Effects of aging on the response of single neurons to amplitude-modulated noise in primary auditory cortex of rhesus macaque

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Overton JA, Recanzone GH. Effects of aging on the response of single neurons to amplitude-modulated noise in primary auditory cortex of rhesus macaque. J Neurophysiol 115: 2911–2923, 2016. First published March 2, 2016; doi:10.1152/jn.01098.2015.—Temporal envelope processing is critical for speech comprehension, which is known to be affected by normal aging. Whereas the macaque is an excellent animal model for human cerebral cortical function, few studies have investigated neural processing in the auditory cortex of aged, nonhuman primates. Therefore, we investigated age-related changes in the spiking activity of neurons in primary auditory cortex (A1) of two aged macaque monkeys using amplitude-modulated (AM) noise and compared these responses with data from a similar study in young monkeys (Yin P, Johnson JS, O’Connor KN, Sutter ML. J Neurophysiol 105: 582–600, 2011). For each neuron, we calculated firing rate (rate code) and phase-locking using phase-projected vector strength (temporal code). We made several key findings where neurons in old monkeys differed from those in young monkeys. Old monkeys had higher spontaneous and driven firing rates, fewer neurons that synchronized with the AM stimulus, and fewer neurons that had differential responses to AM stimuli with both a rate and temporal code. Finally, whereas rate and temporal tuning functions were positively correlated in young monkeys, this relationship was lost in older monkeys at both the population and single neuron levels. These results are consistent with considerable evidence from rodents and primates of an age-related decrease in inhibition throughout the auditory pathway. Furthermore, this dual coding in A1 is thought to underlie the capacity to encode multiple features of an acoustic stimulus. The apparent loss of ability to encode AM with both rate and temporal codes may have consequences for stream segregation and effective speech comprehension in complex listening environments.

aging; auditory cortex; primate; temporal processing; single-unit electrophysiology

AGE-RELATED HEARING LOSS (ARHL) is a complex disorder, commonly thought to arise from deterioration of the peripheral organ, but it can also result from changes in the central auditory pathway and a host of contributing factors, such as environmental noise exposure, hormones, and drugs (Charitidi et al. 2010; Gates and Rees 1997; Guimaraes et al. 2006; Kim and Chung 2013; Willott et al. 2001). A principal complaint of people with ARHL is a difficulty comprehending speech, especially in noisy or challenging environments (Walton 2010; Willott et al. 2001). Whereas elevated audiometric thresholds are often associated with ARHL, elderly subjects with normal hearing can still show impaired speech processing abilities compared with younger counterparts (Frisina and Frisina 1997; Snell et al. 2002; Strouse et al. 1998), as well as other deficits in temporal processing (Barsz et al. 2002; Snell and Frisina 2000; Strouse et al. 1998). Several studies have shown that these speech processing measures are correlated with temporal processing deficits, such as gap-detection thresholds in elderly subjects with normal hearing (Snell et al. 2002) or mild hearing loss (Mazelová et al. 2003). The evidence supports an age-related decline in central auditory processing that results in difficulties processing complex temporal signals, such as speech (Barsz et al. 2002; Walton 2010). Whereas many studies have looked at the aging central auditory pathway, very few have studied auditory cortex, and only one, to our knowledge, has evaluated temporal processing in the nonhuman primate auditory cortex (Recanzone et al. 2011). Our goal in this study is to investigate age-related temporal processing changes in the primate auditory cortex that might shed light on speech processing deficits in humans suffering from ARHL.

Age-related temporal processing deficits have been demonstrated along the auditory pathway in several rodent models. Neurons in the inferior colliculus (IC) of aged CBA/CaJ mice and Long Evans rats showed poor gap detection, as measured by the strength of response to the second stimulus or delay needed to recover an average response (Barsz et al. 2002; Finlayson 2002; Walton 2010; Walton et al. 1998). Gap-detection thresholds, however, were not significantly different in middle-aged C57BL/6 mice with advanced peripheral hearing loss (Walton et al. 2007). Prolonged recovery times in rats were also not related to pure tone hearing thresholds and therefore, not likely due to peripheral hearing loss (Finlayson 2002). These results indicate that age-related processing delays arise at least at the level of the midbrain and are not a result of peripheral damage. Very few studies have investigated the responses of neurons in aging auditory cortex. Recanzone et al. (2011) found that neurons in primary auditory cortex (A1) of aged macaque required a longer gap to recover compared with the perceptual gap-detection thresholds measured in younger monkeys (~4 ms) (Petkov et al. 2003), and some neurons in older animals even failed to recover for the longest gaps tested (Recanzone et al. 2011). These results are consistent with previous rodent work, extending findings to primate auditory cortex.

Human speech is a complex signal, but the amplitude envelope of the signal is a primary component for speech processing (Rosen 1992; Shannon et al. 1995). Shannon et al. (1995) found that speech recognition was 90% preserved, with dramatically reduced spectral information, as long as modulations of the temporal envelope were preserved. This amplitude modulation (AM) also provides information that allows a
listener to identify a sound source in a noisy listening environment (Dollezal et al. 2012; Moore and Gockel 2012), making it an important feature to study in the context of AHRL. Neurons in A1 of young monkeys can encode AM with either a change in firing rate (rate code) or with a phase-locked response to the envelope of the stimulus (temporal code) (Liang et al. 2002; Lu et al. 2001; Yin et al. 2011) or with both codes (Malone et al. 2007; Yin et al. 2011). Evidence suggests a temporal-to-rate transformation in the primate auditory thalamocortical pathway and that this coding transformation is important for the accurate encoding of AM (Bartlett and Wang 2007; Lu and Wang 2004; Niwa et al. 2013, 2015; Wang et al. 2008). Furthermore, neurons in young A1 show some correspondence between rate and temporal tuning measures, suggesting that individual neurons can use both types of codes simultaneously to represent AM (Malone et al. 2007; Yin et al. 2011). Therefore, we set out to determine if natural aging influences the encoding of AM in single neurons in macaque A1.

In this study, the responses of neurons in A1 of aged macaque monkeys to passively presented AM noise across a range of modulation frequencies are compared with responses from young monkeys. We tested the predictions that aged neurons do not represent AM as well as younger neurons by several metrics: a decrease in the overall proportion of neurons that are responsive to the AM stimulus, a decrease in the proportion of neurons that encode AM using a temporal code (in other words, that there will be fewer neurons that phase-lock), and a decrease in the ability of phase-locking neurons to follow the envelope of the stimulus compared with young neurons. Finally, we tested the prediction that the correlation between rate and temporal codes decreases in the population of A1 neurons in aged monkeys.

MATERIALS AND METHODS

Data were collected from two alert male Rhesus macaque monkeys (A and B); data from these animals have been reported in several other studies from this lab (Engle and Recanzone 2012; Juarez-Salinas et al. 2010; Ng et al. 2015; Recanzone et al. 2011). Monkey A was 24.7–25.7 yr old and monkey B was 25.0–27.6 yr old during the course of the study, roughly equivalent to 75 and 79 human yr (Davis and Leathers 1985). These results were compared with those from a similar study of A1 (Yin et al. 2011). The two younger monkeys in that study (V and Y) were aged 8 and 9, corresponding to 24 and 27 human yr. Procedures in the aged monkeys followed those described in the younger monkeys very closely, and differences are highlighted where appropriate. All animals in this study and Yin et al. (2011) were raised at the California National Primate Research Center [University of California, Davis (UC Davis), CA] and had no history of ear infections or exposure to ototoxic drugs or excessive loud noise. All animals (both studies) were housed in the same vivarium in individual cages with access to paired housing when possible. In addition to monkey chow, animals received dried and fresh fruit daily and new toys and puzzles weekly. All procedures were approved by the UC Davis Institutional Animal Care and Use Committees and conformed to Association for Assessment and Accreditation of Laboratory Animal Care International and Society for Neuroscience standards.

Stimulus Presentation and Data Collection

All experiments were conducted in a double-walled sound booth (Industrial Acoustics Company, Hillside, IL), lined with echo-attenuating foam (Sonex). During recording sessions, each monkey sat with its head restrained in an acoustically transparent primate chair in the center of a circular array (1.5 m diameter) of 16 equally spaced speakers. In this experiment, AM noise and AM tones were presented in a randomly interleaved fashion. Only the results from the AM noise stimuli are reported here (described below). Subjects were given diluted fruit juices every three to nine stimulus presentations to keep them alert. Yin et al. (2011), similarly, awarded juice or water intermittently to maintain a consistent level of arousal. An infrared camera in the booth allowed experimenters to monitor their behavior via closed-circuit monitor to ensure that they maintained a consistent state of alertness.

Acoustic stimuli were generated using System 2 hardware (Tucker-Davis Technologies, Alachua, FL), controlled by a personal computer and custom software. Stimuli were 100% sinusoidally AM Gaussian noise of 500 ms duration, presented at ~60 dB sound pressure level (SPL), measured from the center of the speaker array at the monkey’s interaural axis (A-weighted; Bruel & Kjaer, Naerum, Denmark). Stimulus modulation frequencies for old monkeys were 2, 4, 6, 8, 12, 16, 24, 32, 64, and 128 Hz. One trial consisted a 500-ms AM noise stimulus plus a 300-ms poststimulus period. Each of the 10 AM noise stimuli was presented over 12 randomly interleaved trials. For comparison, young monkeys were presented with 400 ms duration 100% AM noise at 65 dB SPL at 7 modulation frequencies with 50 repetitions of each stimulus on randomly interleaved trials [see METHODS of Yin et al. (2011)]. For old monkeys, the 2-Hz AM condition was omitted from analyses to maintain a similar range and number of stimulus frequencies across the two groups.

Recording procedures have been described in detail previously (Juarez-Salinas et al. 2010; Woods et al. 2006). Briefly, monkeys were implanted with a head post and recording chamber (Crist Instruments, Hagerstown, MD) that allowed access to auditory cortex using a vertical approach. Before each recording session, the chamber was cleaned and fit with a grid (Crist Instrument), and a guide tube was inserted into a location in the grid, puncturing the dura. A tungsten electrode (FHC, Bowdoin, ME) was inserted into the brain via the guide tube. Neuronal activity was displayed on an oscilloscope and fed to an audio speaker. The electrode was advanced with a Narishige (Amityville, NY) hydraulic microdrive, until responses to search stimuli (broad-band noise, band-pass noise, tones, and clicks) were observed. Once auditory responses were confirmed, a single waveform (“unit”) was isolated using a window discriminator (DIS-2; Bak Electronics, Umatilla, FL) to trigger spike times, which were recorded with millisecond precision on a personal computer via System 2 hardware (ET1; Tucker-Davis Technologies). Spike times were collected from stimulus onset (without accounting for travel time of the stimulus from the speaker to the monkey) until 300 ms after stimulus onset (800 ms total).

Each neuron’s response to tones, broad-band noise, and Gaussian noise was recorded, as well as the spatial selectivity to Gaussian noise to characterize the physiological response properties before the AM stimuli were presented. Neurons were assigned to A1 based on grid location, characteristic frequency (CF), spatial tuning, and spectral response properties, as previously described (Juarez-Salinas et al. 2010; Recanzone et al. 2000). Neurons isolated from surrounding cortical areas served to define clearly the physiological borders of A1 but will not be presented further in this paper. Recordings were evenly sampled across A1 and into bordering fields. Topography of CFs across A1 for monkeys A and B can be found in Fig. 2 of Juarez-Salinas et al. (2010). The comparison of this figure with Supplemental Fig. S1 of Yin et al. (2011) shows comparable distributions of CFs and an even sampling across field A1. Forty-six neurons were isolated from A1 in the left hemisphere of monkey A, and 110 were isolated from A1 in the left hemisphere of monkey B.
Data Analysis

Firing rate analyses. Data were analyzed using MATLAB (MathWorks, Natick, MA) and Excel (Microsoft, Redmond, WA). Evoked firing rates were calculated from 70 to 500 ms poststimulus onset. Exclusion of the 70-ms onset period in both rate and temporal analyses is consistent with analyses in younger animals (70–400 ms for a 400-ms stimulus duration) (Yin et al. 2011). Spontaneous firing rates were calculated using the first 5 ms and last 20 ms (25 ms total) of each 800 ms trial, including 2 Hz AM noise and AM tone trials whose data are not included in this study. These two periods were chosen as they lie well before the start of any stimulus-driven activity and well beyond the completion of any offset responses, which are generally completed within 100–150 ms from stimulus offset (Engle and Recanzone 2012; Juarez-Salinas et al. 2010; Recanzone et al. 2000). Thus spontaneous spike rate was calculated over a total period of 6,000 ms/neuron: 25 ms (5

RESULTS
We recorded the activity of 156 single units from A1 of two geriatric macaque monkeys (mean age 26 yr) with similar behavioral audiograms compared with young monkeys [see Fig. 1 in Juarez-Salinas et al. (2010)]. Old monkey responses to AM frequencies of 4, 6, 8, 12, 16, 24, 32, 64, and 128 Hz were analyzed. These data are compared with 182 units collected from A1 of young macaques (mean age 8.5 yr) from a previously published study in a neighboring lab using AM frequencies of 5, 10, 15 20, 30, 60, and 120 Hz (Yin et al. 2011). Responses from a representative neuron from an old monkey are shown in Fig. 1. Figure 1A shows a raster plot of spiking responses to each trial sorted by the AM frequency, with the lowest frequency (4 Hz) shown at the top and the highest frequency (128 Hz) at the bottom. Trial-averaged activity is shown in Fig. 1B for the corresponding frequencies. tMTFs and rMTFs are shown in Fig. 1, C and D, respectively. In this example, it is clear that the neuron can follow the envelope of
the stimulus from 4 up to 16 Hz, as there is a strong periodicity in the spike raster. A phase-locked response at 32 Hz is difficult to see in the raster plots but apparent from the tMTF. There is a breakdown of this periodicity in the responses to the highest AM rates tested (64 and 128 Hz) but an equally clear increase in the overall firing rate with increasing AM rate at these high modulation frequencies.

Classifying Responses to AM

Given previous research that showed a deficit in temporal processing with age (Barsz et al. 2002; Snell and Frisina 2000; Strouse et al. 1998; Walton 2010), we sought to determine if this is reflected by a decrease in the proportion of neurons that responded to the AM stimulus. We determined the responsiveness of each neuron based on firing rate and vector strength, where rate-responsive neurons showed a significant change in firing rate from spontaneous for at least one AM stimulus, and synchronous neurons showed significant vector strength (VS or VSPP) for at least one AM stimulus (see MATERIALS AND METHODS). Neurons were then categorized into four mutually exclusive groups: exclusively rate-responsive neurons ("FR only"), which were rate responsive but did not synchronize to any AM stimulus; exclusively synchronous neurons ("VS only" or "VSPP only"), which phase-locked without significant changes in firing rate to any AM stimulus; both rate-responsive and synchronous, which showed a significant firing rate and vector strength for at least one AM stimulus (but not necessarily the same AM stimulus); or neither (not AM sensitive). This analysis was conducted twice using two different temporal criteria: standard VS and RS or trial-by-trial VSPP.

Units were classified as responsive to an AM stimulus with respect to firing rate if there was a significant change in firing rate from spontaneous ($P < 0.01$ with Bonferroni correction for 9 comparisons, $t$-test) for at least one AM rate. For the first temporal coding analysis, units were classified as synchronous if a significant VS was elicited for at least one AM rate (Rayleigh >17.7). Of 156 old A1 neurons analyzed, 5 were excluded, due to lack of spontaneous spikes, leaving 151 units for this analysis and any further analysis that requires a comparison with spontaneous activity. Of those remaining 151
units, only 17 cells (11.3%) were not sensitive to any AM stimulus with respect to firing rate or VS, 16 (10.6%) were firing rate responsive without synchronizing to any stimulus, 21 (13.9%) were synchronous without changing firing rate, and 97 (64.2%) responded to AM noise by synchronizing and changing firing rate. These AM classification results were compared with 182 units from young monkeys and are summarized in Fig. 2A. An analysis ($\chi^2$) showed that the differences between young and old were statistically significant ($P < 0.0001$, df = 3). Whereas differences are subtle, there were relatively more units from old monkeys that were exclusively synchronous and fewer that responded with changes in both firing rate and VS. With the comparison of the number of synchronous units between young and old monkeys, 118/151 (78.1%) units in old monkeys and 142/182 (78.0%) units in young monkeys yielded significant VS to at least one AM rate, as determined by the RS. This similarity in phase-locking between age groups is surprising, given previous results of decreased temporal processing with age (Barsz et al. 2002; Walton 2010; Walton et al. 1998). However, this method of determining synchronization is prone to false positives, as discussed in Yin et al. (2011). Unit classification was, therefore, repeated using a more conservative estimate of phase-locking.

Response categorization analysis was repeated using VS$_{pp}$ instead of the standard VS (see MATERIALS AND METHODS; note that VS$_{pp}$ results are used throughout the remainder of this study). Units were classified as synchronous if there was a significant increase between trial-by-trial VS$_{pp}$ values for at least one AM rate compared with spontaneous activity (two-sample $t$-test). Firing rate responsiveness was determined as above. With the use of this criterion, 27/151 units (17.9%) were not sensitive to AM, 56 (37.1%) were exclusively rate responsive, 11 (7.3%) were exclusively synchronous, and 57 (37.7%) showed significant changes in both VS$_{pp}$ and firing rate compared with spontaneous activity. These results were compared with data from young monkeys using the same criteria and are shown in Fig. 2B; as before, classification results are significantly different between age groups ($P < 0.0001$, $\chi^2$ test, df = 3). The use of VS$_{pp}$ criteria to assess phase-locking made a substantial difference in this AM sensitivity comparison: overall, there were fewer A1 neurons that synchronized in old monkeys (45.0%) compared with young monkeys (69.2%). The ratio of neurons that exclusively synchronize in old (7.3%) was approximately equal to young (8.2%) neurons. Fewer units in old vs. young subjects responded to an AM stimulus with both synchrony and a change in firing rate (37.7% and 61.0%, respectively). More units responded exclusively with changes in firing rate in old (37.1%) compared with young (17.0%), and slightly more neurons were not responsive to AM in old vs. young neurons (17.9% vs. 13.7%, respectively). Whereas there was only a modest decrease in overall AM responsiveness with age, there was a shift from neurons that code AM with changes in both firing rate and synchrony in young to a predominance of rate-responsive neurons in old, due to a decrease in the ability of A1 neurons to synchronize to the stimulus.

Increasing and Decreasing Firing Rates in Response to AM

As reported above, we found fewer synchronous neurons in old A1 and a concomitant increase in the proportion of non-synchronous neurons that responded with a change in firing rate. Yin et al. (2011) observed that more synchronous cells increased firing rate in response to an AM stimulus, whereas nonsynchronous cells were more likely to decrease firing rate or both increase and decrease firing rate. Thus we looked at changes in evoked firing rate relative to spontaneous firing for all neurons. Specifically, we counted the number of neurons that increased firing rate to all AM stimuli or decreased firing rate to at least one AM stimulus to investigate if the loss of
synchronous neurons was related to whether the neurons were increasing or decreasing their firing rate. The results of this analysis are shown in Fig. 3. Overall, fewer neurons showed firing rate suppression to at least one modulation frequency in old monkeys (14/113 = 12.4%) compared with young (31/142 = 21.8%). Only two neurons (1.8%) from the old monkeys showed suppressed firing rate to all AM stimuli compared with 17 (12.0%) neurons in younger animals, whereas a similar proportion of neurons from old and young animals showed both increases and decreases in firing rate to different AM frequencies (10.6% and 9.9%, respectively). Next, we compared neurons with significant temporal coding (“both” neurons of Fig. 2B) with those that did not synchronize (FR only of Fig. 2B) and found that the shift from decreasing to increasing firing rates is not dominated by synchronous (Fig. 3A) or nonsynchronous (Fig. 3B) neurons. These results suggest that A1 neurons in geriatric primates are less able to encode AM rate with rate suppression, and although it is not clear whether this change may be responsible for a decrease in the ability to synchronize effectively, this change is apparent across all neurons whether they synchronize or not.

Spontaneous and Evoked Firing Rates Are Higher in Older Monkeys Than Young and Higher in Synchronous Neurons

Previous studies drawing from the same set of A1 neurons demonstrated significantly greater spontaneous firing rates and evoked firing rates in response to 200 ms noise bursts that varied in spatial location compared with neurons from young monkeys (Engle and Recanzone 2012; Juarez-Salinas et al. 2010). We repeated this analysis for this experiment and compared spontaneous and driven rates with values reported in Yin et al. (2011), who reported data from synchronous (both) and nonsynchronous (FR only) cells separately, as it was thought that these might comprise distinct classes of neurons or neural responses. Figure 4 shows the distributions of spontaneous firing rates for synchronous and nonsynchronous neurons in old animals (mean and median values for young animals are indicated, as distribution data were not available), and Fig. 5 shows the distributions of evoked firing rates for synchronous and nonsynchronous neurons for both young and old neurons. For nonsynchronous neurons, mean spontaneous firing rates were 4.4 times greater, and mean evoked firing rates were 5.6 times greater in old neurons compared with young neurons. Meanwhile, synchronous neurons had 7.5 times greater mean spontaneous and 6.9 times greater mean evoked firing rates in old animals compared with young (refer to Tables 1 and 2). Overall, evoked and spontaneous firing rates are much higher in neurons from old compared with young A1, consistent with the results using shorter, unmodulated stimuli.

In addition to increased stimulus-evoked firing rates, Yin et al. (2011) reported that spontaneous firing rates in young macaques were higher in synchronous cells (mean 7.8 and median 6.5 spikes/s) than nonsynchronous cells (mean 5.5 and median 3.6 spikes/s). Among rate-responsive units in old monkeys, mean and median evoked firing rates for synchronous neurons were also greater (155.9 and 136.3 spikes/s, respectively) than spike counts for nonsynchronous neurons (mean 86.5 and median 48.2 spikes/s). The same pattern is true of spontaneous firing rates for synchronous neurons (mean 58.5 and median 43.7 spikes/s) compared with nonsynchronous neurons (mean 24.0 and median 12.9 spikes/s). These differences between synchronous and nonsynchronous neurons are highly significant among the population of older neurons for both evoked firing rates ($P < 0.005$ for all AM rates) and spontaneous firing rates ($P < 0.0001$). These data are summarized in Tables 1 and 2.

Population MTFs

The previous analysis noted that there were different firing rate distributions of synchronous and nonsynchronous neurons...
Fig. 4. Distribution of spontaneous firing rates from older monkeys. Solid gray lines indicate mean, and dashed gray lines indicate median spontaneous firing rate from young (Yin et al. 2011). A: synchronous neurons from old monkeys (“both” in Fig 2B; n = 57). B: nonsynchronous AM-responsive neurons (“FR only” in Fig 2B; n = 56). Synchronous neurons showed greater spontaneous firing rates (mean = 58.5, median = 43.7 spikes/s) than nonsynchronous [mean = 24.0, median = 12.9 spikes/s; P < 0.0001, Kolmogorov–Smirnov (K-S) test].

(Fig. 5) and a difference in spontaneous and evoked firing rates between young and old neurons (Fig. 4) but did not analyze population responses to different modulation rates. Therefore, we examined the relationship between the rMTFs and tMTFs for each population of AM-responsive neurons. The population tMTF was constructed across all units that had a significant VSPP for at least one AM rate compared with spontaneous activity (Fig. 6A). In A1 neurons from young monkeys, neurons phase-locked well to lower AM rates but showed decreased VSPP values at frequencies greater than ~60 Hz. Compared with young tMTFs, A1 neurons from old monkeys showed similar phase-locking above 60 Hz but synchronize significantly worse (decreased VSPP values) at lower modulation frequencies. These data indicate that not only are there fewer old neurons providing temporally based information about the envelope of the stimulus but those that do are not as temporally precise.

The population rMTF was constructed across all units that had a significant change in firing rate for at least one AM noise stimulus: rMTFs are shown separately for synchronous (Fig. 6B) and nonsynchronous (Fig. 6C) neurons. In these plots, stimulus-evoked firing rates are normalized by spontaneous rate; otherwise, there would be a much greater separation between the rMTFs generated for young and old monkeys. In old monkeys, nonsynchronous neurons had higher normalized firing rates than synchronous neurons, despite synchronous neurons having greater evoked and spontaneous firing rates (see above). The rMTF for synchronous neurons is relatively flat and slightly increasing, whereas the rMTF for nonsynchronous neurons peaks somewhat between 12 and 16 Hz, decreases to 64 Hz, and then increases again at 128 Hz. In young monkeys, the synchronous neuron population rMTF shows an increase in the low-to-midrange frequencies and then decreases steadily after 60 Hz. In contrast, the nonsynchronous neurons in young monkeys show a flatter rMTF profile, slightly decreasing their response up to 20 or 30 Hz and then leveling off. Thus whereas the population rMTFs are fairly similar between young and old synchronous cells, there is a clear increase in evoked activity (relative to the increased spontaneous activity) in old nonsynchronous neurons compared with young neurons. These data reveal that neurons in A1 of aged monkeys that are unable to follow the envelope of an AM stimulus have dramatically increased normalized firing rates, whereas the remaining aged neurons that retain the ability to phase-lock to AM maintain similar signal-to-noise ratios compared with young.

**AM Noise Tuning Functions and BMFs Are Correlated in Young but Not Old Neurons**

Yin et al. (2011) found that rate and temporal population tuning functions were correlated in their data from young monkeys. Therefore, we examined the relationship between population tuning functions in our old neurons. In young animals, tMTFs and rMTFs for synchronized neurons were significantly, positively correlated (r = 0.97, P < 0.005). In old monkeys, however, this relationship is reversed (r = −0.74, P = 0.03), revealing a dramatic change in AM tuning across the population of synchronous neurons in A1. Given the strong relationship between population tuning functions, we next tested whether this relationship held for individual cells. We compared the single neuron rMTFs and tMTFs for each of the synchronous units. The results showed that firing rates and VSPP values are positively correlated for young neurons (r = 0.18, P < 0.0001) but again, are negatively correlated for old neurons (r = −0.45, P < 0.0001).

Finally, we also analyzed the peaks (or “best” values) of the MTFs (rMTF and tMTF using VSPP) for each neuron. Figure 7 shows the distributions of BMFs for firing rate (rBMFs) and VSPP (tBMFs) for both young (Fig. 7A) and old (Fig. 7B) neurons. rBMFs and tBMFs for synchronous neurons are correlated in young monkeys (r = 0.90, P < 0.01) but not old (r = −0.32, P = 0.40). In other words, the distributions of peak rate and temporal responses to AM across the population of AM-responsive A1 neurons are similar in young animals (positively correlated) but dissim-
Fig. 5. Distribution of evoked firing rates for young and old synchronous and nonsynchronous AM-responsive neurons. Cells are categorized as in previous figures. A and B: neurons synchronous to any AM stimulus (“both” in Fig. 2B). C and D: neurons that did not synchronize to any AM stimulus (“FR only” in Fig 2B). Data from young neurons are shown in white bars (top; A and C), and old data are in black bars (bottom; B and D). Older neurons show significantly greater evoked firing rates than young neurons for both synchronous (10 times greater; \( P < 10^{-22} \), K-S test) and nonsynchronous (3.8 times greater; \( P < 0.0001 \), K-S test) cells. Whereas firing rates were greater in nonsynchronous neurons than synchronous in young animals, the reverse was true in older animals, with synchronous neurons almost doubling the firing rate over nonsynchronous neurons.

Table 1. Spontaneous firing rates in young vs. old monkeys

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<td>Young</td>
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<td>Old</td>
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Table 2. Evoked firing rates in young vs. old monkeys

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<td>Young</td>
<td>22.7</td>
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The goal of this study was to investigate whether there were age-related changes in neural processing of the acoustic envelope in A1, reasoning that this could reveal neuronal processes implicated in age-related hearing deficits in the temporal domain. We compared the spiking activity of single neurons to 100% AM noise in aged macaques with a previous study in young monkeys using the same analysis techniques (Yin et al. 2011). For each neuron, we measured firing rate, as well as temporal responses defined by VSPP. We found that neurons in aged macaque A1 were not less AM responsive overall compared with young; however, aged neurons were less likely to synchronize to the stimulus envelope. More specifically, there was a decrease in the proportion of neurons that synchronized and responded to AM noise with a change in firing rate (both), whereas there was a concurrent increase in the proportion of solely rate-responsive (FR only) neurons. Furthermore, neurons in aged monkeys had higher spontaneous firing rates and higher evoked firing rates and were less likely to decrease stimulus-evoked firing rates compared with neurons from young animals. These findings are consistent with previous support for an age-related decrease in inhibition in the auditory pathway (Caspar et al. 1995, 1999, 2008; Gray et al. 2014b). Finally, there was also a disorganization of the relationship between the rMTFs and tMTFs, as well as rBMFs and tBMFs in aged animals, where neurons preferred higher AM rates based on firing rate in older monkeys compared with younger ones. These results suggest that the perturbed balance of excitation and inhibition with aging also affects periodicity coding, which could underlie temporal processing deficits, including speech perception in aged humans (Barsz et al. 2002; Walton 2010).
Potential Effects of Stimulus Intensity

One consideration is in the small difference in intensity between this study and that of Yin et al. (2011). It is common for aged monkeys to have higher auditory brain stem response (ABR) thresholds to click and tone stimuli (Ng et al. 2015), as well as behavioral thresholds (Davis and Leathers 1985). Thus this is an important consideration when comparing neuronal responses between young and aged monkeys, in that there could be a large sensation difference for the same intensity stimulus. In the first report from these geriatric monkeys (Juarez-Salinas et al. 2010), audiograms of the old monkeys (A and B; same as in the present study) were compared with four young macaques from the same colony and with the same history as monkeys from this report and Yin et al. (2011), as well as data from another study of Japanese macaques (Jackson et al. 1999). Noise thresholds were identical between young and old, and tone thresholds were similar up to \( \sim 12 \text{ kHz} \). Note that noise thresholds were between 10 and 15 dB SPL for both young and old monkeys.

**Fig. 6.** Mean modulation transfer functions (MTFs). A: mean temporal MTFs for all units that synchronized to any given stimulus (young, \( n = 126 \); old, \( n = 68 \)). B: mean rate MTFs for synchronous units that had a significant rate response (young, \( n = 123 \); old, \( n = 57 \)). C: mean rate MTFs for units that had a significant rate response but did not synchronize to any stimulus (young, \( n = 37 \); old, \( n = 56 \)).

**Fig. 7.** Distribution of rate BMFs (rBMFs) and VS_{pp} [temporal BMFs (tBMFs)]. A: young monkeys (rBMFs, \( n = 160 \); tBMFs, \( n = 126 \)). B: old monkeys (rBMFs, \( n = 113 \); tBMFs, \( n = 68 \)).

**Potential Effects of Stimulus Intensity**

One consideration is in the small difference in intensity between this study and that of Yin et al. (2011). It is common for aged monkeys to have higher auditory brain stem response (ABR) thresholds to click and tone stimuli (Ng et al. 2015), as well as behavioral thresholds (Davis and Leathers 1985). Thus this is an important consideration when comparing neuronal responses between young and aged monkeys, in that there could be a large sensation difference for the same intensity stimulus. In the first report from these geriatric monkeys (Juarez-Salinas et al. 2010), audiograms of the old monkeys (A and B; same as in the present study) were compared with four young macaques from the same colony and with the same history as monkeys from this report and Yin et al. (2011), as well as data from another study of Japanese macaques (Jackson et al. 1999). Noise thresholds were identical between young and old, and tone thresholds were similar up to \( \sim 12 \text{ kHz} \). Note that noise thresholds were between 10 and 15 dB SPL for both young and old monkeys.
and perceptual performance. Wang and colleagues (Bartlett and Wang 2007; Liang et al. 2002; Lu et al. 2001) have suggested that there is a transformation in the auditory pathway from a temporal code to a firing rate code, which for AM stimuli begins in the medial geniculate and continues at the level of A1. Previous studies in aged rodents have shown that temporal coding of single neurons is diminished in the auditory midbrain (Allen et al. 2003; Barsz et al. 2002; Finlayson 2002; Simon et al. 2004; Walton et al. 1998, 2007). Consistent with a decrease in subcortical temporal processing and therefore, impoverished temporal inputs to cortex, we observe a decrease in the proportion of temporal coding neurons in A1 of aged macaques, presumably at the expense of neurons that previously had used both rate and temporal codes to encode AM (Fig. 2B). Furthermore, the proportion of rate coding neurons is similar between young and old. This suggests that the transformation from temporal to rate coding is more advanced in aged animals. Thus the seemingly inconsistent results regarding rate and temporal coding in the primate auditory pathway (Liang et al. 2002; Lu et al. 2001; Malone et al. 2007; Yin et al. 2011) could be, at least in part, due to the age of animal participants, a factor often ignored (indeed, rarely, if ever, reported) in previous studies.

In addition to a change in the proportion of temporal coding neurons, we found significant differences in synchrony and firing rate measures that might influence perceptual performance. We noted that those neurons that phase-locked to AM had lower VS_{pp} values (Fig. 6A). Furthermore, whereas the population tMTF in young neurons showed peak values below 60 Hz and a steep synchrony cutoff above that value, the average tMTF for old neurons was relatively flat across all AM rates tested (Fig. 6A). Decreased synchrony throughout the auditory pathway necessarily leads to disruptions in rate coding upstream. Furthermore, changes in inhibitory interneurons in A1 (e.g., loss of parvalbumin-positive and somatostatin-positive expression among GABAergic neurons) (Ouellet and de Villers-Sadani 2014) might play a role in changes in AM processing in aging A1. Synchronous neurons maintained similar signal to noise, as measured by stimulus-evoked firing rates normalized to spontaneous rate levels, but population rMTFs for these neurons were flat across AM frequency in old neurons, indicating an overall loss of tuning across the population (Fig. 6B). Nonsynchronous neurons in old had dramatically elevated normalized firing rates compared with young and a population rMTF more similar to synchronous population rMTF in young (Fig. 6, B and C). Whereas the role of rate coding in synchronous and nonsynchronous neurons is not clear for AM perception, it seems likely that the reduced temporal fidelity of neurons that retain the ability to synchronize their firing with the envelope of the stimulus and the much flatter rMTFs of synchronous cells is consistent with a decreased ability to process auditory temporal information, as seen psychophysically in humans (Frisina and Frisina 1997; Palmer and Musiek 2014; Snell and Frisina 2000; Snell et al. 2002; Strouse et al. 1998) and rodents (Barsz et al. 2002). These data, taken together, are consistent with the idea that synchronous encoding of the stimulus envelope is degraded in subcortical areas as a function of aging, leading to decreased temporal fidelity and numbers of synchronous neurons at the level of A1, which cannot be compensated for by shifting to a firing rate code.

**Rate vs. Temporal Coding in A1**

We found significant changes in rate and temporal coding measures in old A1 responses compared with young, which may have a profound effect on temporal envelope processing.

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**Fig. 8. Joint distribution of firing rate (rBMFs) and VS_{pp} or temporal BMFs (tBMFs) for all rate-responsive synchronous cells. A: young monkeys (n = 71). B: old monkeys (n = 108).**

Young and old monkeys (Juarez-Salinas et al. 2010), and the present data were collected using stimuli at 65 and 60 dB SPL, respectively. Whereas noise thresholds are not known for monkeys V and Y from Yin et al. (2011), it is reasonable to assume that they are similar to young monkey data from Juarez-Salinas et al. (2010). ABR thresholds for click stimuli for monkeys A and B were each measured at 30 dB. With the use of these data, we can confirm that 60 dB was well above detection threshold. Furthermore, there is little difference between ABR waveforms for 60 and 70 dB (Ng et al. 2015). Neural activity in response to broad-band noise is similar between 55 and 75 dB for monkeys aged 5–12 yr across auditory cortex (Woods et al. 2006), further indicating that the 5-dB difference in presentation level between age groups should not have had an effect on the results of the present study.

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Given these changes, it is not surprising that there are differences in the relationships among population tuning functions and BMFs that may have meaningful implications for perception. rMTFs and tMTFs for synchronous neurons were positively correlated in young monkey A1 (Fig. 6A) but negatively correlated in old (Fig. 6B). There was also a significant correlation between temporal (VSpp) and firing rate BMFs (tBMFs and rBMFs) in the population of young (Fig. 7A) but not old (Fig. 7B) A1 neurons. Whereas joint BMFs were not significantly coincident in young animals, there was a tendency for neurons to have rBMFs and tBMFs near each other in young (Fig. 8A). Malone et al. (2007) showed a similar pattern of joint BMFs in young macaque A1 using AM tone stimuli, where rBMFs and tBMFs were clustered between 5 and 20 Hz [see Fig. 14 of Malone et al. (2007)]. However, this correspondence was entirely lost among joint BMFs in old neurons (Fig. 8B). This indicates that the way in which these codes are communicated by the same population of neurons is very different between young and aged auditory cortex.

Niwa et al. (2012a,b, 2013, 2015) showed that firing rate activity in A1 is correlated with choice behavior, more so than phase-locking activity; furthermore, phase-locking remains constant regardless of choice behavior, but firing rate becomes stronger and is more associated with AM sensitivity. There were also differences in the timing of choice-probability relative to the task structure, which indicates that phase-locking may be more important for initial encoding of stimulus features, whereas rate coding is more important for decision and action based on those stimulus features. However, Niwa et al. (2012a) suggest that dual rate and temporal coding of AM by A1 neurons may be necessary for processing of various stimulus parameters simultaneously. Whereas our data cannot directly address this issue, because animals were presented with stimuli passively and were not required to attend to the stimulus or make a response, these results suggest that the loss of efficient dual coding in the aging auditory system may lead to a decreased ability to process effectively several features of an auditory stimulus simultaneously. This hypothesis is consistent with age-related deficits in perception of a single speaker with competing babble (two or more speakers superimposed to simulate a noisy environment), observed in elderly subjects with normal hearing (Füllgrabe et al. 2014; Gordon-Salant and Fitzgibbon 2001; Snell et al. 2002).

Changes in Excitatory/Inhibitory Balance in Aged Monkeys

The understanding of the process of aging in the auditory system is a complex challenge. A great deal of important work has been done in rodents to describe age-related changes along the ascending auditory pathway. Previous studies in aged rodents have shown changes in a variety of histological markers throughout the ascending auditory pathway (Caspar et al. 2008). We recently described similar changes in the aged macaque, starting with decreased afferent drive from the cochlea (Engle et al. 2013) and ascending through the cochlear nucleus (Gray et al. 2014a), superior olivary complex (Gray et al. 2013a), IC (Engle et al. 2014), and thalamus (Gray et al. 2013b). Histological results across species indicate that there is an alteration in the excitatory/inhibitory balance throughout the auditory central nervous system as a function of age (Caspar et al. 2008; Gray et al. 2014b). In rodents, many of these changes have been associated with changes in evoked activity in the auditory midbrain. Increases in evoked spike counts have been reported in IC of mice in response to AM noise (Walton et al. 2002) and spontaneous activity in the cochlear nucleus (Caspar et al. 2005, 2006). Other changes in mouse auditory brain stem have been related to disruption of the efferent feedback system, which serves a protective function to the outer hair cells; this effect precedes detectable audiometric changes in mice and humans and may contribute to amplification of peripheral inputs (Jacobson et al. 2003; Kim et al. 2002; Zettel et al. 2007; Zhu et al. 2007).

Our previous studies in the core and belt of auditory cortex noted increases in both spontaneous and driven activity but no differences in signal-to-noise ratio, as measured by stimulus-evoked rates divided by spontaneous firing rates (Engle and Recanzone 2012; Juarez-Salinas et al. 2010). Furthermore, whereas there was little difference in spatial tuning found in A1 between young and old macaques, large differences were found in the caudolateral belt field. The current report, which comprises a significant overlap of individual neurons from those previous studies, also shows an increase in spontaneous firing rates, as well as evoked firing rates in older macaques compared with young for a different stimulus condition (AM as opposed to unmodulated noise). Interestingly, in the present study, there is no change in signal to noise (normalized firing rate) for synchronous cells (see Fig. 6B; “both” neurons, n = 57 old), but there is a dramatic increase in excitability for the nonsynchronous “rate only” population of neurons (see Fig. 6C; n = 56 old). In other words, neurons that maintain the ability to synchronize to the stimulus envelope maintain a similar firing rate relative to the elevated spontaneous rate, whereas those that do not synchronize have significantly elevated firing rates relative to the elevated spontaneous activity. This result supports the idea that there is some compensatory mechanism, whereby a subset of aged neurons is able to maintain the dynamic range necessary to follow a periodic stimulus effectively in spite of increased firing rates. Increased signal-to-noise ratio for nonsynchronous cells may be one way to compensate for the loss of temporal fidelity and decreased numbers of synchronous neurons in an attempt to maintain AM processing abilities.

Conclusions—Implications for Speech Processing

Our results revealing increased spontaneous and evoked firing rates, as well as decreased signal-to-noise ratios in old relative to young monkeys, support previous studies showing age-related decrease in inhibition (Caspar et al. 2008; Gray et al. 2014b). The shift in signal to noise corresponds to both a loss of the ability of many old neurons to synchronize to the AM envelope and decreased phase-locking strength in those remaining cells that are synchronous, compared with the responses in young macaque A1 neurons. These dramatic age-related changes in neuronal excitability have significant ramifications for temporal and rate encoding of AM stimuli in A1 of the nonhuman primate. Specifically, not only do fewer neurons in old monkeys use both rate and temporal codes, but also, there is a breakdown in the relationship between rate and temporal coding metrics at the population and single cell level. Given these results and recent choice-probability results (Niwa et al. 2012a,b), it is very likely that this age-related shift in dual
coding will result in deficits in the ability to process multiple features of a sound effectively, which predicts an increased difficulty in speech comprehension in a complex natural environment. Future studies will be necessary to test the perceptual consequences of these age-related changes explicitly.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.A.O. and G.H.R. conception and design of research; J.A.O. and G.H.R. performed experiments; J.A.O. analyzed data; J.A.O. and G.H.R. interpreted results of experiments; J.A.O. prepared figures; J.A.O. drafted manuscript; J.A.O. and G.H.R. edited and revised manuscript; J.A.O. and G.H.R. approved final version of manuscript.

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