor. *The Synaptic Organization of the Brain*. New York: Oxford Press; 1998. p. 121-157.
- Young, ED.; Oertel, D. Cochlear nucleus. In: Shepherd, GM., editor. *The Synaptic Organization of the Brain*. New York: Oxford University Press; 2004. p. 125-163.
- Yu S, Wang Y, Li X, Zhou Y, Leventhal AG. Functional degradation of extrastriate visual cortex in senescent rhesus monkeys. *Neuroscience* 2006;140:1023–1029. [PubMed: 16678974]
- Zhao HB, Liang ZA. Processing of modulation frequency in the dorsal cochlear nucleus of the guinea pig: amplitude modulated tones. *Hear Res* 1995;82(2):244–256. [PubMed: 7775289]
- Zhao HB, Liang ZA. Temporal encoding and transmitting of amplitude and frequency modulations in dorsal cochlear nucleus. *Hear Res* 1997;106(1–2):83–94. [PubMed: 9112108]
- Zhou J, Shore SE. Projections from the trigeminal nuclear complex to the cochlear nuclei: a retrograde and anterograde tracing study in the guinea pig. *J Neurosci Res* 2004;78:901–907. [PubMed: 15495211]

List of Abbreviations

ABR, auditory brainstem response
 ANOVA, analysis of variance
 BMF, best modulation frequency
 BP, band-pass
 C, complex
 CF, characteristic frequency
 CN, cochlear nucleus
 DCN, dorsal cochlear nucleus
 DP, double-peaked
 FBN, Fischer Brown Norway
 HRP, horseradish peroxidase
 IAC, Industrial Acoustic Company
 IC, inferior colliculus
 IPSCs, inhibitory postsynaptic currents
 LP, low-pass
 LPN, low-pass notched
 PSTH, post-stimulus time histogram
 SAM, sinusoidally amplitude modulated
 SPL, sound pressure level
 TDT, Tucker Davis Technologies
 tMTF, temporal modulation transfer function
 rMTF, rate modulation transfer function
 VCN, ventral cochlear nucleus

Figure 1.

Distribution of tMTF shapes from young and aged fusiform cells at 100%, 50% and 20% modulation depth. A) Young adult tMTF distribution is predominantly band-pass (BP) function at all modulation depths. B) Aged tMTF distribution shows an age-related loss of band-pass function especially at 100% modulation depth. An age-related increase in low-pass (LP), double-peaked (DP) and low-pass notched (LPN) function is seen at 100% depth of modulation. Age-related increases in low-pass and double-peaked functions are seen at 50% and 20% modulation depths.

Figure 2.

Composite modulation transfer functions (Vector Strength) from young (93) and aged (88) fusiform cells at 100%, 50% and 20% depth of modulation. Note the clear band-pass nature of the young tMTFs at 50% and 20% modulation and the flatter and broader comparable tMTFs from aged fusiform cells. Error bars represent the SEM.

Figure 3.

Composite modulation transfer functions (discharge rate) from young (93) and aged (88) fusiform cells at 100%, 50% and 20% depth of modulation. Note the clear monotonic nature of the rate functions for both young aged groups at 50% and 20% modulation depth. At the 100% modulation depth there was a moderate increase in discharge rate with modulation frequency through BMF. Thereafter, discharge rate remained relatively constant. Error bars represent the SEM.

Figure 4.

Vector strength at the peak of the overall tMTF function is depicted for each age group as a function of the CF response type and is shown separately for each modulation depth (4A). As can be seen, wide chopper response type fusiform cells sync more poorly than buildup or pauser-buildup response types. Additionally, the young have greater vector strength than the aged for each response type. The bottom half of the figure (4B) depicts discharge rate at the peak of the overall tMTF function for each age group as a function of the CF response type and is shown separately for each modulation depth. Error bars represent the SEM.