JARO 03: 321–331 (2002)
DOI: 10.1007/s101620020038

JARO
Journal of the Association for Research in Otolaryngology

Paired-Tone Stimuli Reveal Reductions and Alterations in Temporal Processing in Inferior Colliculus Neurons of Aged Animals

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Received: 9 August 2001; Accepted: 10 December 2001; Online publication: 27 February 2002

ABSTRACT

Temporal processing deficits in the central auditory system of aged subjects are apparent from animal and human studies but could be due to peripheral hearing loss. Sequential paired tone stimuli reveal age-related changes in temporal processing properties of neurons in the central nucleus of the inferior colliculus (CIC). A greater proportion of CIC neurons exhibit suppression of excitability following pure tone stimulation in 20 month old (''aged'') compared with 3–6 month ''young'' Long Evans rats. The duration (time constant of exponential curve fit to recovery of excitability) of suppression is also increased in aged compared with young rats, with more neurons exhibiting suppression with time constants over 100 ms. The time course of poststimulatory suppression is not dependent on the duration or intensity of preceding stimuli and is not correlated with either initial magnitude of suppression or best frequency of IC neurons. Although the increase in unit thresholds is greater for high-frequency units in old animals, the largest poststimulatory suppression changes occur in neurons with best frequencies of less than 10 kHz. Since the increase in duration of poststimulatory suppression is not correlated with peripheral hearing loss, the difference is likely attributed to central auditory neuron changes in aging. In addition, the proportion of IC neurons exhibiting other temporal patterns of excitability (poststimulatory facilitation and delayed-maximum excitability) is reduced in aged animals. Therefore, temporal processing of acoustic information is significantly altered in aged animals. The greater poststimulatory suppression of excitability, reduced facilitation, and delayed facilitation is expected to reduce and alter the encoded information passing from the brainstem through the IC to higher structures. These changes correlate with reduced speech understanding in noise, elevated thresholds in noisy conditions, and reduced temporal processing capabilities in the elderly.

Keywords: inferior colliculus, post-stimulatory suppression, aging, temporal processing

INTRODUCTION

Although speech-understanding problems in the elderly are often reduced by hearing aids, unsatisfactory results and additional complaints may be dependent on central auditory neuron changes in the elderly. A better understanding of binaural and temporal processing and aging in the central auditory system may lead to better methods to improve hearing in the elderly. Temporal processing deficits in central neurons are apparent since, for example, the elderly have greater difficulty in understanding time-compressed speech (Konkle 1977). The detection of gaps in stimuli, a measure of auditory temporal resolving power, deteriorates in aged subjects compared with young subjects with matched hearing loss (Frisina and Frisina 1997) and is not correlated with hearing loss in aged subjects (Schneider et al. 1994). Single unit

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recordings in animals reveal that fewer neurons in the inferior colliculus (IC) of old mice respond at the minimal gap threshold (1–2 ms; Boettcher et al. 1996; Walton et al. 1998). Tonal threshold elevation during continuous background noise (simultaneous masking) of IC neurons is also significantly greater in aged C57BL/6J mice (McFadden and Willott 1994b). There is also a prolonged suppression of IC neuronal firing activity to the second of the paired noise burst stimuli (gap-detection stimuli) in aged animals (Walton et al. 1998). Therefore, a decrease in the temporal resolving power in aged human and aged animal subjects may involve central auditory neurons. However, these observations are complicated by those of previous studies which demonstrated that gap detection is affected by high-frequency hearing loss (Salvi and Arehole 1985). Therefore, age-related peripheral cochlear hair cell loss, which typically first affects high-frequency hearing, could also affect gap detection.

Properties of temporal processing can also be examined with pure-tone stimuli. Temporal (poststimulatory) changes in excitability can be determined by varying the interval between tones and examining neuronal responses to the second stimulus. Poststimulatory changes markedly affect excitability in IC neurons in young adult rats and include near-complete suppression, facilitation, and delay-dependent changes in responses to the second tonal ''probe'' stimulus (Finlayson 1999). Age-related changes in temporal properties of responses to pure-tone stimulation may provide information to distinguish between central and peripheral hearing loss effects of aging.

Post-stimulatory changes in excitability of IC and lower-order auditory neurons likely involve central mechanisms, such as activation of ionic conductances or changes in neurotransmission. Neuronal responses to ipsilateral tones are suppressed (decreased excitability) following contralateral tonal stimulation both in the IC and the superior olivary complex (SOC) (Finlayson 1999; Finlayson and Adam 1997a,b). Therefore, poststimulatory suppression is at least in part independent of short-term adaptation of inner hair cell, VIIIth nerve, and cochlear nucleus neuron responses and involves neurons with binaural inputs. The increase in the magnitude (and duration) of post-stimulatory suppression at higher levels of the auditory system also must involve central processing. Responses to probe tones following tonal stimulation of equal intensity exhibit small decreases in ventral cochlear nucleus units (21% reduction at 5 ms delays; Boettcher et al. 1990), whereas SOC units exhibit 50% suppression (Finlayson and Adam 1997a,b) and IC neurons exhibit an average of 77% poststimulatory suppression of responses (Finlayson 1999).

The duration of post-stimulatory suppression is an important temporal property which determines neuronal response level to sequential or continuous stimulation. Poststimulatory suppression of SOC neuron responses decreases with an exponential time course with a mean time constant (τ) of 106 ± 20.0 ms (Finlayson and Adam 1997a,b) and a median of 57 ms (unpublished observation). Poststimulatory suppression in the SOC is comparatively longer than the exponential recovery of VIIIth nerve responses (Chimento and Schreiner 1991; Relkin and Doucet 1991) and linear in log(time) recovery functions of ventral cochlear nucleus neurons (Boettcher et al. 1990; Shore 1995). The median exponential time constant for the recovery from suppression in IC neurons of young adult rats is 72.8 ms (Finlayson 1999). This is similar to the exponential time for the recovery from masking of wave V in the brainstem auditory evoked potential in 1 month old CBA and C57 mice of 82–90 ms (Walton et al. 1995).

Complex modulation of excitability following stimulation is found in some neurons in the dorsal cochlear nucleus (DCN), SOC, and IC. Approximately 11% of IC neurons (Finlayson 1999) and 44% of chinchilla DCN neurons (Palombi et al. 1994) exhibit facilitation (i.e., an increase in responses to probe tones immediately following stimulation). A few DCN ''pauser/buildup'' units (Boettcher et al. 1990), SOC neurons (Finlayson and Adam 1997a), and 10% of IC neurons (Finlayson 1999) exhibit U-shaped (poststimulatory delayed-minimum) recovery functions following best frequency (BF) tones. Other IC neurons (10%) that respond with a long latency to stimulation exhibit maximal responsiveness at 128 ms intertone intervals (poststimulatory delayed-maximum pattern; Finlayson 1999). These more complex poststimulatory temporal modulations of excitability could be important for the detection of complex sound patterns such as found in speech.

In this study, we examined if poststimulatory suppression of IC neuron responses is prolonged in aged animals. Twenty (20) month old Long Evans rats were used since they were expected to have only moderate peripheral hearing loss; this improves the ability to characterize all neurons with acoustic stimuli. The use of pure-tone stimulation avoids confounding interactions of noise and high-frequency hearing loss. In addition, we examined if more complex patterns of temporal excitability changes are also affected in aged animals.

METHODS

Animal preparation and recording techniques

The methods used in this study have been described previously in detail (Finlayson 1999). Aged adult male Long Evans rats (20 months of age) weighing 410–

600 g were anesthetized with sodium pentobarbital (44 mg/kg), xylazine (3 mg/kg), and ketamine (85 mg/kg IM). Supplemental doses of anesthetics were administered as required (every 2–3 h), which was indicated by the presence of toe-pinch or eye-blink reflexes. The doses alternated between 25 mg/kg sodium pentobarbital and 40 mg/kg ketamine (Finlayson and Adam 1997a; Palombi and Caspary 1996). A thermostatically controlled DC heating pad maintained the animal's rectal temperature at 37°C. The care and use of animals in this study was approved by the Canadian Animal Care Committee, and all experiments were conducted at the University of British Columbia.

Animals were held in the normal stereotaxic plane (Paxinos and Watson 1982) with a custom head holder. The tympanic membrane was free of effusion and cerumen in all animals. Hollow couplers attached to stimulus transducers were placed against exposed tympanic rims, forming a closed stimulus delivery system. Standing waves were minimized using cotton and steel wool in the coupling tubes. Electrodes were advanced through the cortex to the IC through an opening made in the right dorsal cranium. Microelectrodes with outer-tip diameters of 1.0 μm or less were filled with 2% wheat germ agglutinin–horseradish peroxidase (WGA–HRP) in 2 M NaCl. Tip resistances were 10–20 MΩ. Recording sites were marked by ejecting WGA–HRP from the recording electrode with 5 μA positive current pulses of 500 ms duration at 1 Hz for 5 min.

Neural signals were amplified by an AM-SYSTEMS 1800 amplifier (AM-SYSTEMS, Everett,WA) and a Kikisui oscilloscope (Kikisui, Japan) and bandpass filtered (between 10 or 100 Hz and 5 kHz). Spikes of single neurons were identified on an oscilloscope, converted to pulses using a WPI 121 window (voltage threshold) discriminator, and spike time stored with a 10 μs resolution.

At the end of each experiment, the animal was overdosed with sodium pentobarbital and perfused through the heart with 0.9% saline followed by 40% paraformaldehyde in 0.1 M phosphate buffer with 10% sucrose. Frozen sections were cut in the plane of the electrode track, mounted, and reacted for peroxidase (tetramethylbenzidine/glucose oxidase chromogen reaction). Sections were counterstained with thionin. Tracings of relevant sections and electrode depth were used to identify locations of the neurons studied.

Stimulus generation and delivery

Binaural stimuli digitally generated on a Macintosh computer (Apple, Cupertino, CA) were low-pass filtered, amplified by an Amcron (Crown PowerLine Two) amplifier (Amcron, Elkhart, IN), and attenuated under computer control (MA919 attenuators, Medical Associates, East Fairfield, VT). Stimuli were transduced by impedance-matched headphones (Beyer-Dynamic 600 Ω, B4-132.01, frequency range = 0.05–35 kHz, Beyerdynamic North America, Farmingdale, NY). Output of the stimulus system was calibrated offline using a coupler with a 0.3 cc air space. All puretone bursts had rise/fall ramps of 5 ms. Brainstem auditory evoked potentials to monaural clicks were recorded for both ears of all animals. Search stimuli were binaural clicks with ipsilateral stimuli delayed by 20 ms relative to contralateral stimuli.

Unit classification

Extracellularly recorded single auditory units were initially characterized using 50 ms tone bursts. Best frequencies (BF, the frequency producing the greatest spike count) were determined from contralateral monaural isointensity curves at 50 dB SPL. Thresholds at BF were determined from monaural rate–intensity functions.

Temporal changes in excitability

As in previous experiments (Finlayson 1999), sequential tones were used to study temporal changes in IC neuron responses. This study compares recorded responses to monaural contralateral stimulation. Neuronal excitability following 200 ms ''masker'' stimuli was estimated from responses to 50 ms ''probe'' tones. Both stimuli were presented at the unit's BF and at equal intensity of 20–40 dB above threshold. Intertone intervals were 2, 8, 32, 128, 512, and 2048 ms between the end of the masker offset ramp and the beginning of the probe ramp. The duration of the initial tone was varied from between 12 and 100 ms in some experiments. Thirty individually randomized series of tone pairs with intertone intervals of 2–2048 ms were presented. Stimuli were presented with intertrial intervals of 2.5 s.

Data analysis

Post-stimulatory changes (magnitude and exponential time constants) are expressed as mean ± standard deviation (SD). The percent change in responses was determined from spike counts during probe stimuli for intertrial intervals of 2 ms (or other delays) and 2048 ms:

$$\%\ \text{suppression/facilitation} = \left(\frac{\text{probe response @2 ms delay}}{\text{probe response @2048 ms delay}} - 1\right) * 100$$

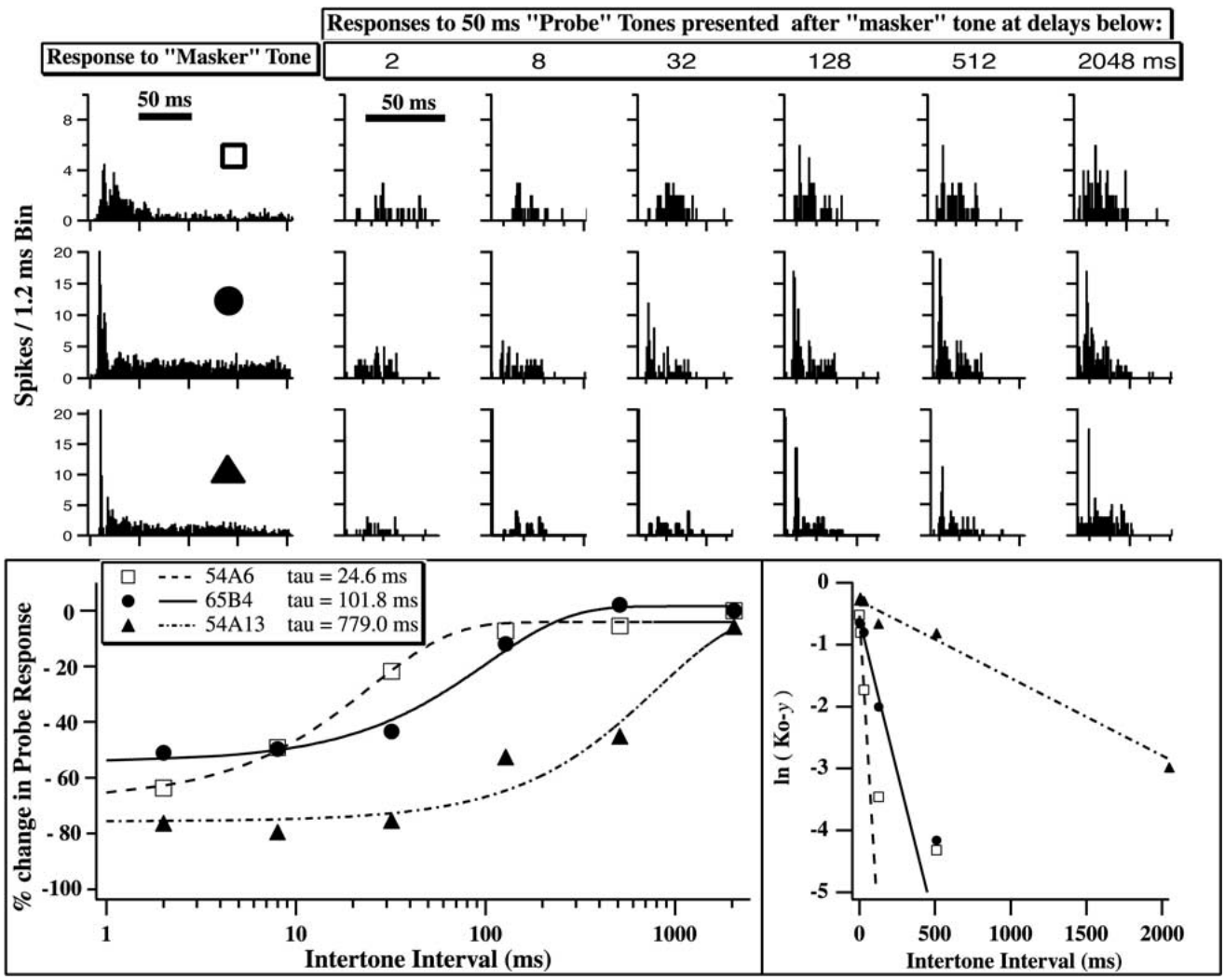

FIG. 1. Post-stimulatory suppression in three CIC neurons from 20 month old rats. The response to best frequency (BF) tones presented after a BF tone of equal intensity is suppressed for varying periods. Peristimulus histograms of responses to ''probe'' tones show initial suppression of activity to tones presented 2 ms after the initial ''masker'' tone, which decreases over time. The duration of suppression can be represented by exponential curves (lower panels). Responses to probe tones are normalized to the response to the probe tone after a 2048 ms intertone interval (lower left). The time course of recovery for these three neurons represents a fast recovery (open boxes), a cell with a median time of recovery (filled circles), and a long duration of recovery (filled triangles). The recovery follows an exponential time course, as the $\ln(k_0 - y)$ plotted against the intertone interval (right lower panel) is linear (lines are theoretical curves calculated from the exponential curve fit).

and the criteria for facilitation was greater than a +20% change and for suppression greater than −20% change.

The recovery from suppression was quantified as a function of the intertone interval. Recovery data were fitted with a single exponential expression:

$$y = k_0 + k_1 e^{-t/\tau_1}$$

The asymptotic response magnitude is k_0 The difference in the final response level to the initial peak is k_1, t is the intertone interval (ms), and τ is the time constant. Least-squares curve fitting of data was performed using a Levenberg-Marquardt algorithm (IGOR Pro, WaveMetrics, Lake Oswego, OR). Data were also transformed and plotted as the natural logarithm of $(k_0 - y)$ versus time, which should form a linear relationship if the recovery is exponential. Typically, at longer intertone intervals, $y \geq k_0$ and $\ln(k_0 - y)$ is not a real number. Therefore, these points were not plotted. Values of k_0 and τ from exponential curve fits were used for the calculation of $\ln(k_0 - y)$ and to calculate the expected.

Properties of suppression (magnitude and recovery time constant) were compared with previous data on poststimulatory changes in young adult (3–6 month old) Long Evans rats (Finlayson 1999). Data were grouped by the type of poststimulatory excitability

TABLE 1

Summary of properties of post-stimulatory suppression in 3–6 month (young) and 20 month old (aged) Long Evans rat inferior colliculus

	Median	*Mean*	*SD*	*SE*	*n*	*Min*	*Max*
% Suppression—young	80.5	77.5	15.4	2.3	43	37.0	100
% Suppression—aged	85.5	77.3	20.1	2.8	51	28.0	100
τ Recovery—young*	**71.6**	**271.7**	607.6	92.7	43	6.8	3565
τ Recovery—aged*	**101.1**	**441.4**	730.2	108.8	45	6.8	3323

% suppression *t*-test = 0.122, DF = 95, $p = 0.9030$.

*Significantly different (Wilcoxon: $p < 0.05$).

change (suppression, facilitation, delayed-minimum, and delayed-maximum) observed at 20–40 dB above threshold. The distribution of values was tested for normality. Significant group effects of age on post-stimulatory effects were assessed with Student *t* tests and one-way analysis of variance (ANOVA), employing Bartlett's index to examine homogeneity of variance. Tukey–Kramer honestly significant difference (HSD) *post hoc* analysis was then conducted for significant main effects obtained in the ANOVA to disclose pairwise differences. For data which did not conform to a normal distribution, nonparametric tests were used. Criterion for significance levels was $p < 0.05$.

RESULTS

Animal cohort

A cohort of 18 animals, which were siblings of animals studied at 3–6 months (Finlayson 1999), was maintained in our colony and examined at 20 months of age. Animals with choleostoma ($n = 1$), no evokable auditory brainstem responses (ABR, $n = 1$), or severe weight loss ($n = 8$) were not used. Weight loss and decreased water intake in the later animals were usually associated with pituitary adenomas. Data were collected on the remaining 8 healthy animals which had no evidence of adenomas or other diseases.

IC unit responses

Responses to monaural contralateral paired-tone stimuli were collected from IC single-unit recordings in 20 month old animals. All units ($n = 62$) located in the central nucleus of the IC (CIC), as demonstrated by horseradish peroxidase marking of recording sites, were included in this study. Data from 20 month old animals were compared with data previously collected from 82 CIC neurons in 3–6 month old animals (Finlayson 1999).

Best frequency and threshold changes

The average BF of single units in the CIC of 20 month old animals was slightly lower [l0.9 ± 7.4 kHz (mean ± SD), $n = 62$] than in young animals (12.8 ± 8.3 kHz, $n = 82$), but this was not significant (*t* test: $p = 0.15$, DF = 142; Wilcoxon: $p = 0.17$). Thresholds were significantly increased in old animals. Thresholds of ABR were increased (ANOVA $p < 0.001$) by an average of 14.0 dB in aged animals ($n = l2$ ears) compared with young animals ($n = 37$ ears). Thresholds of single units grouped by BF in octave ranges were significantly increased in 20 month old animals for units with BF between 4 and 8 kHz (by an average of 10.4 dB, *t* test: $p = 0.03$), 8–16 kHz (by 16.1 dB, $p = 0.0014$), and 16–32 kHz (by 26 dB, $p < 0.0001$).

Responses to monaural contralateral paired tones

The pattern, duration, and magnitude of poststimulatory changes in responses to probe tones that followed 200 ms tones were examined in 60 neurons in the central nucleus of the IC in 8 aged animals and compared with previous data. The most common post-stimulatory change was a suppression of responses to the second tone presented at short intertone intervals (Fig. 1). Poststimulatory suppression was observed in 67.1% and 85.0% of neurons in young and aged animals, respectively.

The magnitude of post-stimulatory suppression did not depend on the age of the animal. Responses to probe tones presented 2 ms after a 200 ms tone of equal intensity (20–40 dB above threshold) and frequency were suppressed by an average of 77.3% ± 20.1% ($n = 43$) in aged animals. There was no significant difference in the magnitude of suppression at short delays when comparing units of young (77.5% ± 15.4%, $n = 51$) and aged animals [Student *t* test: $p = 0.62$; Wilcoxon (rank sum) test: $p = 0.38$; see Table 1, Fig. 2]. Post-stimulatory suppression was evident following tones as short as 12 ms (including 5 ms rise/fall ramps) in all cells ($n = 17$) tested in aged animals (Fig. 3). This was also observed for suppression of IC unit activity in young animals (Finlayson 1999). Responses of IC neurons in 20 month old animals to probe tones presented 2 ms after stimulation were decreased by 54.7% ± 17%

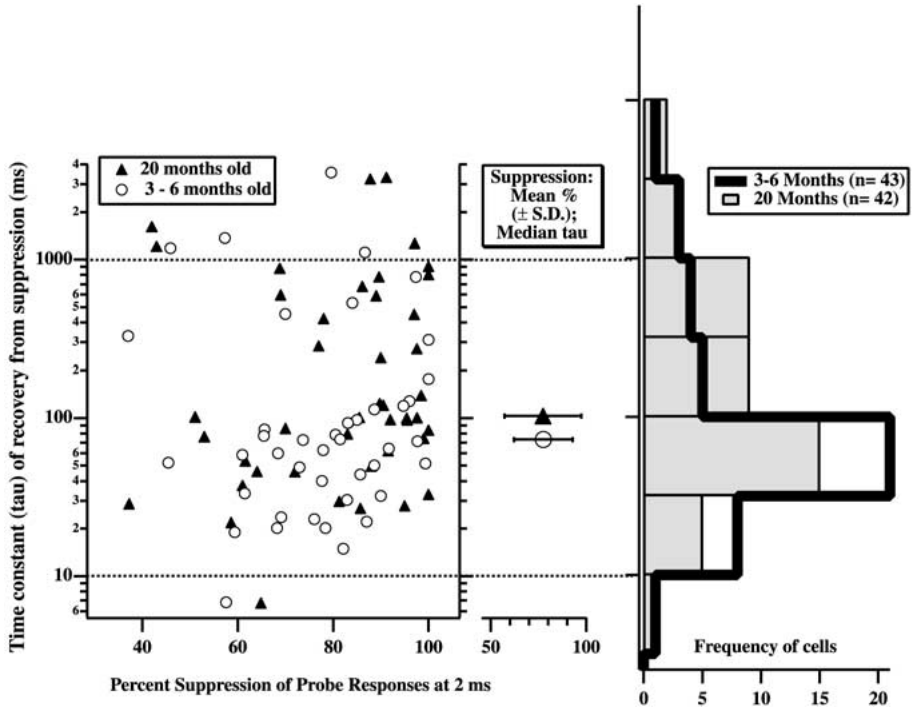

FIG. 2. The duration of poststimulatory suppression was not correlated with the magnitude of suppression (left panel). The magnitude of suppression was similar in 3–6 month (circles) and 20 month old rats (center panel), but there was an increase in the median duration (exponential time constant) of suppression, and an increase in the proportion of IC neurons in 20 month old animals where the suppression had a time constant of recovery of over 100 ms (right panel).

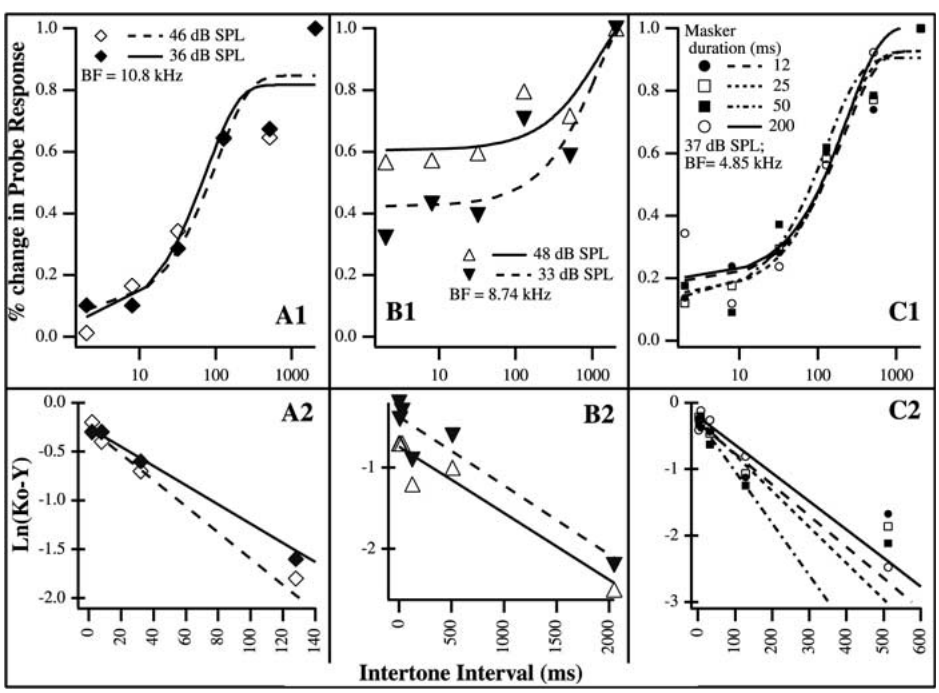

FIG. 3. The duration of poststimulatory suppression was similar when either the intensity of masker and probe tone was increased (**A1, A2, B1, B2**) or the duration of the masker was decreased (**C1, C2,**). The recovery from suppression again follows exponential time courses, as the $\ln(k_0 - y)$ plotted against the intertone interval is linear (**A2, B2, C2**).

following 12 ms tones ($n = 11$) and by 62% ± 20% following 25 ms tones ($n = 17$).

Increased duration of poststimulatory suppression in old animals

More IC neurons were suppressed for long durations in 20 month old animals. Poststimulatory suppression decreases as the duration between the masker and probe tones increases and can be represented by exponential curves for IC neurons in young (Finlayson 1999) and aged animals (Figs. 1 and 3). The exponential time constant (τ) is the time for 63% recovery of responses and is used to measure the duration of recovery. The time course of recovery from suppression was similar following shorter (25 and 50 ms) tones in aged animals ($n = 17$; Fig. 3), as was found in young animals (Finlayson 1999). For IC units with

monotonic rate–intensity functions, the time course of recovery was similar over a 30 dB range (10–40 dB above threshold) in neurons in young animals ($n = 15$; Finlayson 1999). We also examined and found no effect of intensity (10 dB shift) on the time course of recovery for 7 units tested in aged animals (Fig. 3). The range of time constants (3–3000 ms) of exponential curves representing the recovery from poststimulatory suppression was similar in 3–6 and 20 month old animals. The mean duration of suppression, as determined from time constants of exponential curve fit to the recovery, was considerably longer in aged animals (Table 1; Figs. 1 and 2). However, the distribution of recovery time constants was statistically significantly skewed [skewness = 3.35, kurtosis = 12.28, Shapiro–Wilk W test for normal distribution: $p < 0.0001$; recovery τ's are graphed using a logarithmic scale (Figs. 2 and 4). Therefore, nonparametric tests were used to test for significant differences. The duration of suppression in older animals was significantly longer in aged animals [Wilcoxon (rank sum) test: $p = 0.01$], with a median time constant for recovery from suppression of 71.6 ms in young animals and 101.1 ms in 20 month old animals. This was also reflected in the distribution of time constants with more IC neurons in old animals exhibiting poststimulatory suppression with exponential time constants greater than 100 ms (Fig. 2). The time course of recovery was not correlated with BF of units ($r = 0.04$, Fig. 4A). Data were grouped into two groups by BF above and below 10 kHz to determine if there was a relationship between high-frequency hearing loss and duration of suppression. There was a significant increase in the duration of suppression in aged animals for units with BFs < 10 kHz (Wilcoxon test: $p = 0.024$). Although there was also a similar trend for units with BFs 10 kHz, this was not significant (Wilcoxon test: $p = 0.37$). Threshold increases in aged animals were greater for units with BFs > 10 kHz (Fig. 4D). In addition, the time course of recovery was not correlated with threshold in either young or aged animals (Fig. 4C). Therefore, the increased duration of suppression does not appear to be related to high-frequency hearing loss.

Changes in other poststimulatory response patterns with age

There was a significant shift in the pattern of excitability following stimulation, with a significantly greater proportion of neurons exhibiting poststimulatory suppression compared with other poststimulatory patterns in 20 month old animals (likelihood ratio χ^2: $p = 0.0167$; Fischer's exact test, two-tail: $p = 0.0243$; Fig. 5). Facilitation of probe tones following stimulation, as observed in 10% ($n = 8$) of IC (pauser/buildup) neurons in young animals, was not observed in aged animals. A poststimulatory delayed-maximal response in 11.4% of IC neurons in young animals was associated with long-latency peristimulus time histogram (PSTH) response patterns but was observed in only one cell in aged animals. The total proportion of neurons exhibiting facilitation and delayed-maximal responses to probe tones following stimulation also significantly decreased in 20 month old animals compared with the combined proportion of cells exhibiting poststimulatory suppression and delayed-minimal responsiveness (likelihood ratio χ^2: $p = 0.0012$; Fischer's exact test, two-tail, $p = 0.0031$; Fig. 5). On the other hand, the portion of IC neurons exhibiting a delayed-minimal response was not significantly greater (Fischer's exact test, two-tail, $p = 0.79$) in aged animals (13.3%) than in young (11.4%) animals (Fig. 5). In these neurons, a minimal response level to probe tones was observed when the delay between two tones was around 32 ms (Fig. 6). IC neurons exhibiting nonmonotonic rate–intensity functions exhibited poststimulatory suppression at low sound intensities but poststimulatory delayed-minimal responses at higher stimulation levels (Fig. 5).

PSTH pattern and post-stimulatory response pattern

The PSTH response patterns in aged animals were characterized by an initial peak. This peak was followed by a second (and occasionally a third blunted) peak (On-chopper) in 22 neurons. Other neurons exhibited a low sustained activity after the initial peak ($n = 25$). A primarily like response pattern was observed in 6 neurons. A typical pauser/buildup response pattern was observed in only 1 neuron, but 5 other neurons exhibited a clear pause in the PSTH response pattern. Unlike in younger animals (Finlayson 1999), there was no relationship between PSTH response and poststimulatory response pattern in aged animals. The latency to the first peak in the PSTH (Fig. 1) was significantly shorter (t test: $p < 0.01$) in aged animals [10.9 ± 3.3 ms (mean ± SD), $n = 62$] than in young animals (16.2 ± 7.9 ms, $n = 71$). This difference partially reflects fewer neurons in 20 month old animals with long latency and buildup temporal response patterns.

DISCUSSION

This study supports and extends prior work which showed decreased temporal processing capability, increased threshold elevation in noisy conditions, and shifts in inhibitory neurotransmission in the IC of aged subjects.

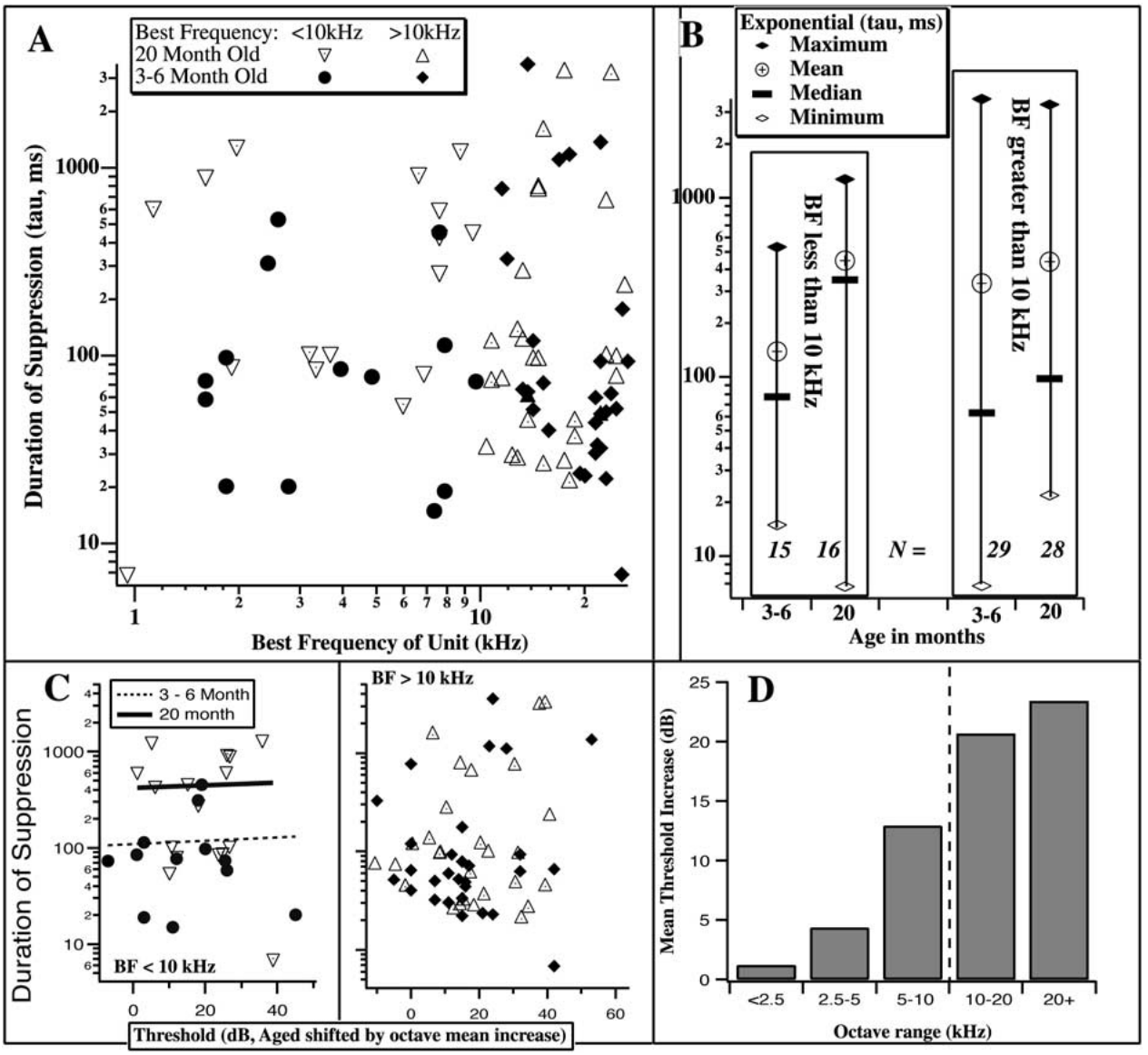

FIG. 4. The duration of post-stimulatory suppression was not correlated with the BF of units (**A**, $r = 0.04$). The duration of suppression was significantly increased for neurons in 20 month old rats with BFs < 10 kHz (**B**). A similar trend is observed for neurons with BF > 10 kHz (**B**). The duration of suppression was not correlated with unit threshold at BF [**C**, aged data plotted with threshold shifted by mean threshold increase (**D**) of units grouped in octaves]. Thresholds of IC units in aged animals increased with frequency and were pronounced above 10 kHz (**D**).

Poststimulatory suppression of excitability

Examination of temporal properties in IC neuron excitability, using sequential monaural contralateral pure-tone stimuli, revealed changes in post-stimulatory suppression of responses and other changes in temporal processing of aged subjects. A predominant effect of prior stimulation on neurons in the inferior colliculus is a temporary suppression in responses to subsequent stimuli. This effect is robust, reducing CIC neuronal excitability to tonal stimuli of equal intensity by over 75% in both young and old animals. The length of time that excitability is suppressed will have a profound effect on auditory information flow from the periphery to the cortex. This duration is well represented by single exponential curves for neurons in the SOC and IC (Finlayson 1999; Finlayson and Adam 1997a,b). The exponential time constants describing the recovery from suppression of CIC neurons vary over a wide range (10–3000 ms) in both young and old animals. There is also a high variability between neurons in the duration of suppression of IC neuron responses to click pairs (Yin 1994) and to paired noise bursts (Walton et al. 1998). However, the time course of recovery to pure-tone pairs is constant in each cell and is largely independent of the dura-

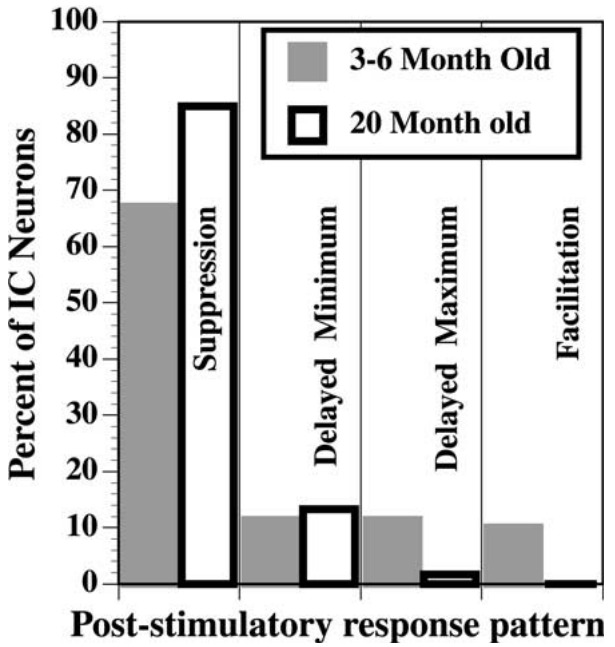

FIG. 5. Pattern of post-stimulatory suppression changes with more units exhibiting suppression in 20 month old rats and fewer cells exhibiting post-stimulatory facilitation and delayed-maximal response patterns than in 3–6 month old rats.

tion or relative intensity of prior stimulation. In 20 month old animals, there is a shift in the distribution but not in the range of the durations of suppression. The increase in the proportion of IC neurons with time constants of recovery over 100 ms is reflected in the shift in the median time constant from 71.6 ms in 3–6 month old rats to 101.1 ms in 20 month old rats. The recovery function time constant masking of masking of the ABR to 12 kHz, 5 ms tones (determined from exponential curves of the masker intensity reducing wave V by 50% as a function of the intertone interval Δt) also increased from 81.9 ms in 1 month old to 198.3 ms in hearing-impaired 8 month old C57BL/6 mice (Walton et al. 1995). A similar increase in duration of masking was observed in hearing-impaired humans (Nelson and Freyman 1987) and in old humans with normal hearing (Walton et al. 1999). A complete suppression of activity could result in an apparent increase in the duration of suppression, as responses would not be elicited for a period following stimulation. However, the increased duration of suppression is not correlated with the magnitude of suppression, and suppression is not greater in aged animals.

Progressive age-related sensorineural hearing loss, which generally starts at high frequencies, may affect central processing in addition to auditory sensitivity (thresholds). Age-related peripheral hearing loss (McFadden and Willott 1994a; Willott et al. 1988a,b) and spiral ganglion lesion (Snyder et al. 2000) result in more sensitive low-frequency tails of tuning curves. It has been suggested that this is due to broader tuning of inhibitory compared with excitatory inputs to IC (Snyder et al. 2000). Such relative changes in inhibition do not readily account for the increase in gap-detection thresholds (to noise bursts) and longer duration of suppression following high-frequency sensorineural hearing loss (Salvi and Arehole 1985) or in aging (Walton et al. 1998). An increased duration of forward masking of pure-tone evoked potentials in the IC of chinchillas, following noise-induced hearing loss (of over 25 dB), was directly related to the frequencies affected by the lesion (2–8 kHz; Arehole et al. 1989). The increased duration of poststimulatory suppression in aged animals is not correlated with hearing loss and, therefore, has a different etiology than similar changes in response following hearing loss. Therefore, processes related to aging in the central auditory system are likely factors in temporal processing changes.

Other post-stimulatory changes in excitability

The sequential tone paradigm reveals other temporal processing properties of IC neurons, which also change with age. There was a significant decrease in the proportion of neurons with post-stimulatory facilitation and delayed-maximal excitability. In addition, there was a slight increase in the proportion of neurons with post-stimulatory delayed minimum excitability. These changes also would affect the temporal resolving power of the auditory system.

Poststimulatory delayed maxima or minima in CIC neuron excitability could be due to many factors including excitatory and inhibitory interactions. IC neurons receive many excitatory and inhibitory inputs, including projections from lower and higher auditory neurons and from other IC neurons. As the duration of poststimulatory suppression is highly variable between neurons, post-stimulatory suppression of excitatory and inhibitory inputs to IC neurons is likely to exhibit different temporal properties. A very simplistic model summing excitation and inhibition and assuming different recovery rates can easily account for delayed poststimulatory changes in excitability (Finlayson 1999). An indication that inhibition affects poststimulatory responsiveness is the post stimulatory delayed-minimum pattern in neurons with nonmonotonic rate–intensity functions, only during higher-intensity stimuli where inhibition is expected to be greater. Inhibition is clearly decreased in the IC of aged rats with decreased GABAergic neurons, GABA levels, GABA release, glutamic acid decarboxylase activity, GABA positive presynaptic terminals (Raza et al. 1994), and $GABA_B$ receptor binding (Milbrandt et al. 1994) and changes in

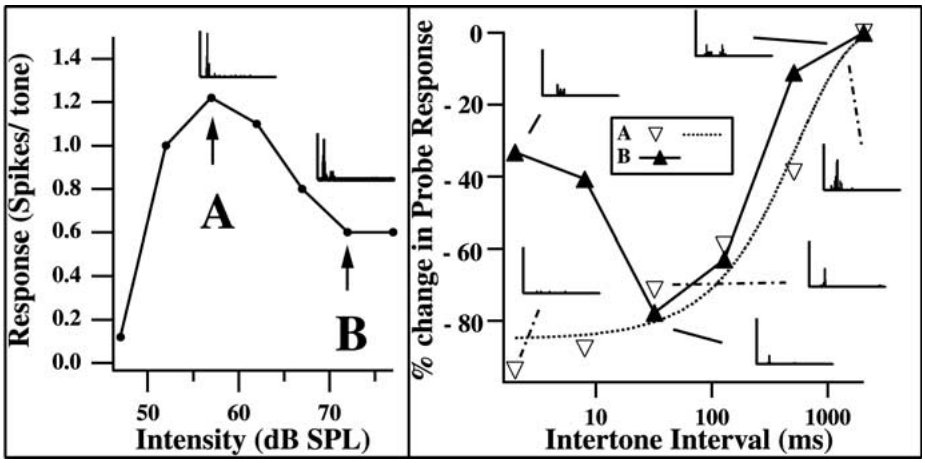

FIG. 6. A CIC neuron from a 20 month old animal with a nonmonotonic rate–intensity function (left panel) exhibits post-stimulatory suppression for tones at the maximal response level (**A**, downward triangles, right panel). At higher intensities (**B**), where more inhibition is expected, a post-stimulatory delayed minimum is observed (right panel, filled triangles). PSTHs for representative responses are shown in each panel.

$GABA_A$ receptor subtypes (Milbrandt et al. 1996). In addition, there are fewer neurons with nonmonotonic rate–intensity functions in the CIC of middle-aged C57 mice (Willott 1986) and the F-344 rat (Palombi and Caspary 1996), which may be a result of decreased inhibition. IC neurons with long latencies in their responses exhibit post-stimulatory delayed-maxima responses, and their long latencies are dependent on GABAergic inhibition (Le Beau et al. 1996). The decrease in neurons with post-stimulatory delayed maxima is likely related to local loss of inhibition in aged subjects.

The decrease in IC neurons exhibiting post-stimulatory facilitation could be due to age-related changes in DCN neurons or similar changes in IC neurons. In responses to gap stimuli, there was also a delayed facilitation of responses in young but not in old animals (Walton et al. 1998). Post-stimulatory facilitation to tonal stimuli is observed in neurons with pauser/buildup PSTH response patterns, in both the IC of young animals (Finlayson 1999) and DCN neurons (Palombi et al. 1994). A preliminary study found fewer pauser/buildup and more Chopper-W cells in the DCN of aged rats (Schatteman et al. 2001). Post-stimulatory response patterns have not been examined in the DCN of aged animals. Since a group of IC neurons, at least in very young animals, have membrane properties which can produce a pauser/buildup response pattern (Sivaramakrishnan and Oliver 2001), post-stimulatory facilitation changes could be related to properties in either DCN or IC pauser/buildup neurons.

Age-related changes in temporal processing in the IC may have many sources. Changes in lower auditory structures, such as for post-stimulatory facilitation of DCN neurons, will affect IC neuron temporal response patterns. Decreased intrinsic inhibition may also affect post-stimulatory facilitation, delayed excitability changes, and post-stimulatory suppression. There is a prolonged decreased excitability following stimulation of hippocampal neurons in aged animals (Disterhoft et al. 1996). This results from increased activation of calcium-activated potassium conductances (I_{KCa}, SK subtype) due to increased calcium influx through L-type calcium channels (Disterhoft et al. 1996; Landfield 1996). Prolonged calcium influx, in turn, may be due to decreases in delayed rectifier and A-type potassium channels in aged hippocampal neurons (Alshuaib et al. 2000). The wide variation in the duration and age-related changes in post-stimulatory suppression of IC neurons could depend on the number, type, or activation of ion channels expressed in IC neurons or neurons impinging on them.

In summary, temporal processing in aged animals is significantly altered and is expected to reduce the capability of the auditory system to encode information of sequential or simultaneous sound stimuli. Central deficits contributing to age-related hearing problems are anticipated, as there are many age-related neurological syndromes and neurophysiological changes. However, we are just beginning to understand the nature and extent of changes in the central auditory system. At least some of these changes are not apparently directly related to peripheral hearing loss but may be complex, involving reorganization of afferents, loss of inhibition, and other changes such as in ionic conductances.

ACKNOWLEDGMENTS

This research was supported by the Deafness Research Foundation.

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