Title: Effects of aging on the response of single neurons to amplitude modulated noise in primary auditory cortex of Rhesus macaque

Running Head: Effect of aging on AM encoding in A1

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ABSTRACT

Temporal envelope processing is critical for speech comprehension, which is known to be affected by normal aging. While the macaque is an excellent animal model for human cerebral cortical function, few studies have investigated neural processing in the auditory cortex of aged non-human primates. We therefore 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 to data from a similar study in young monkeys (Yin et al., 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, while 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. Further, 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.

KEYWORDS

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

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 (Gates and Rees, 1997; Willott et al., 2001; Guimaraes et al., 2006; Charitidi et al., 2010; Kim and Chung, 2013). A principal complaint of people with ARHL is a difficulty comprehending speech, especially in noisy or challenging environments (Willott et al., 2001; Walton, 2010). While elevated audiometric thresholds are often associated with ARHL, elderly subjects with normal hearing can still show impaired speech processing abilities compared to younger counterparts (Frisina and Frisina, 1997; Strouse et al., 1998; Snell et al., 2002) as well as other deficits in temporal processing (Strouse et al., 1998; Snell and Frisina, 2000; Barsz et al., 2002). 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). While 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 non-human 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 AHRL.

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 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 (Walton et al., 1998; Barsz et al., 2002; Finlayson, 2002; Walton, 2010). Gap-detection thresholds, however, were not significantly different in middle-aged C57 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 A1 of aged macaque required a longer gap to recover compared to the perceptual gap detection thresholds measured in younger monkeys (~4ms; 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 (Dolležal et al., 2012; Moore and Gockel, 2012), making it an important feature to study in the context of AHRL. Neurons in primary auditory cortex 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) (Lu et al., 2001; Liang et al., 2002; 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 (Lu and Wang, 2004; Bartlett and Wang, 2007; Wang et al., 2008; Niwa et al., 2013, 2015). Further, neurons in young primary auditory cortex (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). We, therefore, 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 primary auditory cortex of aged macaque monkeys to passively presented AM noise across a range of modulation frequencies are compared to responses from young monkeys. We tested the predictions that aged neurons do not represent AM as well as younger neurons by several metrics: by a decrease in the overall proportion of neurons that are responsive to the AM stimulus, by 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 to 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.

**SUBJECTS & METHODS**

Data were collected from two alert male Rhesus macaque monkeys, A and B; data from these animals has been reported in several other studies from this lab (Juarez-Salinas et al., 2010; Recanzone et al., 2011; Engle and Recanzone, 2012; Ng et al., 2015). Monkey A was 24.7 to 25.7 and monkey B was 25.0 to 27.6 years old during the course of the study, roughly equivalent to ~75 and ~79 human years (Davis and Leathers, 1985). These results were compared to those from a similar study of primary auditory cortex (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 years. 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, and had no history of ear infections or of 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 IACUC and conformed to AAALAC and Society for Neuroscience standards.

*Stimulus presentation and data collection*
All experiments were conducted in a double-walled sound booth (IAC) 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-meter diameter) of 16 equally spaced speakers. In this experiment, AM noise and AM tones were presented in 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 in order 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 Tucker Davis Technologies System 2 hardware controlled by a PC and custom software. Stimuli were 100% sinusoidally amplitude modulated (AM) Gaussian noise of 500ms duration presented at approximately 60 dB SPL measured from the center of the speaker array at the monkey’s interaural axis (Bruel & Kjaer, A-weighted). Stimulus modulation frequencies for old monkeys were 2, 4, 6, 8, 12, 16, 24, 32, 64, and 128 Hz. One trial constituted a 500ms AM noise stimulus plus a 300ms post-stimulus period. Each of the 10 AM noise stimuli were presented over 12 randomly interleaved trials. For comparison, young monkeys were presented with 400ms duration 100% AM noise at 65 dB SPL at seven modulation frequencies with 50 repetitions of each stimulus on randomly interleaved trials (see Methods, Yin et al., 2011). For old monkeys, the 2 Hz AM condition was omitted from analyses in order to maintain a similar range and number of stimulus frequencies across the two groups.

Recording procedures have been described in detail previously (Woods et al., 2006; Juarez-Salinas et al., 2010). Briefly, monkeys were implanted with a head post and recording chamber (Crist Instruments) that allowed access to auditory cortex using a vertical approach. Before each recording session, the chamber was cleaned, fit with a grid (Crist Instruments) and a guide tube was inserted into a location in the grid, puncturing the dura. A tungsten electrode (FHC) 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 hydraulic microdrive until responses to search stimuli (broadband noise, bandpass noise, tones and clicks) were observed. Once auditory responses were confirmed, a single waveform (“unit”) was isolated using a window discriminator (Bak DIS-2) to trigger spike times which were recorded with millisecond precision on a PC via TDT System 2 hardware (AD2, ET1). Spike times were collected from stimulus onset (without accounting for travel time of the stimulus from the speaker to the monkey) until 300ms after stimulus onset (800ms total).

Each neuron’s response to tones, broadband noise, and Gaussian noise was recorded, as well as the spatial selectivity to Gaussian noise in order to characterize the physiological response properties before the AM stimuli were presented. Neurons were assigned to the primary auditory cortex (A1) based on grid location, characteristic frequency, spatial tuning and spectral response properties as previously described (Recanzone et al., 2000; Juarez-Salinas et al., 2010). Neurons isolated from surrounding cortical areas served to clearly define 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 characteristic frequencies across A1 for monkeys A and B can be found in Figure 2 of Juarez-Salinas, et al. (2010).

Comparing this figure to supplemental figure S1 of Yin et al. (2011) shows comparable distributions of CFs and an even sampling across field A1. Forty-six (46) 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 and Excel. Evoked firing rates were calculated from 70ms to 500ms post-stimulus onset. Exclusion of the 70ms onset period in both rate and temporal analyses is consistent with analyses in younger animals (70-400ms for a 400ms stimulus duration; Yin et al., 2011). Spontaneous firing rates were calculated using the first 5ms and last 20ms (25ms total) of each 800ms 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 - 150ms from stimulus offset (Recanzone et al., 2000; Juarez-Salinas et al., 2010; Engle and Recanzone, 2012). Thus, spontaneous spike rate was calculated over a total period of 6000ms per neuron: 25ms (5+20ms windows) per trial times 240 stimulus trials (10 AM rates x 2 AM conditions x 12 trials per stimulus). Twelve trials of spontaneous spike trains were constructed by concatenating spikes from the same intervals described above (first 5ms and last 20ms) in order to compare against twelve trials of evoked firing rates for each AM rate, and for temporal trial-by-trial statistical analyses (see below). To validate our spontaneous activity estimation and to ensure that we were not inadvertently including driven activity, rate analyses were repeated excluding neurons that showed a significant correlation between evoked firing rates and spontaneous estimates across trials ($p < 0.05$; $n = 20$). Exclusion of these neurons made no appreciable difference in the reported results so they were included for the remainder of the study. Neurons with zero spontaneous spikes ($n = 5$ cells) were excluded from all analyses.

Each neuron was classified as AM-responsive by firing rate if at least one AM rate elicited a significantly different firing rate compared to spontaneous activity (two-tailed unpaired t-test, $p < 0.01$) in order to directly compare these data to the statistical tests of Yin et al. (2011). Resulting rate-responsive cells were included in all further rate analyses. Rate modulation transfer functions (rMTFs) were constructed by plotting average firing rates as a function of AM rate. The best modulation frequency with respect to rate (rBMF) for each neuron was defined as the AM rate that elicited the peak firing rate or the most significant difference from spontaneous (including significant decreases in firing rate).

Temporal analyses. Both vector strength (Goldberg and Brown, 1969) and phase-projected vector strength (Yin et al., 2011) were used to determine phase-locking to the AM stimulus. Phase-projected vector strength ($VS_{PP}$) provides an advantage over the standard vector strength metric because it allows trial-by-trial measurements, even for trials with low spikes counts that produce spuriously high vector strength values. There is little difference between these two variations of the metric for trials with more than seven spikes (M.L. Sutter, personal communication). While low spike counts were not an issue in
our old monkey sample, we used VSPP primarily to keep our analyses consistent with young data from Yin et al. (2011). Significant phase-locking was determined using both metrics for comparison. The standard vector strength (VS) is calculated as follows:

(Equation 1)

\[
VS = \sqrt{\left(\frac{\sum_{i=1}^{n} \cos(\theta_i)}{n}\right)^2 + \left(\frac{\sum_{i=1}^{n} \sin(\theta_i)}{n}\right)^2}; \quad \theta_i = \frac{2\pi}{t_1 \text{ modulo } p} \]

Where \( n \) is the number of spikes over all trials, \( \theta_i \) is the phase of each spike in radians, and \( p \) is the modulation period of the stimulus in milliseconds. Vector strength was calculated using 70 to 500ms of each trial, excluding the initial 70ms onset period. For standard VS analyses, the Rayleigh test of uniformity (RS, equation 2), which approximates a chi-square test constrained with 2 degrees of freedom and is appropriate for circular distributions (Mardia and Jupp, 2009) was used to determine if the calculated VS was statistically significant.

(Equation 2)

\[
RS = 2n(VS^2)
\]

Each neuron was classified as significantly synchronized to the stimulus if Rayleigh \( \geq 18.2 \) for at least one AM rate (see Figure 2A). A Rayleigh value of 18.2 (17.7 for young) corresponds to \( p < 0.001 \) after Bonferroni correction for nine (seven) comparisons per cell (Mardia and Jupp, 2009; Yin et al., 2011). An additional test of phase-locking was conducted using VSPP (as in Figure 2b), along with all subsequent temporal analyses. VSPP is calculated for each trial as follows:

(Equation 3)

\[
VSPP = VS_t \cos(\phi_t - \phi_c)
\]

Where \( VS_t \) is the standard vector strength per trial, and \( \phi_t \) is the trial-by-trial phase angle in radians and \( \phi_c \) is the mean phase angle for each AM rate stimulus condition. The formula for phase angle is:
(Equation 4)

\[ \phi = \arctan2 \frac{\sum_{i=1}^{n} \sin \theta_i}{\sum_{i=1}^{n} \cos \theta_i} \]

Where \( n \) is the number of spikes in each trial for \( \phi_1 \) or the number of spikes per condition for \( \phi_c \). The function \( \text{arctan2} \) (Matlab function “atan2”) is an arctangent function that takes the sign of the sine and cosine inputs and determines the appropriate quadrant for the output. Cases with zero spikes were assigned a \( \text{VS}_{PP} \) of zero. Standard VS ranges from 0 (random spike distribution, e.g.) to 1 (all spikes occur at the same phase with respect to the stimulus). \( \text{VS}_{PP} \), on the other hand, ranges from -1 (all spikes 180 degrees out of phase with mean phase) to 1 (all spikes in phase with mean phase), where 0 corresponds to a random or circularly symmetric distribution as with VS. Yin et al. (2011) reported that, except in cases with low spike counts, there was very good agreement between VS and \( \text{VS}_{PP} \) measures. All temporal analyses were done using \( \text{VS}_{PP} \), except where otherwise noted.

To determine if a response phase-locked significantly, \( \text{VS}_{PP} \) values to each AM rate were compared to \( \text{VS}_{PP} \) calculations using spontaneous trials (see above) with an unpaired two sample t-test at a criterion level of \( p = 0.01 \). Neurons were considered synchronous if there was a significant \( \text{VS}_{PP} \) for at least one AM stimulus condition. These “synchronous” neurons were used in all following temporal analyses.

Temporal modulation transfer functions (tMTF) were constructed by calculating \( \text{VS}_{PP} \) across all 12 trials for each AM rate in each neuron. The temporal best modulation frequency (tBMF) was defined as the AM rate that elicited the maximum \( \text{VS}_{PP} \).

Joint BMF analyses. For neurons that were responsive to AM noise with respect to both firing rate and vector strength (\( \text{VS}_{PP} \)), rate and temporal BMFs for each neuron were used to plot joint BMF distributions. Cells with more than one BMF in either category (\( n = 12 \)) were excluded from this analysis.
RESULTS

We recorded the activity of 156 single units from primary auditory cortex (A1) of two geriatric macaque monkeys (mean age 26 years) with similar behavioral audiograms compared to young monkeys (see Figure 1 in (Juarez-Salinas et al., 2010). Old monkey responses to amplitude modulation (AM) frequencies of 4, 6, 8, 12, 16, 24, 32, 64, and 128 Hz were analyzed. These data are compared to 182 units collected from A1 of young macaques (mean age 8.5 years) 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 Figure 1: The left panel of Fig. 1 shows a raster plot of spiking responses to each trial sorted by the AM frequency with the lowest frequency analyzed (4 Hz) at the top and the highest frequency (128 Hz) at the bottom. Trial-averaged activity is shown in the right panel for the corresponding frequencies. Temporal and rate MTFs are shown in in the bottom panel in Fig. 1C and 1D, respectively. In this example, it is clear that the neuron can follow the envelope of the stimulus from 4 Hz 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 temporal MTF. There is a break-down 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 AMPLITUDE MODULATION

Given previous research that showed a deficit in temporal processing with age (Strouse et al., 1998; Snell and Frisina, 2000; Barsz et al., 2002; 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 for at least one AM stimulus (see Methods). Neurons were then categorized into four mutually exclusive groups: exclusively rate-responsive neurons
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(“FR only”), which were rate-responsive but did not synchronize to any AM stimulus; exclusively synchronous neurons (“VS only” or “VS$_{PP}$ 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 vector strength (VS) and the Rayleigh statistic (RS), or the trial-by-trial phase-projected vector strength (VS$_{PP}$).

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 (t-test, $p < 0.01$ with Bonferroni correction for 9 comparisons) for at least one AM rate. For the first temporal coding analysis, units were classified as synchronous if a significant vector strength was elicited for at least one AM rate (Rayleigh >17.7). Of 156 old A1 neurons analyzed, five 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 vector strength, 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 to 182 units from young monkeys and are summarized in Figure 2a. Chi-square analysis showed that the differences between young and old were statistically significant ($p < 0.0001$, df = 3). While 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 vector strength. Comparing the number of synchronous units between young and old monkeys, 118/151 (78.2%) units in old monkeys and 142/182 (78.0%) units in young monkeys yielded significant vector strength to at least one AM rate as determined by the Rayleigh statistic. This similarity in phase-locking between age groups is surprising given previous results of decreased temporal processing with age (Walton et al., 1998; Barsz et al., 2002; Walton, 2010). However, this method of
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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 phase projected vector strength ($VS_{PP}$) instead of the
standard vector strength (see 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 to spontaneous activity (two-sample t-test). Firing rate
responsiveness was determined as above. Using this criterion, 27 of 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 to spontaneous activity. These
results were compared to data from young monkeys using the same criteria and are shown in Figure 2b; as
before, classification results are significantly different between age groups ($X^2$ test, $p < 0.0001$, df = 3).

Using $VS_{PP}$ 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
to 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 versus 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 to young (17.0%) and slightly
more neurons were not responsive to AM in old vs. young neurons (17.9% vs 13.7%, old vs. young,
respectively). While 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 nonsynchronous 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 while 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 in order 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 Figure 3. Overall, fewer neurons showed firing rate suppression to at least one modulation frequency in old monkeys (14/113 = 12.4%) compared to young (31/142 = 21.8%). Only two neurons (1.8%) from the old monkeys showed suppressed firing rate to all AM stimuli, compared to 17 (12.0%) neurons in younger animals, while 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) to 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 while 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 primary auditory cortex neurons demonstrated significantly greater spontaneous firing rates and evoked firing rates in response to 200-ms noise bursts that varied in spatial location compared to neurons from young monkeys (Juarez-Salinas et al., 2010; Engle and Recanzone, 2012). We repeated this analysis for this experiment and compared spontaneous and driven
rates to values reported in Yin et al. (2011). Yin et al. (2011) 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 Figure 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 to young neurons. Meanwhile, synchronous neurons had 7.5x greater mean spontaneous and 6.9x greater mean evoked firing rates in old animals compared to young (refer to Tables 1.1 and 1.2). Overall, evoked and spontaneous firing rates are much higher in neurons from old compared to young A1, consistent with the results using shorter, unmodulated stimuli.

In addition to increased stimulus-evoked firing rates, Yin et al. (2011) also reported that spontaneous firing rates in young macaques were higher in synchronous cells (mean 7.8 spikes/sec, median 6.5 spikes/sec) than nonsynchronous cells (mean 5.5 and median 3.6 spikes/sec). Among rate-responsive units in old monkeys, mean and median driven firing rates for synchronous neurons were also greater (155.9 and 136.3 spikes/sec, respectively) than spike counts for nonsynchronous neurons (mean 86.5; median 48.2 spikes/sec). The same pattern is true of spontaneous firing rates for synchronous neurons (mean 58.5; median 43.7 spikes/sec) compared to non-synchronous neurons (mean = 24.0, median 12.9 spikes/sec). 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 1.1 and 1.2.

**POPULATION MODULATION TRANSFER FUNCTIONS**

The previous analysis noted that there were different firing rate distributions of synchronous and nonsynchronous neurons (Fig. 5), and a difference in spontaneous and evoked firing rates between young and old neurons (Fig. 4), but did not compare the population response to different modulation rates. We
therefore examined the relationship between the rate and temporal modulation transfer functions (MTFs) for each population of AM responsive neurons. The population temporal MTF (tMTF) was constructed across all units that had a significant VSPP for at least one AM rate compared to 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 about 60 Hz. Compared to 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 rate MTF (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 to 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, while 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 to 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, while the remaining aged neurons that retain the ability to
phase-lock to AM maintain similar signal-to-noise ratios compared to young.

\textit{AM Noise Tuning Functions and Best Modulation Frequencies 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. We therefore examined the relationship between population tuning functions in our
old neurons. In young animals, temporal and rate MTFs 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 vector strength ($\text{VS}_{pp}$) 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 modulation transfer functions (rMTF and
tMTF using $\text{VS}_{pp}$) for each neuron. Figure 7 shows the distributions of best modulation frequencies for
firing rate (rBMFs) and $\text{VS}_{pp}$ (tBMFs) for both young (7a) and old (7b) neurons. Rate and temporal BMFs
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 dissimilar in old.
To determine if this pattern holds up at the single cell level we plotted the joint distributions of rate and
temporal BMFs for each individual neuron. These joint BMF distributions are depicted in Figure 8. Here
we see a distinctively different pattern of BMFs in old neurons compared to young ones. Specifically, in
young neurons there is a dearth of BMFs at the higher modulation rates, particularly for the tBMFs. In
aged monkeys, the bulk of rBMFs are to the highest AM rate tested (29% of old rate-responsive cells had an rBMF of 128 Hz, see Fig. 7), as opposed to the lowest AM rate tested for the young animals. There is also a shift from the majority of neurons having similar tBMFs and rBMFs for the lower AM rates in young neurons, to a less organized and more scattered distribution of BMFs in older animals.

**DISCUSSION**

The goal of this study was to investigate whether there were age-related changes in neural processing of the acoustic envelope in primary auditory cortex, 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% amplitude modulated (AM) noise in aged macaques to 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 the phase-projected vector strength (VS<sub>pp</sub>). Using firing rate and VS<sub>pp</sub>, we found that neurons in aged macaque A1 were not less AM-responsive overall compared to 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”), while 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, higher evoked firing rates, and were less likely to decrease stimulus-evoked firing rates compared to neurons from young animals. These findings are consistent with previous support for an age-related decrease in inhibition in the auditory pathway (Caspary et al., 1995, 1999, 2008; Gray et al., 2014c). Finally, there was also a disorganization of the relationship between the rate and temporal modulation transfer functions as well as rate and temporal best modulation frequencies in aged animals, where neurons preferred higher AM rates based on firing rate in older monkeys compared to younger ones. These results suggest that the perturbed balance of excitation and inhibition with aging also affects
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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 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 & B, same as in the present study) were compared to 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 ~12 kHz. Note that noise thresholds were between 10 and 15 dB SPL for both young and old monkeys (Juarez-Salinas et al., 2010) and the present data were collected using stimuli at 65 and 60 dB SPL, respectively. While noise thresholds are not known for monkeys Y and V 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 & B were each measured at 30dB. Using this data, we can confirm that 60dB was well above detection threshold. Further, there is little difference between ABR waveforms for 60dB and 70dB (Ng et al., 2015). Neural activity in response to broadband noise is similar between 55dB and 75dB for monkeys aged 5-12 years across auditory cortex (Woods et al., 2006), further indicating that the 5dB difference in presentation level between age groups should not have had an effect of the results of the present study.

Rate vs. temporal coding in primary auditory cortex
We found significant changes in rate and temporal coding measures in old A1 responses compared to young that may have a profound effect on temporal envelope processing and perceptual performance.

Wang and colleagues 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 (Lu et al., 2001; Liang et al., 2002; Bartlett and Wang, 2007). Previous studies in aged rodents have shown that temporal coding of single neurons is diminished in the auditory midbrain (Walton et al., 1998; Simon et al., 2004; Walton et al., 2007; Barsz et al., 2002; Allen et al., 2003; Finlayson, 2002). 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). Further, 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, it could be that seemingly inconsistent results regarding rate and temporal coding in the primate auditory pathway (Lu et al., 2001; Liang et al., 2002; Malone et al., 2007; Yin et al., 2011) could be due at least in part by 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, while the population temporal MTF in young 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 primary auditory cortex (e.g., loss of PV+ and SOM+ 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, indicating an overall loss of tuning across the population (Fig. 6B). Nonsynchronous neurons in old had dramatically elevated normalized firing rates compared to young and a population rMTF more similar to synchronous population rMTF in young (Fig. 6B, 6C). While 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 are consistent with a decreased ability to process auditory temporal information as seen psychophysically in humans (Frisina and Frisina, 1997; Strouse et al., 1998; Snell and Frisina, 2000; Snell et al., 2002; Palmer and Musiek, 2014) 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 sub-cortical 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.

Given these changes, it is not surprising that there are differences in the relationships among population tuning functions and best modulation frequencies (BMFs) that may have meaningful implications for perception. Rate and temporal MTFs 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 (VS_{pp}) and firing rate best modulation frequencies (tBMFs and rBMFs) in the population of young (Figure 7A), but not old (7B) A1 neurons. While joint BMFs were not significantly coincident in young animals, there was a tendency for neurons to have rate and temporal BMFs 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 20Hz (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 these codes are communicated by the same population of neurons is very different between young and aged auditory cortex.
Niwa et al. (Niwa et al., 2012a, 2012b, 2013, 2015) showed that firing rate activity in A1 is correlated with choice behavior more-so than phase-locking activity; also 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 that indicate that phase-locking may be more important for initial encoding of stimulus features, while rate-coding is more important for decision and action based on those stimulus features. However, Niwa et al. suggest that dual rate and temporal coding of AM by A1 neurons may be necessary for processing of various stimulus parameters simultaneously. While 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 effectively process 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 (Gordon-Salant and Fitzgibbons, 2001; Snell et al., 2002; Füllgrabe et al., 2014).

Changes in excitatory/inhibitory balance in aged monkeys

Understanding 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 (Caspary et al., 2008). We recently described similar changes in the aged macaque, starting with decreased afferent drive from the cochlea (Engle et al., 2013) ascending through the cochlear nucleus (Gray et al., 2014a), superior olivary complex (Gray et al., 2014b), inferior colliculus (Engle et al., 2014) and thalamus (Gray et al., 2013). 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 (Caspary et al., 2008; Gray et al., 2014c). 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 inferior colliculus of mouse in response to AM noise (Walton et al., 2002) and spontaneous activity in the cochlear nucleus (Caspary et al., 2005, 2006). Other changes in mouse auditory brainstem 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 (Kim et al., 2002; Jacobson et al., 2003; 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 (Juarez-Salinas et al., 2010; Engle and Recanzone, 2012). Further, while there was little difference in spatial tuning found in A1 between young and old macaques, large differences were found in belt field CL. 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 to 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 Figure 6B, “both” neurons, n = 57 old), but there is a dramatic increase in excitability for the non-synchronous “rate only” population of neurons (see Figure 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 are 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 (Caspari et al., 2008; Gray et al., 2014c). 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 to 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 primary auditory cortex of the nonhuman primate. Specifically, not only do fewer neurons in old monkeys use both rate and temporal codes, 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, 2012b), 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.

ACKNOWLEDGMENTS

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REFERENCES


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FIGURE CAPTIONS

Figure 1

Example single neuron responses. A: Raster plot of a single unit showing spiking activity for each trial for each AM rate. B: Trial-averaged activity for each AM rate. Black line shows a moving window average of spikes over all trials (Δt of 20ms). Grey lines represent trial-to-trial variability in firing (standard deviation). C: Temporal MTF for this unit; mean phase-projected vector strength (VS_{PP}) is indicated in black and conventional vector strength (VS) in grey. Bold black stars indicate significant VS_{PP} values (t-test, \( p < 0.01 \)), and light gray stars indicate significant VS (Rayleigh > 17.7). Differences between the two metrics are subtle: according to VS with Rayleigh criterion this cell synchronized to AM rates up to 64 Hz and had a temporal BMF of 6 Hz; using this cell synchronized up to 32 Hz and had a tBMF of 4 Hz. D: Rate MTF for this unit shows significant firing rate response above spontaneous firing for all AM rates measured, and a rate best modulation frequency (BMF) of 128 Hz.

Figure 2

AM noise response categorization in single neurons in young and old monkeys. Units were classified as responsive to AM by only their firing rate (“FR only”), only the ability to phase-lock to the stimulus (“VS_{PP} only”), both metrics (“both”) or neither (“not AM sensitive”). A: Units were classified as synchronous using the Rayleigh statistic, a commonly used vector strength metric. B: Units were classified as synchronous if the phase-projected vector strength (VS_{PP}) values for stimulus evoked periods were significantly greater than VS_{PP} for spontaneous period (T-test, \( p < 0.05 \)). VS_{PP} was used for all further temporal analysis to be able to compare directly to Yin et al. 2011. While there was no difference in the proportion of AM responsive neurons between young and old (>80%; \( \chi^2 = 1.08, p = 0.30 \)), fewer older neurons had significant temporal responses to the stimulus (\( \chi^2 = 19.9, p < 0.0001 \)). Also, there was a decrease in the proportion of “both” synchronous and rate-responsive neurons, but no decrease in the proportion of “VS_{PP} only” neurons between young and old.

Figure 3

Percentage of neurons that increased or decreased firing rate in response to AM noise. A: Percentage of synchronous cells (corresponding to “both” in Figure 2B) that responded with an increase in firing rate to all AM noise stimuli (“all FR >= spont”, left) or decreased firing rate to at least one stimulus (“any FR < spont”, right). B: Percentage of nonsynchronous cells (corresponding to “FR only” in Figure 2B) that increased firing rate to all AM noise presentations or decreased firing rate to at least one stimulus. Fewer synchronous neurons decreased firing rate in response to AM noise compared to nonsynchronous neurons. Older neurons were less likely to show suppression in response to AM than younger neurons (\( \chi^2 \) test, \( p < 0.05 \)), particularly among synchronous neurons.

Figure 4
Distribution of spontaneous firing rates from older monkeys. Solid grey lines indicate mean and dashed grey lines indicate median spontaneous firing rate. 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/second) than nonsynchronous (mean = 24.0, median = 12.9 spikes/second; K-S test *p* < 0.0001).

Figure 5

Distribution of evoked firing rates for young and old synchronous and nonsynchronous AM-responsive neurons. Cells are categorized as in previous figures. A: Neurons synchronous to any AM stimulus (“both” in Fig. 2B). B: Neurons that did not synchronize to any AM stimulus (“FR only” in Fig 2B). Older neurons show significantly greater evoked firing rates than young neurons for both synchronous (10x greater, K-S test *p* < 10^-22) and nonsynchronous cells (3.8x greater, K-S test *p* < 0.0001). While 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.

Figure 6

Mean modulation transfer functions. 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).

Figure 7

Distribution of best modulation frequencies for firing rate (rBMFs) and phase-projected vector strength (temporal BMF or tBMF). A: Young monkeys (rBMFs, n = 160; tBMFs, n = 126). B: Old monkeys (rBMFs, n = 113; tBMFs, n = 68). Abscissae are drawn to the same log scale with modulation frequency of stimuli used in each group denoted on the respective axis.

Figure 8

Joint distribution of firing rate and phase-projected vector strength or temporal best modulation frequencies (rBMFs and tBMFs) for all rate responsive synchronous cells. A: Young monkeys (n = 71). B: Old monkeys (n = 108).
### Table 1.1

<table>
<thead>
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<th>Spontaneous firing rates (spikes/sec)</th>
<th>Nonsynchronous</th>
<th>Synchronous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>median</td>
</tr>
<tr>
<td>YOUNG</td>
<td>5.5</td>
<td>3.6</td>
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<tr>
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<td>24.0 (4.4x)</td>
<td>12.9 (3.6x)</td>
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### Table 1.2

<table>
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<tr>
<td></td>
<td>mean</td>
<td>median</td>
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<tr>
<td>YOUNG</td>
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<td>33.5</td>
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<tr>
<td>OLD</td>
<td>86.5 (5.6x)</td>
<td>48.2 (1.4x)</td>
</tr>
</tbody>
</table>
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**EQUATIONS**

Equation 1:

\[ VS = \sqrt{\left(\Sigma_{i=1}^{n} \cos \theta_i \right)^2 + \left(\Sigma_{i=1}^{n} \sin \theta_i \right)^2} ; \theta_i = 2\pi \left(\frac{t_i \mod p}{p}\right) \]

Equation 2:

\[ RS = 2n(VS^2) \]

Equation 3:

\[ VS_{pp} = VS_t \cos(\phi_t - \phi_c) \]

Equation 4:

\[ \phi = \arctan2 \left( \frac{\Sigma_{i=1}^{n} \sin \theta_i}{\Sigma_{i=1}^{n} \cos \theta_i} \right) \]
A  
\text{synchronous cells by Rayleigh}

\begin{itemize}
\item young
\item old
\end{itemize}

\begin{itemize}
\item FR only
\item VS only
\item both
\item not AM sensitive
\end{itemize}

\begin{itemize}
\item synchronous cells by VSpp
\item young
\item old
\end{itemize}

\begin{itemize}
\item FR only
\item VSpp only
\item both
\item not AM sensitive
\end{itemize}
A young, n=111
old, n=57

B young, n=31
old, n=56

synchronous cells

nonsynchronous cells

% synchronous neurons

% nonsynchronous neurons

all FR >= spont
any FR < spont

% synchronous neurons

% nonsynchronous neurons

all FR >= spont
any FR < spont
A synchronous cells ("both")

B nonsynchronous cells ("FR only")
**A**

**temporal MTF**

![Graph showing temporal MTF](image)

**vector strength**

- young
- old

**modulation frequency (Hz)**

2 8 32 128

---

**B**

**rate MTF (synchronous cells)**

![Graph showing rate MTF for synchronous cells](image)

**firing rate (evoked/spont)**

2 8 32 128

---

**C**

**rate MTF (nonsynchronous cells)**

![Graph showing rate MTF for nonsynchronous cells](image)

**firing rate (evoked/spont)**

2 8 32 128
A

Young BMFs

% significant neurons

fsr

VSpp

modulation frequency (Hz)

B

Old BMFs

% significant neurons

firing rate

VSpp

modulation frequency (Hz)
A

Young

rate BMFs

B

Old

temporal BMFs

%cells

11% 10% 9% 8% 7% 6% 5% 4% 3% 2% 1% 0%