Coding of Amplitude Modulation in Primary Auditory Cortex

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Abstract:
Conflicting results have led to different views about how temporal modulation is encoded in primary auditory cortex (A1). Some studies find a substantial population of neurons that change firing rate without synchronizing to temporal modulation, while other studies fail to see these non-synchronized neurons. As a result, the role and scope of synchronized temporal and non-synchronized rate codes in amplitude modulation (AM) processing in A1 remains unresolved.
We recorded A1 neurons’ responses in awake macaques to sinusoidal AM noise. We find most (37-78%) neurons synchronize to at least one modulation frequency (MF) without exhibiting non-synchronized responses. However, we find both exclusively non-synchronized neurons (7-29%) and "mixed-mode" neurons (13-40%) that synchronize to at least one MF and fire non-synchronously to at least one other. We introduce new measures for modulation encoding and temporal synchrony that can improve the analysis of how neurons encode temporal modulation. These include comparing AM responses to the responses to unmodulated sounds, and a vector strength measure that is suitable for single-trial analysis. Our data support a transformation from a temporally-based population code of AM to a rate-based code as information ascends the auditory pathway. The number of mixed-mode neurons found in A1 indicates this transformation is not yet complete, and A1 neurons may carry multiplexed temporal and rate codes.

Keywords:
Audition, Synchronization, Awake
INTRODUCTION

Amplitude modulation (AM), the change over time of a sound’s amplitude envelope, is an important information-bearing parameter carried by communication sounds such as syllabic features in speech (Nelken et al. 1999; Steinschneider et al. 1999; Shannon et al. 1995; Füllgrabe et al. 2009; Drullman et al. 1994) and is thought to be of particular use in segregating sound sources during auditory scene analysis (Bregman 1990; Yost 1991; Hu and Wang 2004). AM also can carry important information about pitch and has been used to investigate how pitch relates to temporal as opposed to spectral aspects of the stimulus (Burns and Viemeister 1976, 1981;). The sound amplitude envelope has been treated as a fundamental property of sound (Attias and Schreiner 1997, 1998; Joris et al. 2004; Smith et al. 2002), and in marmoset AI some neurons only respond to tones if they are amplitude modulated (Liang et al. 2002), suggesting that this is a feature highly selected upon by the auditory system. There is a great deal known about neuronal responses to temporally modulated sounds throughout the auditory system in a number of species (e.g. Creutzfeldt et al. 1980; Rose and Capranica 1985; Langner and Schreiner 1988; Frisina et al. 1990; Preuss and Müller-Preuss 1990; Joris and Yin 1992; Eggermont 1994; Grothe 1994; Burger and Pollak 1998; Kuwada and Batra 1999; Caspary et al. 2002; Klump et al. 2004; Nelson and Carney 2007; Zheng and Escabí 2008; reviewed in Joris et al. 2004), including humans (Brugge et al. 2009). In this paper we will resolve some outstanding issues in the processing of AM in non-human primates.

Studies using AM and other time-varying stimuli consistently suggest that the ability of cells to phase lock to high frequency stimuli declines at successive auditory stages (Joris et al. 2004; Rhode and Greenberg 1994; Krishna and Semple 2000; Schreiner and Urbas 1988; Creutzfeldt et al. 1980). Auditory nerve fibers will phase lock to envelopes as high as 3-4 kHz,
but in cochlear nucleus phase-locking cutoffs lie between 750-1500 Hz (Rhode and Greenberg 1994; Zhao and Liang 1997). In the inferior colliculus (IC) the phase-locking limit is reduced to 500 Hz, with a majority of cells losing the ability to follow the envelope above 200 Hz (Rees and Palmer 1989; Krishna and Semple 2000). Studies using AM in auditory thalamus are rare, but suggest peak phase locking sensitivity below 50 Hz and phase-locking limits on the order of 100 Hz (Preuss and Müller-Preuss 1990). Using click trains in the MGB, phase-locking limits were between 100 and 300 Hz with peak sensitivity between 25 and 125 Hz (Rouiller et al. 1981; Bartlett and Wang 2007).

In cortex, phase-locking cutoffs are somewhat variable. Anesthetized preparations generally yield synchronization cutoffs below 50 Hz (Schreiner and Urbas 1986, 1988; Eggermont 1991; Gaese and Ostwald 1995) while awake preparations can show synchronization cutoffs up to and beyond 100 Hz (Bieser and Müller-Preuss 1996; Lu et al. 2001; Liang et al. 2002; Anderson et al. 2006; Malone et al. 2007; Fitzpatrick et al. 2009). Despite this, most cortical neurons only strongly phase lock to lower modulation frequencies with a median synchrony cutoff usually in the 10-30 Hz range (e.g. Ter-Mikaelian et al. 2007; Fitzpatrick et al. 2009). More recently at higher levels of the marmoset auditory system, starting in A1, Lu et al (2001) report finding a population of non-phase-locking (non-synchronized) neurons tuned for modulation frequency. These results have led to the hypothesis that the creation of distinct synchronized and non-synchronized populations of neurons is an emergent property of A1 (Lu et al. 2001, Liang et al. 2002; Bartlett and Wang 2007), and support a transformation from a temporal code at lower levels of the auditory system to a rate-based code in higher areas. The finding of non-synchronized neurons, however, has not been verified outside of marmosets, and a recent study in macaques fails to find a population of such neurons (Malone et al 2007). This
leads to the possibility that non-synchronized neurons in A1 are unique to marmosets. We hypothesize that it is not species differences that lead to these differing reports but rather other experimental factors.

We examined the responses of A1 neurons, in awake macaque monkeys, to sinusoidal amplitude modulated (AM) wideband noise. Modulation frequency tuning was narrower in neurons that did not exhibit any phase locking, but remained broad overall. We found both synchronized and non-synchronized neurons, as well as neurons that share both characteristics. Contrary to previous studies, we did not find strong evidence that synchronized and non-synchronized neurons comprise independent populations, and we suggest that both response types may be simultaneously present in the same neuron to a varying degree, as has been reported previously in thalamus (Bartlett and Wang 2007) and IC (Zheng and Escabí 2008).

METHODS

Overview

The general methods (surgical procedures, animal care, etc,) were similar to those described in previous studies (O’Connor et al. 2005). A general summary of the methods follows with any differences in methods explained in more detail.

Subjects

All procedures conformed to the PHS policy on experimental animal care and were approved by the UC Davis animal care and use committee. Experiments were performed on two (one male, one female) adult rhesus monkeys (Macaca mulatta) weighing 6-8 kg. The monkeys sat in an acoustically “transparent” primate chair (custom made, Crist Instruments) in a double-
walled, sound-attenuated, foam-lined booth (IAC: 9.5’ x 10.5’ x 6.5’) during physiological recording. They received diluted fruit juice or water during daily recording sessions, and monkey chow, fruits and supplementary water in their individual home cages. Each monkey was implanted with a head post and recording chamber for chronic access to auditory cortex. Recordings were made while the monkeys sat quietly in the primate chair in the sound booth with the head restrained, with diluted juice or water given intermittently. A plastic grid (Crist Instruments) was fit into the recording chamber to guide the electrode penetrations. High-impedance tungsten microelectrodes (FHC) were driven into the cortex by a remotely controlled hydraulic microdrive (FHC), through guide tubes held by the plastic grid.

Stimulus Generation and Data Collection

The sinusoidal amplitude modulated (AM) stimuli had a “frozen” broadband noise carrier and were 400 ms in duration. Neurons at each recording site were assessed with an unmodulated noise burst and 100% modulation depth AM noise (Fig. 1A) at seven different modulation frequencies (5, 10, 15, 20, 30, 60 and 120 Hz), for a total of 8 stimuli. Each stimulus was presented fifty times. For each repetition, the entire set of eight stimuli was presented in random order, without replacement, before the next repetition began.

The sound signals were generated by a digital signal processor (AT&T DSP32C) and a digital-to-analog converter (TDT Systems DA1). They then passed through a programmable attenuator (TDT Systems PA4), and a passive attenuator (Leader LAT-45). The signal was amplified (Radio Shack MPA-200) before being delivered to a speaker (Radio Shack PA-110, 10-in. woofer and piezo-horn tweeter, 0.038-27 kHz) positioned at ear level 1.5 m in front of the subject.
The auditory stimuli were presented at a sampling rate of 50 kHz and were cosine ramped at onset and offset (5.0 msec rise/fall time). Stimulus intensity was adjusted to ~65 dB SPL (<2 dB variation). Extracellular potentials were amplified and filtered (0.3-5 kHz; AM Systems 1800), sampled at 50 kHz, and were stored on hard disk for later analysis. Spikes were re-sorted offline using SPIKE2 software (CED).

Location of Recording

The determination that our recordings were in A1 was based on the tonotopic gradient and latency information (maps in Supplementary material) obtained from physiological recording as well as post-mortem histology. The frequency tuning at each recording site was assessed by using a set of pure tones with a combination of frequencies and intensities. In frequency, this set spanned a three-octave range with 1/5 octave increments around a center frequency that was estimated by hand-tuning. In intensity the set spanned 63 dB with a 7 dB increment between 15-78 dB SPL for a total of 150 combinations of frequency and intensity. Tone duration was 0.1 second. During data collection, the 150 tone combinations were presented in a random order and repeated at least 5 times. The neuronal responses to each combination were represented by the average spike counts within the first 75 ms window following stimulus onset. A two-dimensional response matrix (intensity x frequency) was obtained from these responses. The neuron’s frequency tuning curve was estimated by the contour line at the mean spontaneous response (spike count in a 75 ms window before the onset of each frequency-intensity combination) plus two standard deviations using Matlab’s “contourc” function. The best frequency (BF) and threshold were determined from the obtained frequency tuning curve.
Upon termination of the experiments electrode locations were marked at several key border points, in one animal with tracking lesions (Monkey Y) and in another by inserting an electrode dipped in biotinylated dextran amine (Monkey V). One hour later the animal was given an overdose of sodium pentobarbital and perfused with 4% paraformaldehyde in 0.1M phosphate buffer. In the monkey with the tracking lesions the perfusion was poor, leading to difficulty in lesion identification. In the monkey with the biotinylated dextran markers track reconstruction was reliable. Examination of Monkey V’s histology confirmed that all of the recordings took place in A1. In monkey Y, due to the bad perfusion we were forced to rely on tonotopic gradient and latency information exclusively. There was a large low frequency border between A1 and more rostral fields (most likely R), so we defined a border which conservatively estimated the extent of A1. The other recordings, which could belong to A1 or R, are excluded from the analysis. A comparison between A1 and the border area is included in the supplementary material.

Data Analysis

Modulation Transfer Functions

Modulation transfer functions (MTFs) were determined for 182 A1 neurons using both a spike rate measurement (spike count, rMTF) and a measure of temporal phase locking (phase-projected vector strength, tMTF) at each modulation frequency tested. Phase-projected vector strength ($VS_{PP}$, see next section below) is a variation of vector strength that allows trial-by-trial calculations of $VS$ without the problems contributed by low spike counts. For both rMTFs and tMTFs, the value reported at each modulation frequency is the mean of the trial-by-trial values. All calculations were made using only spikes from 70-400 ms post-stimulus onset to exclude
onset responses (the same general pattern of results holds if onsets are included). A cell was considered AM-sensitive if the response to at least one modulation frequency was significantly different than the response to the unmodulated noise using either rate or temporal measures (see below for statistical details). The best modulation frequency (BMF) of a cell was defined as the modulation frequency that resulted in the largest mean spike count or mean phase-projected vector strength ($VS_{PP}$).

Vector Strength and Phase-Projected Vector Strength

The standard, non-phase-projected formula for vector strength is

$$VS = \sqrt{\frac{\sum (\cos(\theta_i))^2 + (\sin(\theta_i))^2}{n}}$$

(1)

where $VS$ is the vector strength, $n$ is the number of spikes over all trials, and $\theta_i$ is the phase of each spike in radians, calculated by

$$\theta_i = 2\pi \frac{t_i \mod p}{p}$$

(2)

where $t_i$ is the time of the spike in ms relative to the onset of the stimulus and $p$ is the modulation period of the stimulus in ms (Goldberg and Brown 1969, Mardia and Jupp 2000). One weakness of the standard $VS$ measure is that it may give spuriously high values at low firing. This can be a problem when using single trial measurements for statistical purposes. An example of this is illustrated in Fig. 1B where at high modulation frequencies (especially 30 and 60 Hz in this example) low spike counts lead to high vector strength values (dotted line). Another shortcoming of $VS$ is that because it is usually calculated on the summed or averaged cycle
histogram in order to perform statistical tests various assumptions have to be made, most of
which confound firing rate with phase locking (e.g., Rayleigh test of uniform distribution,

To avoid these two problems, phase-projected vector strength ($VS_{pp}$) was used. Conceptually, $VS_{pp}$ compares the mean phase angle for each trial with the mean phase angle of all trials at that MF and penalizes single-trial $VS$ values if they are not in phase with the global response. $VS_{pp}$ was calculated on a trial-by-trial basis as follows:

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

(3)

where $VS_{pp}$ is the phase-projected vector strength per trial, $VS_t$ is the vector strength per trial, calculated as in Eq. 1, and $\phi_t$ and $\phi_c$ are the trial-by-trial and mean phase angle in radians, calculated for each stimulus condition

$$\phi = \text{arctan2}\left(\frac{\sum_{i=1}^{n} \sin \theta_i}{\sum_{i=1}^{n} \cos \theta_i}\right)$$

(4)

where $n$ is the number of spikes per trial (for $\phi_t$) or across all trials (for $\phi_c$) and arctan2 is a modified version of the arctangent that determines the correct quadrant of the output based on the signs of the sine and cosine inputs (Matlab, $\text{atan2}$). For all $VS_{pp}$ calculations, a cell that fired no spikes was assigned a $VS_{pp}$ of zero, but there was no minimum spike count because the condition-wide mean phase acted as an external phase reference. Whereas $VS$ may range from 1 (all spikes occur at the same stimulus phase) to 0 (spikes times occur in any circularly symmetric pattern including random with regard to stimulus phase), $VS_{pp}$ may range from 1 (all spikes in phase with the population mean phase) to $-1$ (all spikes 180 degrees out of phase with population mean phase) with 0 corresponding to random or circularly symmetric phase with regard to the
population mean phase. Except for the cases where there were low spike counts (e.g. Fig. 1B),
the two VS measures were in good agreement. In all examples we show both for reference, but
except where otherwise noted, all statistical analysis was done using VS\textsubscript{PP}.

To measure the reliability of a neuron to follow every cycle of modulation we calculated
cycle-by-cycle vector strength (VS\textsubscript{CC}, gray line in Fig. 1B). This measures how reliably a neuron
follows the stimulus as well as how precise the timing of firing is. This is contrasted with VS\textsubscript{PP}
which only quantifies timing precision but not reliability. VS\textsubscript{CC} is calculated in a similar fashion
to VS\textsubscript{PP} except it is calculated on a cycle-by-cycle basis rather than on the cycle histogram. One
VS\textsubscript{PP} value is calculated for each cycle of the stimulus and then all these values are averaged
together to arrive at VS\textsubscript{CC} for a given trial. On any cycle where no spike is fired a value of 0 is
used.

\textit{Statistical Testing}

To determine whether a neuron’s response was influenced by AM, two-tailed \textit{t}-tests were
performed comparing the distributions of either trial-by-trial spike count (SC) or trial-by-trial
VS\textsubscript{PP} at each modulation frequency against the same measure for an unmodulated noise burst. It
is important to note that this tests whether the responses can distinguish AM from its
unmodulated carrier and not whether the neuron responds to AM sounds. (Note that the VS\textsubscript{PP}
measure is fundamentally tied to a modulation frequency. When we refer to the VS\textsubscript{PP} of an
unmodulated stimulus, this is a control measurement made assuming the same modulation
frequency as the corresponding test stimulus – instead of a single VS\textsubscript{PP} value for the unmodulated
stimulus, the value depends on the modulation frequency of the experimental group under
investigation. Control measures of VS\textsubscript{PP} are not always zero because some cells exhibit
temporally structured firing to unmodulated stimuli.) To determine if a neuron responded to AM sounds, SC distributions were also compared against spontaneous firing (100-ms pre-stimulus, collected across all trials regardless of stimulus type). All t-tests were performed with \( p < 0.05 \) after Bonferroni correction for 7 comparisons (one for each modulation frequency) per cell.

We also determined whether there was significant synchronization relative to a random distribution of spikes without regard to a comparison distribution of the unmodulated sound. This was accomplished with the Rayleigh statistic (Mardia and Jupp 2000), which evaluates whether the cycle histogram (time histogram relative to each period of modulation) significantly differs from a flat distribution in time:

\[
RS = 2\pi(VS_{A}^2)
\]

(5)

where \( RS \) is the Rayleigh statistic and \( VS_{A} \) is a single vector strength value calculated over all trials. In all cases we considered \( RS \geq 17.7 \) to be statistically significant, which corresponds to \( p < 0.001 \) after Bonferroni correction for 7 comparisons per cell (this differs from the 13.8 that is usually used when there is no multiple comparison correction).

Because Bonferroni correction controls the experiment-wide error rate (i.e. the probability that any null hypothesis is falsely rejected) it becomes very conservative for high numbers of multiple comparisons. Consequently, we used the False Discovery Rate (FDR, Benjamini and Hochberg 1995) method, which for large numbers of neurons more accurately determines the number of significant neurons in a population while correcting for multiple comparisons.
Joint Distribution Analysis

We performed a joint distribution analysis to determine whether the rate BMF (rBMF) and temporal BMF (tBMF) were related within cells. To test the hypothesis that the rBMF and tBMF measures coincided, we designed an ad hoc Monte Carlo permutation analysis (100,000 permutations). We first determined the observed count of cells that had coincident rBMF and tBMF \( (o_c) \) and the observed count of cells where the rBMF and tBMF were adjacent (e.g. rBMF = 20, tBMF = 30, \( o_a \)) from the joint distribution \( o_{ij} \). Then, for each repeat a random joint distribution \( \hat{o}_{ij} \) was created by randomly pairing (without replacement) the observed rBMF and tBMF classifications, from which we determined the randomized coincident count \( \hat{o}_c \) and the randomized adjacent count \( \hat{o}_a \). The p-value of this analysis was taken as the probability that \( \hat{o}_c \) exceeded \( o_c \). Because the BMFs of band-reject cells may not indicate the regions of greatest sensitivity, we restricted our analysis to cells that had both rMTFs and tMTFs classified into low-pass, band-pass, or high-pass categories (a single peak in the MTF that was significantly different than the minimum), a total of 71 cells.

Bandwidth Analysis and Fitting Procedure

For the bandwidth analysis, we used three different functions to fit the MTFs, a logistic (sigmoid) function (Eq. 6), a Gaussian (Eq. 7), and a log-transformed Gaussian (Eq. 8).

\[
y = \alpha + \beta \cdot \frac{e^{-\frac{(x - \mu)^2}{2\sigma^2}}}{\sigma \sqrt{2\pi}}
\]

(6)

\[
y = \alpha + \beta \cdot \frac{e^{-\frac{(x - \mu)^2}{2\sigma^2}}}{\sigma \sqrt{2\pi}}
\]

(7)
All three functions have four free parameters determining the y-offset ($a$), the height ($b$), the x-center ($\mu$), and the slope ($s$). Fitting was performed using Matlab’s “fmincon” function.

Constraints on the parameters were set as follows:

Logistic: $a \geq 0; b \geq 0; b \leq 1.3 \cdot (\text{max-min of data}); \mu \geq 0; -33 \leq s \leq 33$. The constraint on the height parameter prevented the fit from extrapolating too far beyond the observed data. A negative slope parameter would allow the logistic to fit a high-pass MTF rather than a low-pass MTF, but this was not observed.

Gaussian: $a \geq 0; b \leq 1.3 \cdot (\text{max-min of data}); \mu \geq 0; 3 \leq s \leq 50$. The slope factor restricted the full width at half height (FWHH) of the Gaussian fit to lie between about 7 and 120 Hz.

Log-Transformed Gaussian: $a \geq 0; b \leq 1.3 \cdot (\text{max-min of data}); \mu \geq 0; s \leq 2.36$. The slope factor restricted the FWHH of the log-transformed Gaussian fit to a maximum of about 8 octaves.

For each MTF, a fit was attempted for all three curves and the significance of each fit was calculated. If no fit was significant at the $p < 0.01$ level, all fits were rejected. Otherwise, of the significant fits the one with highest correlation coefficient value was selected. For logistic fits, a high-pass cutoff was calculated as the half-height point of the curve. For both Gaussian fits, low-pass and high-pass cutoffs were selected as the two half-height points on the curve, and the bandwidth was calculated as the full width at half height (FWHH). Low-pass cutoff and bandwidth values were rejected for any Gaussian fit where the low-pass cutoff was less than zero. Additionally, in cases where the MTF value at 5 Hz was either the largest value in the
MTF or was within 90% of the full height of the fit, we felt that there was insufficient evidence of a reduction in response at low frequencies to justify a Gaussian fit. In these cases, we accepted the sigmoid fit when the sigmoid fit was significant at a 0.01 level, regardless of the significance of the Gaussian fits. If the sigmoid fit was not significant, we calculated a high-pass cutoff (but no low-pass cutoff or bandwidth values) from the most significant Gaussian fit ($p < 0.01$ required). For most MTFs there was no major difference between the Gaussian fit and the log-Gaussian fit. The general difference is in the tails – the log-Gaussian has a heavier right-hand tail while the regular Gaussian has a heavier left-hand tail. However, the log-transformed Gaussian was used because for some MTFs the log-Gaussian fit was notably better than the Gaussian fit – generally in cases where the Gaussian fit failed to appropriately capture the floor of the MTF.

Fourier Transform of Population Spike Train

To reduce low frequency noise when investigating frequency aspects of the spike train, the overall spike train (across all trials, 70 ms onset removed) was binned into 10 µs bins and convolved with a Gaussian of the same resolution with $\mu = 0$, $\sigma = 0.33$, and a total width of 3 ms. The result of the convolution was then analyzed via FFT.

RESULTS

Data Set

Responses to the full set of modulation frequencies were determined for 182 isolated single neurons recorded from two awake macaque monkeys. Of those 13 neither phase locked significantly (Rayleigh test with Bonferroni correction) nor yielded spike counts (SC)
significantly different than spontaneous or the unmodulated noise (t-test with Bonferroni correction), yielding 169 neurons that responded to at least one sound in the stimulus set.

Although there was a tendency for both spike-count (rate) and phase-locked (vector strength, temporal) based response measures to prefer low modulation frequencies and for responses to span a broad range of modulation frequencies, a large variety of response properties were encountered. We also saw a mixture of synchronized and non-synchronized response properties often observing both within an individual neuron.

The results can strongly depend on the metrics used. One difference in metrics is whether significance of phase locking is determined by the Rayleigh statistic or trial-by-trial $VS_{pp}$. The Rayleigh statistic, which has traditionally been used to test significance of phase locking, has some shortcomings that lead to high sensitivity and high false positives. Trial-by-trial $VS_{pp}$ is less sensitive so might underestimate phase locking, but is less likely to result in falsely identifying phase locking (See Discussion). Another important distinction is whether AM responses are compared to spontaneous activity or to the responses to unmodulated sounds. This is not a trivial distinction because a cell whose firing rate differs from spontaneous activity is able to signal the presence of an auditory stimulus relative to no event, while a cell whose firing rate differs from the firing rate in response to an unmodulated stimulus is able to signal the presence of modulation in that stimulus.

Synchronized and exclusively non-synchronized neurons: prevalence

To compare across studies we divided our cells into synchronized and exclusively non-synchronized categories. We defined a cell to be synchronized if it exhibited significant phase
locking to at least one of the tested modulation frequencies, and exclusively non-synchronized if it exhibited a significant change in firing rate without significant phase locking.

Examples of exclusively non-synchronized neurons are shown in Fig. 2. The neuron of Fig. 2A responded to unmodulated noise with a firing rate significantly greater than the spontaneous firing rate. The response to 5 Hz modulation was similar to the response to unmodulated noise. Between 10 and 30 Hz the neuron fired at levels significantly below that evoked by the unmodulated noise. At high frequencies (60 and 120 Hz), non-synchronized increases in activity emerge that are absent for middle frequencies, and the temporal structure of these responses appears to be different than that to unmodulated noise. The neuron of Fig. 2B shows non-synchronized responses that were strongest at the lowest modulation frequencies and declined as modulation frequency increased. We found this pattern of declining, exclusively non-synchronized response in 8% of our neurons (15/182). The increase in response at 5 Hz, and decreases at 60 and 120 Hz, were significantly different from that to unmodulated noise, and, except for 120 Hz, all responses were greater than the spontaneous level. Depending on whether the Rayleigh statistic or $V_{SP}$ are used to define synchrony and whether spike counts are compared against spontaneous or unmodulated activity, 4-20% of A1 neurons were classified as exclusively non-synchronized.

Most neurons synchronized to at least one modulation frequency. Some synchronized well to all modulation frequencies tested (Fig. 3A). In this example cycle-by-cycle $VS (V_{S_{CC}})$ decreases at higher modulation frequencies, indicating that the neuron fires precisely but not reliably to the modulation cycle. Other neurons phase locked to low frequencies and then had non-synchronized responses at higher modulation frequencies (Fig 3B).
Figure 4 shows the results of the categorization when synchrony is measured by comparing $VS_{pp}$ distributions between modulated sounds and the unmodulated carrier controls (see Methods for an explanation of the calculation of $VS_{pp}$ on unmodulated sounds) with a Bonferroni correction for multiple comparisons. When comparing *spike counts to spontaneous activity* (Fig. 4A) 31 (17% of total) neurons were *exclusively* non-synchronized – showing significant changes in *spike count* from *spontaneous* activity but no significant phase locking. There were 15 (8%) exclusively *synchronized* neurons – showing significant phase locking but no change in firing rate from spontaneous. Another 111 (61%) neurons both changed their *spike count* and phase locking significantly relative to spontaneous. When comparing *spike counts to the unmodulated noise carrier* (Fig. 4C) 37 (20%) neurons were exclusively non-synchronized, there were only 3 (2%) exclusively synchronized neurons, and 123 (68%) neurons changed both their *spike count* and phase locking significantly relative to the unmodulated noise response.

Using the Rayleigh statistic to define synchrony resulted in a substantial increase in the reporting of synchrony. Relative to spontaneous and the response to the unmodulated noise carrier control, only 7 (4%) and 12 (7%) neurons were exclusively non-synchronized using the Rayleigh statistic while the number of neurons showing synchronized responses increased to 156 (86%).

### Synchronized and exclusively non-synchronized neurons: coding with increases and decreases

Neurons encode modulation with either increases or decreases in activity, but the manifestation of these spike-count based results are different for synchronized and exclusively non-synchronized neurons. This is at least partially because response to the unmodulated carrier tended to be greater than spontaneous, so that an apparent increase in activity relative to
spontaneous for AM might actually be solely due to the response to the carrier rather than the modulation. We find that for non-synchronized neurons both decreases and increases in activity were commonly seen whether analyzed relative to the unmodulated noise response or spontaneous activity. Synchronized neurons, on the other hand, encoded modulation with both decreases and increases in activity relative to the unmodulated noise carrier control, but they primarily increased activity relative to spontaneous for encoding an event (Fig. 4B, D).

Relative to spontaneous, 142 neurons (78%) signaled the presence of a stimulus with a significant change in firing (t-test with Bonferroni correction for seven comparisons; Fig. 4A, SC significant). For exclusively non-synchronized neurons, 2 increased activity at one MF and decreased at another, 20 increased activity at some MFs and never decreased, and 9 decreased at some MFs without increases (Fig. 4B, white hatched bars). The majority of the synchronized neurons showed an increase in firing rate relative to spontaneous with a much smaller number showing decreases (91 increased, 8 decreased, and 12 did both depending on MF, Fig. 4B gray hatched bars). Therefore for non-synchronized neurons both decreasing and increasing codes relative to spontaneous are common, but for synchronized neurons increases in spike counts relative to spontaneous were much more likely than decreases.

When responses to AM were compared to responses to the unmodulated carrier, different results were obtained for the synchronized neurons. Unlike the coding of the presence of a stimulus where synchronized neurons were more likely to increase firing rate relative to spontaneous, when looking at modulation coding the majority of synchronized neurons decreased firing rate relative to the unmodulated carrier (Fig. 4D, gray hatched bars, 55 neurons decreased and 39 increased, and 29 both depending on MF). Non-synchronized neurons were also more
likely to decrease relative to the unmodulated carrier (Fig. 4D, white hatched bars, 23 neurons decreased and 10 increased, and 4 both depending on MF).

A few other properties shed light on these differences. It might be that synchronized neurons have lower spontaneous rates, and a floor effect is preventing them from firing significantly below spontaneous. This is not the case; in fact both the mean and median spontaneous rates are higher for synchronized neurons (7.8 and 6.5 spikes per second) than non-synchronized neurons (5.5 and 3.6 spikes per second). Another possibility is that the carrier is primarily excitatory relative to spontaneous and rarely drives the neurons below spontaneous. This appears to be the case. We found that 6/182 neurons’ responses to the unmodulated carrier significantly decreased relative to spontaneous and 105/182 had significantly increased activity relative to spontaneous (t-tests). This suggests that changes in activity that are observed relative to spontaneous for synchronized neurons might be more reflective of responsiveness to the carrier rather than the modulation.

Synchronized and exclusively non-synchronized neurons: Coding both an event and AM

A single neuron’s response is more informative if it is able to distinguish a modulated stimulus from both the unmodulated carrier and from no sound (spontaneous). For this to be meaningful it must happen for the same stimulus (i.e., be measured at the same modulation frequency). A total of 116 of our neurons were capable of doing this, but very few of them (17, 46% of the exclusively non-synchronized neurons) were exclusively non-synchronized. For the synchronized neurons a total of 99 neurons (79% of the synchronized neurons) had the same property. These proportions were significantly different (p = 0.001, z-test for independent proportions). This result suggests that if individual neurons in A1 are specifically encoding the
presence of an AM stimulus at or below 120 Hz, it is achieved chiefly through synchronized
neurons.

Synchronized and exclusively non-synchronized neurons: Modulation frequency tuning

Tuning for modulation frequency tended to be broad (Fig. 5A). Because of the small
number of neurons that had a significant Gaussian fit in combination with the small number of
non-synchronized neurons, statistical power for comparing bandwidth would be very low. To
improve the statistical power for this analysis only we pooled the 182 conservatively defined A1
neurons with 50 neurons on the A1/R border (these border neurons tend to have low BFs, see
Supplemental Data for more on these neurons). Relative to the unmodulated carrier, the mean
rate bandwidth of cells that were exclusively non-synchronized was 1.59 octaves (11 cells with a
defined bandwidth), while the mean rate bandwidth of synchronized cells (65 cells with a
defined bandwidth) was 2.67 octaves ($t$-test, $p = 0.03$), indicating that exclusively non-
synchronized cells have narrower bandwidths. It should be noted that neither within
conservatively defined A1 nor the border region did these differences reach significance,
suggesting that this effect is not due solely to one of the two regions. The mean temporal
bandwidth based on $VS_{PP}$ was 2.13 octaves (67 cells with significant fits), which was not
significantly different than the bandwidth of non-synchronized neurons but significantly less
than the rate-based code for synchronized neurons. To aid in comparison to other studies the
bandwidths measured relative to spontaneous for non-synchronized, rate-synchronized and $VS_{PP}$-
synchronized is 1.16, 2.67, 2.13 respectively, and all comparisons are significantly different ($t$-
test, $p < 0.05$).
Relationship of temporal and rate based best modulation frequencies (BMFs)

For both temporal and rate measures, BMFs were more commonly found at low than at high frequencies (Fig 5B). BMFs >= 60 Hz were more common for rate (31/160, 19%) than temporal measures (6/126, 5%, p = 2.8 x 10^{-5} proportion test).

We asked whether rate and temporal BMFs were coincident by performing a joint distribution Monte Carlo analysis (See Methods). The observed joint distribution of BMF is depicted in Fig. 5C. For both coincident BMFs (e.g., rBMF = 60 Hz and tBMF = 60 Hz) and adjacent BMFs (e.g., rBMF = 30 Hz and tBMF = 20 Hz) we were unable to reject the null hypothesis of independent distribution of BMF (coincident p = 0.41, adjacent p = 0.14). This result does not support the prediction that rBMF and tBMF are closely related.

Mixed synchronized/non-synchronized neurons

We encountered many neurons that appeared to have both synchronized and non-synchronized responses (e.g. Fig. 3B, also Fig 7B). As one estimate of the size of this mixed-mode population, we counted the number of cells that exhibited significant phase locking at one frequency, and a significant non-synchronized increase in firing rate at a different modulation frequency. We looked at increases in firing rate so as to exclude cells whose firing rate at some modulation frequencies is suppressed to near zero, because synchrony cannot be validly measured in these cases. This method would necessarily exclude any cells with significant non-synchronized decreases, so our estimate of the mixed-mode population may be low. Using VS_{pp} as our measure of phase locking, we found 82 cells (45%) that showed this pattern when firing rate was compared against spontaneous activity and 37 cells (20%) when compared against an unmodulated stimulus. A total of 32 cells (18%) had a non-synchronized firing rate to at least
one modulation frequency that was higher than that to both unmodulated noise and the spontaneous rate, but also had significant phase locking to at least one different modulation frequency. Altogether, we found 87 cells (48%) with mixed-mode responses – capable of using a temporal code to signal the presence of modulation at one frequency and a non-synchronized rate code to signal the presence of a stimulus and/or modulation at another frequency.

A previous study using click trains in marmosets (Lu et al. 2001) found that neurons segregated neatly into two categories based on phase locking at low modulation frequencies and spike count at high modulation frequencies. This appears to differ from our finding of mixed synchronized/non-synchronized responding neurons. To facilitate comparison with the previous study, we developed a metric similar to the one used there. In Fig. 6 we show the relationship between phase locking at low frequencies (best $VS_{pp} \leq 30$ Hz) and a cell-normalized measure of firing rate at high frequencies (standard deviations above spontaneous firing rate, $\geq 60$ Hz) for all 182 cells. Cells plotted in black showed significant phase locking for at least one modulation frequency ($Rayleigh statistic > 17.7, p < 0.001$ after correction for 7 comparisons per cell) while those plotted in gray did not.

If our cells segregated into previously reported synchronized and non-synchronized categories, we would expect the non-synchronized cells (gray) to cluster in the lower right (weak phase locking at low modulation frequencies, increased firing at high modulation frequencies) and the synchronized cells (black) to cluster towards the upper left (strong phase locking at low modulation frequencies, low firing rates at high modulation frequencies) as they did in Fig. 3 from Lu et al. 2001. We do not see this pattern. In fact, only 2 of 54 cells that exhibited spike counts more than 1 S.D. above spontaneous at the highest modulation frequencies did not also have significant phase locking at lower modulation frequencies.
Phase locking at high modulation frequencies

High-frequency phase-locking cutoffs decline in successive auditory stages. In Fig. 3B we show a neuron that loses phase locking ability at high MFs but that simultaneously shows an increase in firing rate. Figure 7 plots two other cells which show a decrease in phase locking at high MFs with different rate profiles. Of the 121 neurons that showed decreased phase locking at the highest modulation frequencies, the majority (75) showed a concomitant decrease in spike count (Table 1). In Fig. 7A, the neuron’s firing rate is suppressed below spontaneous at 60 and 120 Hz, and the absence of firing results in a loss of phase locking. In the neuron in Fig. 7B, we see a sharp decline in phase locking between 30 and 60 Hz with no concomitant change in firing rate, as if the neuron is being driven at the same rate but no longer is able to synchronize. These three modes of synchrony loss – increase of nonsynchronized activity, loss of activity, and desynchronization of activity – are suggestive of the idea that the gradual loss of synchrony at successive auditory stages is not merely due to the inability to follow a temporal envelope (e.g. due to accumulated temporal jitter in the inputs) but rather that the transformation from a temporal to a rate code may be due to multiple means of desynchronization at higher modulation frequencies.

Regardless of the mode of synchrony loss, there is a large drop in the population mean vector strength (i.e., the mean of the vector strength values of each individual cell) in synchronized cells at 60 and 120 Hz. In Figure 8 we plot the population mean rate modulation transfer functions (rMTFs, in terms of firing rate normalized by spontaneous rate) for both synchronized (126 cells with significant phase locking as measured by $V^P$) and exclusively non-synchronized cells (37), as well as the population mean temporal modulation transfer
function (tMTF) for synchronized cells. The firing rate of synchronized cells is highly correlated with vector strength \(r = 0.97\), though at the higher modulation frequencies, phase locking declines more precipitously than firing rate, suggesting that there is a tendency for synchronizing cells to maintain a non-synchronized response at modulation frequencies higher than they can reliably follow. For MFs between 10-30 Hz, the normalized firing rate of synchronized cells is greater than that of non-synchronized cells (t-test, \(p < 0.05\) in each case), but at high MFs the normalized activity of the two populations becomes indistinguishable. It is notable that the non-synchronized population shows the most activity at those modulation frequencies with the least synchrony (120, 60 and 5 Hz), but that relative to spontaneous the synchronized population fired more at all modulation frequencies.

A primary reason for the loss of population mean synchrony at high modulation frequencies is that many synchronizing cells lose synchrony as mentioned above. Even so, we do see substantial phase-locked activity at and above 60 Hz. Using \(VS_{PP}\) as the measure of phase locking, we found that 41 of our 182 neurons (23\%) significantly phase locked to either 60 or 120 Hz stimuli: 21 cells which phase lock to 60 Hz but not 120 Hz, 7 cells that phase lock to 120 Hz but not to 60 Hz, and 13 cells that phase lock to both. If we use the more sensitive Rayleigh statistic (\(RS > 17.7\)), we find 56 cells (31\%) with significant phase locking: 29 to 60 Hz, 7 to 120 Hz, and 20 to both. Figure 9 plots the population mean vector strength broken down by the presence or absence of synchrony at individual modulation frequencies. Although a drop in vector strength is present at high MFs, it is not as drastic as that seen in Fig. 8 when only cells that significantly synchronize at each MF are included, and the resulting vector strength remains substantially higher than that of the neurons that do not synchronize at the same MFs. For reference, the mean \(VS_{PP}\) is also shown for the unmodulated stimulus control (these values differ
as a function of MF because the frequency used in the $VS$ analysis differs at each point). It is clear from Fig. 9A that there is a small amount of synchronized activity that is not being picked up by our $VS_{pp}$ measure, because the not-significant $VS_{pp}$ values are slightly greater than the unmodulated $VS_{pp}$ control values. However at MFs of 10 Hz and above the Rayleigh statistic (Fig. 9B) appears to capture all synchronous responses – the mean $VS_{pp}$ of activity considered to be non-synchronized is no different than that for the unmodulated control stimulus. However, at the lowest modulation frequency tested (5 Hz) the Rayleigh statistic appears to overreach and produce false positives in identifying synchrony ($VS_{pp}$ for unmodulated noise $>$ $VS_{pp}$ for non-Rayleigh significant).

The presence of synchronous firing does not indicate, however, that the cells are firing on every cycle (e.g. see Fig. 7). For the neuron of Fig 7A at 15 Hz there does not appear to be consistent phase locking to every cycle of modulation in the raster plots. Yet, according to the tMTF (with $VS$ and $VS_{pp}$) 15 Hz is this neuron’s best modulation frequency. The high $VS$ value at 15 Hz reflects the precision of spikes occurring within any cycle, but not the reliability of firing for every cycle or every stimulus presentation. While for some cycles the response is quite reliable (see precise vertical aligning of spikes just shy of 200 ms into the stimulus), for the other cycles of modulation responses are often missing. However when spikes are fired in these other cycles they still are tightly vertically aligned; that is, when spikes occur they fall in a narrow time window. To obtain high $VS$ values precision within a cycle, rather than the ability to follow every cycle, is important. As a result, although the cell in Fig. 7A is more reliable in firing during each stimulus cycle to the 10 Hz stimulus, the number of spikes which occur at extraneous phases of the stimulus cycle results in a slight reduction in vector strength relative to the less reliable, but less noisy, firing evoked by the 15 Hz stimulus. To address this we derived
cycle-by-cycle vector strength ($V_{SCC}$), which requires cycle-by-cycle reliability to reach higher values (see Methods). $V_{SCC}$ is simply an average of the $V_{SP}$ calculated on a cycle-by-cycle basis with the assignment of 0 for any cycle with no spikes. The MTF for $V_{SCC}$ is shown in light gray on the temporal MTF plot. In this example, $V_{SCC}$ demonstrates a lower cutoff frequency than for vector strength proper.

Of the neurons that had significant $V_{SP}$ at 60 or 120 Hz none had higher $V_{SCC}$ than $V_{SP}$.

Furthermore, the neurons that show significance at 60 or 120 Hz show the largest drop in $V_{SCC}$ relative to $V_{SP}$ (table 2). This indicates that even the neurons that had high $V_{SP}$ values at 60 and 120 Hz had low reliability on a cycle-by-cycle basis. At and above 30 Hz, fewer than half of the cycles across the synchronized population have synchronized activity (as seen by a drop of $V_{SCC}$ to $<\frac{1}{2}$ of $V_{SP}$), dropping to fewer than one in six cycles ($V_{SCC} / V_{SP} < 0.167$) at 120 Hz, demonstrating that cycle-by-cycle reliability of firing drops off more quickly at high MFs than synchrony.

This loss of reliability while maintaining some measure of synchrony suggests that at higher modulation frequencies, a synchronized temporal code could potentially still be read out from the pooled activity of multiple synchronized neurons using volley principle coding. The volley principle hypothesizes that when one neuron misses firing in a cycle other neurons fire on that cycle at the same phase, such that if you summed activity across neurons every cycle could be followed. In Fig. 10 we investigate whether this could work in A1 for the higher modulation frequencies tested (30-120 Hz). Figure 10A plots a population-wide spike histogram for all cells (thick black line) and for only cells with significant synchrony at the given MF (gray line), with the representation of the stimulus envelope on the bottom for reference. Remarkably, synchronized activity is strong and coherent enough that the population spike histogram follows
every envelope cycle at 60 Hz, even when non-synchronizing cells are included. At 120 Hz this
synchrony is less clear, but the FFT of the spike histogram (Fig. 10B, see Methods) shows a clear
peak at the stimulus modulation frequency for all three MFs, suggesting that extracting the
dominant temporal frequency of the population spike train at the level of A1 can in general
recover the modulation frequency of the stimulus, even when non-synchronized cells are
included.

Synchrony and tonotopy

To investigate whether the degree of synchrony in a neuron is related to its best
frequency (BF), we performed a linear regression of the base-2 logarithm of BF with VSPP for
each modulation frequency, once using all neurons and separately using only those combinations
of neuron and modulation frequency that were significantly synchronized using VSPP. The
modulation frequency with the highest overall correlation coefficient (r = 0.51) is shown as a
scatter plot in Fig. 11A (60 Hz modulation, only neurons synchronizing to 60 Hz). Here we see
a small but statistically significant trend (p = .006) for neurons with high BFs to synchronize
better to 60 Hz modulation (slope of regression line = 0.045 VSPP units/octave). Fig. 11B
summarizes this relationship over the entire data set. When restricting the analysis to neurons
that significantly phase locked at each modulation frequency, we find that neurons with higher
BFs phase lock better than neurons with low BFs for a range of modulation frequencies between
20-60 Hz (dashed gray line). Over this range of modulation frequencies, phase-locking cells
improve their phase locking by about 0.04 VSPP units per octave of BF. We do not see a similar
trend when we look at all cells, where slopes are generally flat and correlation coefficients are
near zero except for at the lowest modulation frequency tested (5 Hz), where cells with low BFs
tended to phase lock better than cells with high BF values. Overall, there appears to be a trend for high-BF cells to phase lock well to high MFs and for low-BF cells to phase lock better to low MFs, but it is weak enough that it is not evident in a topographic representation.

**DISCUSSION**

Implications for Neural Coding of Amplitude Modulation

Choosing the Correct Comparisons

By comparing responses to AM with responses to the unmodulated carrier, we have gained new insight into how cortex encodes temporal modulation. To evaluate how AM is encoded, it is important to choose appropriate stimuli for comparing responses. Usually, AM responses have been compared to spontaneous activity. This reveals whether the neuron can detect the presence of the sound versus no sound being present. It does not reveal whether the neuron detects the modulation.

Using synchrony measures such as VS or measuring modulation transfer functions (MTFs) can provide more information about modulation encoding. Because VS correlates the neural activity to the modulation frequency (known *a priori*), it reveals response properties linked to the modulation. MTFs, by showing different responses to different modulation frequencies (MFs) can also provide information about encoding MF.

In the present study we use an additional approach by comparing AM responses to unmodulated carrier responses. This comparison tells us whether the neuron can distinguish a
modulated from an unmodulated sound. This approach has been common in psychophysics (e.g. Humans: Viemeister 1979; Bacon and Viemeister 1985; Forrest and Green 1987; Eddins 1999; Ewert and Dau 2004; Animals: Salvi et al. 1982; O’Connor et al. 2000; Kelly et al. 2006; Langemann and Klump 2007), and to a lesser degree in modeling of neural responses (Lorenzi et al. 1995). Despite the psychophysical precedent, comparing AM to the unmodulated (or nearly unmodulated) carrier in neurophysiology is rare (Gleich and Klump 1995; Nelson and Carney 2007; Malone et al. 2007).

A novel result was obtained by comparing responsiveness to modulated and unmodulated sounds. Synchronized neurons encoded modulation with both decreases and increases in activity relative to the unmodulated noise carrier, but they primarily increased activity relative to spontaneous for encoding an event. The increase relative to spontaneous might suggest that the modulation causes increased activity. An observation of how synchronized neurons respond to the unmodulated carrier would suggest that this is incorrect. When presented by itself, the unmodulated carrier also evokes responses greater than spontaneous activity, and rarely causes decreases relative to spontaneous in exclusively synchronized neurons. This suggests that the propensity for synchronized neurons to increase activity relative to spontaneous when using modulated stimuli does not reflect an effect of modulation, but rather reflects how these neurons respond to the carrier. This insight would not be possible without incorporating the responses to the unmodulated carrier into the analysis.

Neural Multiplexing in A1

Our data supports the idea that A1 neurons are capable of carrying multiple signals with regard to AM. We reported numerous neurons with mixed-mode responses – synchronized
responses at some modulation frequencies and non-synchronized responses at others. Previously this response type has not been reported in large numbers in cortex. Inspection of earlier studies reveals that mixed-mode responses can be seen in the inferior colliculus although they were not specifically pointed out (Zheng and Escabí 2008; Krebs et al. 2008). A recent study using periodic click trains in marmosets finds mixed-mode responses in thalamus but not in cortex (Bartlett and Wang 2007), which led to the conclusion that the segregation of synchronized and non-synchronized neurons is an emergent cortical property. However upon inspecting a recent paper (Malone et al. 2007), data implicating mixed mode responders using AM-tones in A1 of macaques can be seen. Our data explicitly addresses this and suggests any synchrony to rate transformation should be completed further along the sensory pathway than A1.

Often mixed-mode responding neurons are non-synchronized at higher modulation frequencies and synchronized at lower ones. This implies that the neurons carry separate signals for high and low modulation frequencies, which can be decoded by post-synaptic neurons. This leads to a picture of single A1 neurons carrying multiple signals and being involved in processing many sounds as opposed to being specialized tuned feature detectors.

In addition to carrying separate information in synchronized and non-synchronized responses, neurons also might carry separate information more generally in temporal and rate codes. That there was no clear relationship between temporal and rate MTFs in this study argues that information about modulation frequency may be separately represented by two distinct codes. Relatively simple mechanisms can allow two different post-synaptic neurons to extract these two different types of information. The ability to represent different modulation frequencies with temporal, rate, and non-synchronized codes suggests that at the level of A1 multiple codes are maintained, possibly to pass on to separate parallel pathways.
Implication of Broad MTFs on Neural Codes

The broad MTFs imply that A1 might not operate as an array of sharply tuned modulation frequency feature detectors creating a sparse MF code. Mean bandwidths overall were > 2 octaves indicating that for beyond an octave on either side of the best modulation frequency (BMF), the responses are at least half as strong/synchronous. For the synchronous neurons there are deeper implications. The broad temporal MTFs suggest that at any given MF numerous neurons phase lock well. In addition it appears that many neurons are synchronously firing in phase with each other (Fig. 10). This means that the BMF might be far less of an important contributor to information about modulation than how well the population synchronizes. In such a scheme neurons with the best phase locking, highest synchronized rate, and most coherence with the population will drive the encoding of the AM, rather than necessarily those with the closest BMF to the stimulus. In other words, it might not be which neurons are firing (place code), but the frequency at which they are firing.

The Quantification of Phase Locking and Synchrony

The Rayleigh Statistic

In this study, we used phase-projected vector strength ($V_{SP}$) instead of the Rayleigh statistic as our preferred metric to statistically quantify phase locking. $V_{S}$ is a measure of effect size. The Rayleigh statistic, on the other hand, is a test statistic (i.e., a measure of statistical significance) which conveys how confident we are that a response is synchronized, rather than how synchronized the response is. A cell with a low firing rate and strong synchrony can have
the same Rayleigh value as a cell with a high firing rate and weak synchrony. We are more concerned with the degree of synchrony, so vector strength is a more appropriate measure.

A further potential problem with the Rayleigh statistic is its extreme sensitivity. Any temporal structure in the response, even if not locked to the modulation, can inflate the Rayleigh statistic under certain conditions. For example, onset responses are typically excluded from VS calculations because they can cause the Rayleigh statistic of an otherwise non-synchronized response to become significant. At high MFs this is less of a problem because the onset response is averaged over many stimulus cycles, but at lower MFs where there are fewer total cycles in the stimulus over which to average the onset response, the problem can be quite severe.

Additionally, the responses to unmodulated sounds may exhibit temporal structure above and beyond simple onset responses. As an example, note the non-onset temporal structure in the response to unmodulated noise in Fig. 3A. This structure may result either from sensitivity to the fine temporal structure of the stimulus or from a cell’s inherent temporal pattern of firing. In our study, relatively short stimuli (400 ms) compared to some other studies could have allowed such structure to have a large influence on our Rayleigh statistic (RS) analysis. To quantify this we calculated the RS of the responses to the unmodulated noise carrier for each modulation frequency used in our study. We found that 100/182 neurons had significant synchrony (Bonferroni corrected for 7 comparisons) in their responses to unmodulated control stimuli. Most of this effect was limited to lower modulation frequencies (5 Hz had 88 false positives, 10 Hz had 44 false positives; there was only one false positive at 120 Hz), and the consequences can be seen in Fig. 9B, where the mean $VS_{PP}$ of non-Rayleigh-significant responses at 5 Hz is artificially lower than the mean $VS_{PP}$ of the unmodulated control stimuli due to the removal of false positives from the pool of responses.
Vector Strength ($VS$)

While the Rayleigh statistic is imperfect, limitations of measuring phase locking with $VS$ have come to the forefront recently. As $VS$ is calculated by creating a histogram with a period of the cycle of the modulation, it is maximal ($VS = 1$) if spikes occur at only one precise time in the cycle. However if a given neuron has a broad temporal response or fires for both the rising and falling phase of AM (e.g. Malone et al. 2007), $VS$ values will decrease dramatically. Some authors have introduced methods to try to work around these problems: Kajikawa and Hackett 2005 use an entropy-based analysis, Malone et al. 2007 calculate the correlations between modulation period histograms, and Kajikawa et al. 2008 use linear discriminant analysis. Because these focus more on general temporal coding than synchronization to the modulation frequency these approaches were not used in this study. Another promising approach is an interspike interval analysis as performed by Imaizumi et al. 2010, but this may be more applicable to periodic click train analysis with discrete stimulus events rather than a continuous carrier.

In addition to this problem $VS$ does not lend itself well to trial-by-trial statistics because trials with low spike counts can result in spuriously high values (for example a trial with one spike will always yield a $VS$ value of 1). To get around both the problem of low spike counts and false-positives with the Raleigh statistic we compared the distributions of $VS_{pp}$ between each modulated noise and the unmodulated carrier control. By projecting the $VS$ of individual trials onto the population mean phase, the low spike count problem goes away. The false positive problem for the Rayleigh statistic is resolved by comparing the distribution of $VS_{pp}$ values evoked by modulated and unmodulated noise. By eliminating these two issues we feel that $VS_{pp}$
(rather than simply $VS$, or Rayleigh statistic without reference to the unmodulated response) gives us the best estimate of the number of cells that fire synchronously to our AM stimuli. While $VS$ (or $VS_{pp}$) provides a good metric of the temporal precision of a neuron’s responses, it is not a good measure of how reliably the neuron fires to each cycle. Often in discussing results about $VS$ it is inadvertently implied that high $VS$ results from the neuron accurately following the stimulus. This often leads to the misinterpretation that phase locking is limited to frequencies at which the neuron can follow the stimulus on a cycle-by-cycle basis. While it has long been known that cells can fire in proper phase while completely missing cycles (Wever 1949) this caveat is sometimes forgotten. A population of neurons can still reliably follow the modulation if different neurons fire all at the correct phase but in different cycles by a pooling principle called the volley principle. In this paper we introduced cycle-by-cycle vector strength ($VS_{CC}$) to disentangle volley principle firing from reliable tracking of the modulation frequency. The results (Table 2, Fig. 9) indicate that at relatively low modulation frequencies reliability ($VS_{CC}$) starts dropping off, yet a large number of neurons continue to phase lock. The population response (Fig. 10) shows synchronized activity to every cycle at 60 Hz, and the population FFT shows that even at 120 Hz the population of neurons follows the MF. This suggests the volley principle is viable even at relatively high MFs, and that across the population the synchronized neurons are generally in phase with each other.

AM Sound Perception

Human perception of AM sounds is complex. AM noise evokes a weak, non-spectral pitch percept which is strongest between MFs of ~50-500 Hz (Burns and Viemeister 1976), while MFs below this typically lack pitch and are perceived as a “flutter” (Krumbholz et al.)
2000), though other intermediate categories such as “roughness” are also reported (Fishman et al. 2000). The boundaries between these are subjective and difficult to define, highly variable between subjects and studies (Burns and Viemeister 1976) and the frequency range overlap for these perceptions is large. For animals, these boundaries are unavailable. In addition, spectral AM sensitivity in macaque is shifted to higher MFs than in humans (O’Connor et al. 2000), so any AM perceptual boundaries likely would not map directly to human boundaries. For these reasons, comparing responses in macaque A1 to the perception of pitch, roughness, and flutter is highly speculative. It has been suggested that in marmoset A1 exclusively non-synchronized neurons underlie pitch perception and low MF synchronized neurons underlie flutter, and that in the rostral field flutter is represented by low MF exclusively non-synchronized neurons (Bendor and Wang 2007). Our data are somewhat at odds with this hypothesis as we see clear population-wide synchrony up to 120 Hz (the highest frequency we tested), well above the typically reported flutter/pitch boundary, and clear non-synchronized activity at lower modulation frequencies in A1 (e.g. Fig. 2, Fig. 10). This does not rule out the possibility that different response types underlie different perceptions, but suggests that other possibilities, such as synchronized neurons in A1 representing pitch, are viable.

Comparison to Previous Studies

Contributing Factors to Conflicting Results

A major aim of this paper is to move towards resolving the differing published results about the degree to which neurons synchronize to temporally modulated sounds in auditory cortex. We will focus on three papers that emphasize the amount of synchronized and non-synchronized activity in A1 of awake monkeys: Lu et al. 2001 (“Lu”), Liang et al. 2002
(“Liang”), and Malone et al. 2007 (“Malone”). Throughout the discussion it will be important to remember that Lu reports the least synchrony and the most non-synchronized responses while Malone reports the most synchrony and the fewest non-synchronized responses. Experimental and analytical differences (Table 4) can lead to differing results. We believe the most relevant are the following: the stimuli, how synchronization is quantified, the recording location within the brain, biases in the sampling of neurons, the species studied, the range of modulation and durations of sounds used, and the age of the animals.

The definition of a synchronized response will greatly impact both the percentage of neurons that synchronize to AM and the percentage of exclusively non-synchronized neurons. The more strictly synchronization is defined the less likely it is that a neuron will be identified as synchronizing to AM and the more likely it is to find exclusively non-synchronized neurons. The paper reporting the least synchronization and the largest proportion of exclusively non-synchronized neurons (Lu) had a fairly stringent criterion requiring independent significant phase locking (RS > 13.8) for both the first and second half of a 10 Hz stimulus. The report having the most synchronization and fewest non-synchronized responses (Malone) had the least strict criterion: RS >13.8 for any one of 9 frequencies. Our RS criterion falls somewhere between the two looking at 7 modulation frequencies (with a Bonferroni correction), and our $V_{SP}$ criterion comparing modulated and unmodulated control responses is stricter than our RS criteria.

The stimuli used can have a large impact on the results. Lu (less synchrony) used broad- and narrow-band click trains. This detail is important because neurons are not only sensitive to the modulation/repetition rate, but also to the amplitude envelope and duty cycle (Eggermont 1994; Eggermont 2002; Heil 1997; Heil 2001; Heil and Neubauer 2003; Krebs et al. 2008). The
broad-band click trains were reported not to drive cortical cells well (Lu). The other studies, which reported more synchrony and stronger responses, used sinusoidal AM with tone (Liang, Malone) or noise carriers (present study).

In addition to the type of stimulus used, stimulus parameters can have a large impact. For example, studies extending their stimuli to lower modulation frequencies are more likely to report a high percentage of synchronization than studies that use only higher modulation frequencies. Malone had the lowest frequency tested (0.7 Hz), while Lu’s range started at 10 Hz (which was the highest of all studies), and our study falls between. The highest modulation frequency can also influence the number of non-synchronized responses seen because non-synchronized responses are often associated with high modulation frequencies. The duration of the stimulation can also have an influence, particularly if RS is used. Shorter durations, because they have fewer cycles, are more likely to give false positive RS values (See section titled: The Rayleigh Statistic). Longer stimuli (e.g. Malone uses 10 second stimuli) have the disadvantage that psychophysically, most temporal integration occurs over less than 1 second and therefore neural responses to stimuli longer than that are less comparable to psychophysical performance.

Neuronal sampling biases, the recording location within the brain, the species and age of the subjects all might also contribute to the degree of synchrony and non-synchronized responses. Of the studies being compared, those that targeted superficial layers of cortex (Lu, Liang) report less synchrony and more non-synchronized responses than those that targeted all layers. However, the studies targeting superficial layers were in marmosets and those targeting all layers were in macaques. Also, Lu searched for units based on spontaneous activity, Malone searched based on responses to the tonal carrier, and our study on a wide variety of sounds. Therefore the Malone study is the most likely to include neurons highly responsive to their
stimuli, including low spontaneous neurons, whereas Lu is more likely to record from high spontaneous neurons that are less responsive to their stimuli. On a final note, AM sensitivity during development is influenced by age (Eggermont 1993), and a recent study by Recanzone (personal communication) has found that geriatric macaques have severely impaired phase locking to AM. Age is not a known factor for the current comparisons we are making, but might be worth considering in the future.

Prevalence of Synchrony

It is quite clear from this study that most A1 neurons synchronize to some modulation frequencies at or below 120 Hz – 69% of our cells show some synchrony as measured by phase-projected vector strength, and 86% show the presence of synchrony using the more sensitive Rayleigh statistic (with Bonferroni, Rayleigh > 17.7). Using SAM tones Liang reports 64% of cells with Rayleigh values above 13.8 for at least two modulation frequencies. Using the same criteria, 80% of our cells would be considered synchronized. Malone found at least 87% of neurons exceed a Rayleigh value of 13.8 for at least one modulation frequency. The number of synchronizing cells we found can be considered roughly similar to Malone and Liang when taking into account the different stringency in classification criteria.

In the first paper to define synchronized and non-synchronized as two separate classes of neurons (Lu), a much smaller proportion of synchronized responses was found (36/190, 19%, Table 3). The conservative nature of Lu’s quantification of synchrony seems to be one likely source of the differences between our results and theirs. Lu required neurons to have significant RS to the 10 Hz stimulus for both the first 450 msec and the last 450 msec to classify the neuron as synchronized. If we had used a longer stimulus and our neurons phase locked more weakly or
stopped phase locking in the second half, by Lu’s criteria these neurons would not be classified as synchronized, but by ours they would. The impact of using only one modulation frequency (10 Hz) can be further explored by reanalyzing our data. When we re-perform our analysis only using 10 Hz and Rayleigh (>13.8, \(p < 0.001\)), then the number of neurons with synchronized responses drops from 86% to 68%. A more dramatic effect is observed when we compare \(V/s_{pp}\) values for modulated sounds vs. unmodulated control sounds. Here significance goes from 69% with all frequencies and a Bonferroni correction to 42% using only 10 Hz (and no Bonferroni correction). Thus, much of the difference in percentage of synchronized neurons reported could be due to neurons that synchronize to frequencies other than 10 Hz and Lu’s stricter statistical criterion.

We also find that the average strength of synchrony, particularly when only synchronized responses (rather than “synchronized” cells) are included (Fig. 9), only begins to drop off above 60 Hz. Many (23%) of our cells exhibit significant phase locking at or above 60 Hz using \(V/s_{pp}\). This cutoff is in line with other studies that used the Rayleigh statistic in awake preparations (Malone, 36% phase lock > 50 Hz; Liang, 25% > 63.6 Hz; Middlebrooks 2008, electrical stimulation SAM pulse train 42% phase lock >= 60 Hz; Lu, 26% > 50 Hz; Ter-Mikaelian et al. 2007, ~23% > 50 Hz), but higher than studies using half height of synchronized rate (Fitzpatrick et al. 2009, 15% > 64 Hz). This is consistent with the notion that synchrony cutoff boundaries are sensitive to the measure used (Eggermont 1991) and anaesthetic state (Goldstein et al. 1959; Lu et al. 2001; Fitzpatrick et al. 2009; Ter-Mikaelian et al. 2007; Creutzfeldt et al. 1980; Steinschneider et al. 1998; Anderson et al. 2006).

Proportion of Exclusively Non-synchronized Neurons
There is some disagreement as to (1) whether there are separate classes of synchronized and non-synchronized neurons, and (2) the degree to which there is non-synchronized activity in cortex. Lu reports a substantial number of exclusively non-synchronized neurons (53%); Malone only finds 2%. This leads to two very different interpretations of how AM is encoded. Our data falls in between with 17% of neurons being classified as exclusively non-synchronized when using $VS_{pp}$ and comparing spike counts to spontaneous.

However, in our opinion it might be more informative to compare the non-synchronized response to the response to the unmodulated carrier because the neurons could be responding in a sustained manner to the carrier and blind to the modulation. With this definition we find 20% of neurons are exclusively non-synchronized. It is possible that additional non-synchronized responses would emerge above 120 Hz in our sample, considering that about 50% of the non-synchronized neurons in Lu only appeared above 100 Hz.

*Non-synchronized Neurons vs. Non-synchronized Responses*

Previous studies (Lu, Liang) have suggested that a large majority of neurons in A1 fall into one of two well-separated classes, cells which exhibit synchronized responses to periodic stimuli and those which only exhibit non-synchronized responses. The strongest argument for a categorical distinction between synchronized and non-synchronized neurons in primate A1 may be found in Lu where the authors found two well-separated clusters of neurons. Synchronized neurons exhibited significant phase locking at 10 Hz and also fired more strongly below 30 Hz than they did above 200 Hz. Non-synchronized neurons did not show significant phase locking at or below 10 Hz, and fired more strongly above 200 Hz than below 30 Hz. Only 9% of their neurons did not fall into one of these two categories. Notably, in the main results it was reported
that no cells fired more strongly at low modulation frequencies than at high without also showing significant phase locking at low modulation frequencies, although there is evidence for such neurons in the rostral fields of auditory cortex in a later paper (Bendor and Wang 2007).

While Lu found evidence for two classes of neurons, Malone failed to see such categories. We also do not see these distinct classes. In our data (Fig. 6), neurons with and without synchronized responses appear to lie on a continuum. There are several potential differences in our design that might account for this discrepancy. We used slightly different measures to quantify phase locking at low modulation frequencies and the relative strength of firing at high modulation frequencies. For instance, to measure phase locking, we used the largest temporal responses at modulation frequencies up to 30 Hz instead of only up to 10 Hz. Since there is often good synchrony up to 30 Hz, a 10 Hz cutoff may result in the misclassification of cells with some synchronized activity as non-synchronized. Our analysis also included responses to 5 Hz, so neurons that only phase locked below 10 Hz might also be captured in our experiments.

Another argument against two classes of neurons is the presence of mixed mode responders. We find up to 48% of neurons have synchronized responses at some modulation frequencies and non-synchronized responses at others. Malone reports that 16% of neurons exhibited statistically significant increases in activity beyond their synchronization boundaries. These might correspond to our mixed-mode responders. Taken together the results suggest that in macaques it is probably better to talk about two types of responses (synchronized and non-synchronized) rather than two different types of neurons, with different mixtures of these response types capable of existing within the same cell.
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REFERENCES


FIGURE LEGENDS:

Figure 1: A) Amplitude modulated stimuli with a noise carrier. The stimuli were created from broadband white noise bursts (top), which were also used as “unmodulated” stimuli. The amplitude of AM stimuli (bottom) is modulated by a sinusoidal envelope. In this study all AM stimuli were 100% modulated. B) Raster plot (top) and modulation transfer function (MTF, bottom) of a sample cell. Standard VS measures (MTF) indicate very strong phase locking at 30/60 Hz, whereas phase-projected VS suggests a lesser degree of synchrony. Cycle-by-cycle vector strength suggests low reliability of firing throughout. BF of cell in B) is 1800 Hz.

Figure 2: Nonsynchronized neurons. The top left of each plot are the spike rasters. The top right is the peristimulus time histogram (bin size = 5 ms). The middle plot is the temporal modulation transfer function (tMTF) shown for phase-projected vector strength (VS_pp, the primary temporal analysis for these experiments), cycle-by-cycle vector strength (VS_CC), and regular vector strength (VS, displayed for reference). The lower plot is the spike rate modulation transfer function (rMTF) with both the spontaneous and unmodulated spike rates shown. Symbols indicate significant departures of the modulated firing rate from spontaneous (open triangles) and unmodulated (filled triangles). BF of cell in A) is 800 Hz and BF of cell in B) is 2800 Hz.

Figure 3: Synchronized neurons showing high activity at high modulation frequencies. Plot details are as in Figure 2. The cell in A) phase locks well to all modulation frequencies presented. The cell in B) increases its spike count at the highest modulation frequencies, but loses synchrony above 30 Hz. BF of cell in A) is 26000 Hz and BF of cell in B) is 7500 Hz.
Figure 4: Comparison of synchronized and non-synchronized neurons: Synchrony measured by $VS_{pp}$. In all plots, gray shading to a bar indicates cells which have significant $VS_{pp}$ responses, and diagonal hatching indicates cells which have significant spike count (SC) responses. In upper plots, SCs are compared to spontaneous firing (difference signals the presence of a stimulus). In the lower plots, SCs are compared to the response to an unmodulated stimulus (difference signals the presence of modulation). Left plots, all cells. Right plots, only SC-significant cells included. SC > spon: at least one MF greater than spontaneous, no MFs below spontaneous. SC < spon: at least one MF below spontaneous, no MFs greater than spontaneous. SC < and > spon: at least one MF below spontaneous and at least one MF greater than spontaneous.

Figure 5: Bandwidth and best modulation frequency for rate and temporal measures of response. A) Distribution of full-width at half-height bandwidth, in octaves, for all cells in which the MTF could be well fit by a Gaussian function (see Methods). For 5A only, 50 cells from the A1/R border area are included. B) Best modulation frequencies (BMF) for all AM-sensitive cells. C) Joint distribution of rate and temporal BMFs for synchronized cells. Only cells with single-peaked MTFs for both rate and temporal measures (n=71) included.

Figure 6: Scatter plot comparing temporal and rate response measures for synchronized and non-synchronized cells. Cell spike count at high modulation frequencies (x-axis) is plotted against a measure of synchrony at low modulation frequencies (best $VS_{pp} \leq 30$ Hz, y-axis). Cells with significant synchronization at low modulation frequencies (black) do not neatly segregate from cells without synchronization (gray) at low modulation frequencies in terms of spike count activity at high modulation frequencies.
Figure 7: Synchronized neurons showing loss of phase locking at high modulation frequencies. Plot details are as in Figure 2. The cell in A) is inhibited below spontaneous at high modulation frequencies. The cell in B) shows a marked drop in phase locking above 30 Hz which is not accompanied by a significant change in spike count.

Figure 8: Mean modulation transfer functions for synchronized and non-synchronized cells. *Left axis* shows mean firing rate, normalized to the spontaneous firing rate, for each cell (both rMTFs). *Right axis* shows phase-projected vector strength (tMTF). Error bars indicate standard error of the mean. MTFs are slightly offset on the x-axis to improve readability.

Figure 9: Phase-projected vector strength values for synchronized and non-synchronized responses. *Left:* Significance determined by *t*-test of VS_{PP} distributions for modulated and unmodulated stimuli (*p* < 0.05, corrected for 7 comparisons per cell). *Right:* Significance determined by Rayleigh statistic (RS > 17.7). All responses with significant phase locking are indicated in *black*. Responses without significant phase locking at a particular modulation frequency are indicated in *gray* (regardless of whether that cell showed phase-locked activity at another modulation frequency). VS_{PP} for the unmodulated response is included (dotted line). Error bars indicate standard error of the mean. Unmodulated response is slightly offset on the x-axis to improve readability of error bars.

Figure 10: Population phase locking at high modulation frequencies. A) Spike histograms of overall response collapsed across cells (2 ms bins, 70 ms onset removed). All cells (thick black) and only cells that significantly phase locked at each MF (gray) are shown. The stimulus envelope (thin black) is provided for reference. B) Fourier transform of signals in A (see Methods).
Figure 11: Relationship between best frequency (BF) and phase locking. A) Scatter plot of BF against $VS_{PP}$ at 60 Hz for all cells with significant phase locking at 60 Hz, with regression line in gray (slope = 0.045 units/octave). B) Left axis: Regression slope (solid lines) of BF against $VS_{PP}$ at each tested modulation frequency for all cells (black) and only synchronizing points (gray). Right axis: Correlation coefficient (dashed lines) of BF and $VS_{PP}$ at each tested modulation frequency for all cells (black) and only synchronizing points (gray). Significant correlations (p < 0.05, Bonferroni corrected for 7 comparisons) are marked with circles.

Figure S1: Tonotopic maps of recording sites. A) Tonotopic map of Monkey V. Each dot represents the best frequency (BF) of a single cell for which a valid tuning curve was obtained; most recording sessions had 2-3 isolated cells recorded simultaneously. Some recordings used to generate the tonotopic map were made during experiments not reported here. The gray background area represents locations of recordings used for this study. For Monkey V, the characteristic change in BF from high frequencies in caudomedial sites to low frequencies in rostrolateral sites was present, confirming histological data that recordings were made in A1 (see Methods). B) Tonotopic map of Monkey Y. Details are the same, with the addition of a pink background area, which denotes sites which were recorded for this experiment and subsequently excluded. Because of the bad perfusion in Monkey Y, we were forced to rely on BF and latency (Fig. S2) data to determine the borders of A1. The tonotopic map shows a frequency reversal which seems to occur about 5 mm anterior to the most caudal site recorded, but the exact point of the reversal is somewhat ambiguous. Based on this data, we believe the sites in pink lie in the A1/R border region, and they have been excluded from analysis in the main body of the paper.
Figure S2: Latency maps of recording sites. Recordings from presentations of the unmodulated noise stimulus were histogrammed into 2 ms bins, and latency was defined as the time of the first bin which exhibited a firing rate four standard deviations above the spontaneous rate. Latency was obtained for 213 of the 232 cells recorded. A) Latency map of Monkey V. Each dot represents the latency of a single cell recorded during the present experiments. Latencies are generally low throughout the recorded area (mean latency = 17.4). B) Latency map of Monkey Y. The pink area denotes sites which were deemed to lie in an A1/R border region and were excluded from analysis in the main body of the paper. Latencies are slightly higher in the border region (mean latency = 31.6 ms) than in the A1 region (mean latency = 25.4 ms), but the difference is not statistically significant (p = 0.14, t-test).

Figure S3: Comparison of phase locking in A1 and border area, Monkey Y. The mean phase-projected vector strength for synchronized neurons is plotted at each modulation frequency for A1 (black) and the border area (gray). Error bars are ± SEM. Points that are significantly different (p < 0.05, t-test) are marked with a circle. There is a clear shift towards phase locking at lower modulation frequencies in the border area relative to A1, consistent with the inclusion of neurons from area R in the border area.
Table 1. Changes in phase locking and spike count measures at high modulation frequencies relative to low modulation frequencies

<table>
<thead>
<tr>
<th>VS&lt;sub&gt;pp&lt;/sub&gt; decrease</th>
<th>VS&lt;sub&gt;pp&lt;/sub&gt; no change</th>
<th>VS&lt;sub&gt;pp&lt;/sub&gt; increase</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC decrease</td>
<td>75</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>SC no change</td>
<td>29</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>SC increase</td>
<td>17</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>60</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are in cell count. Two distributions of trial-by-trial phase-projected vector strength (VS<sub>pp</sub>) and spike count (SC) were created for each cell, one that combined all the responses to high (>= 60 Hz) modulation frequencies, and one that combined all the responses to low (<= 30 Hz) modulation frequencies. The two distributions were compared using the False Discovery Rate method (Benjamini and Hochberg, 1995) to determine how many cells exhibited an increase, decrease, or no change in each measure at high modulation frequencies relative to low modulation frequencies.
Table 2. Change in phase locking vs. reliability as a function of modulation frequency

<table>
<thead>
<tr>
<th>Number of cells</th>
<th>VS&lt;sub&gt;CC&lt;/sub&gt;</th>
<th>VS&lt;sub&gt;PP&lt;/sub&gt;</th>
<th>VS&lt;sub&gt;CC&lt;/sub&gt; / VS&lt;sub&gt;PP&lt;/sub&gt;</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 Hz</td>
<td>21</td>
<td>0.052</td>
<td>0.332</td>
<td>0.15</td>
</tr>
<tr>
<td>60 Hz</td>
<td>35</td>
<td>0.103</td>
<td>0.424</td>
<td>0.24</td>
</tr>
<tr>
<td>30 Hz</td>
<td>73</td>
<td>0.219</td>
<td>0.472</td>
<td>0.44</td>
</tr>
<tr>
<td>20 Hz</td>
<td>80</td>
<td>0.276</td>
<td>0.487</td>
<td>0.57</td>
</tr>
<tr>
<td>15 Hz</td>
<td>85</td>
<td>0.316</td>
<td>0.483</td>
<td>0.65</td>
</tr>
<tr>
<td>10 Hz</td>
<td>85</td>
<td>0.358</td>
<td>0.477</td>
<td>0.75</td>
</tr>
<tr>
<td>5 Hz</td>
<td>67</td>
<td>0.347</td>
<td>0.438</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Average of trial-by-trial phase-projected vector strength (VS<sub>PP</sub>, phase locking) and trial-by-trial cycle-by-cycle vector strength (VS<sub>CC</sub>, reliability) for all combinations of cell and modulation frequency with significant phase locking as measured by VS<sub>PP</sub>. The two distributions were compared using two-tailed t-tests (p < 0.05) to determine if mean reliability values were different than mean phase locking values.
Table 3. *Comparison of AM studies, distribution of cell types*

<table>
<thead>
<tr>
<th>Study</th>
<th>Exclusively nonsynchronized</th>
<th>Synchronized</th>
<th>Mixed mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu et al. 2001</td>
<td>53*</td>
<td>19†</td>
<td>4†</td>
</tr>
<tr>
<td>Liang et al. 2002</td>
<td>NR</td>
<td>64</td>
<td>NR</td>
</tr>
<tr>
<td>Malone et al. 2007</td>
<td>2</td>
<td>87</td>
<td>16</td>
</tr>
<tr>
<td>VSPP, spike count vs. spontaneous</td>
<td>17</td>
<td>69</td>
<td>18</td>
</tr>
<tr>
<td>VSPP, spike count vs. unmodulated</td>
<td>20</td>
<td>69</td>
<td>36</td>
</tr>
<tr>
<td>Rayleigh, spike count vs. spontaneous</td>
<td>4</td>
<td>86</td>
<td>21</td>
</tr>
<tr>
<td>Rayleigh, spike count vs. unmodulated</td>
<td>7</td>
<td>86</td>
<td>44</td>
</tr>
</tbody>
</table>

Values are in percentage of total cells. NR, not reported.

*percentage of exclusively non-synchronized neurons based on total of 94 neurons presented in the results; the 96 excluded neurons may have had nonsynchronized responses. †percentage of neurons that synchronized to the click train is based on a total of 190 neurons reported in the methods; 96 of these neurons did not synchronize to 10 Hz click trains but were excluded from presentation in the results for other reasons.
Table 4. Comparison of AM studies, experimental and analysis parameters

<table>
<thead>
<tr>
<th></th>
<th>Lu et al. 2001</th>
<th>Liang et al. 2002</th>
<th>Malone et al. 2007</th>
<th>Yin et al. 2010, Rayleigh</th>
<th>Yin et al. 2010, VSPP</th>
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</thead>
<tbody>
<tr>
<td>Stimulus</td>
<td>Click Train</td>
<td>Tone AM</td>
<td>Tone AM</td>
<td>Noise AM</td>
<td>Noise AM VSPP AM &gt; VSPP carrier @</td>
</tr>
<tr>
<td>Definition of</td>
<td>Rayleigh&gt;13.8</td>
<td>Rayleigh&gt;13.8 @</td>
<td>Rayleigh&gt;13.8 @</td>
<td>Rayleigh&gt;17.7 @</td>
<td>VSPP AM &gt; VSPP carrier @</td>
</tr>
<tr>
<td>Synchrony</td>
<td>@10 Hz for BOTH first and last 450 ms</td>
<td>2 mod freq</td>
<td>1 mod freq</td>
<td>1 mod freq</td>
<td>1 mod freq</td>
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<tr>
<td>Frequency Range</td>
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<td>1-512 Hz</td>
<td>0.7-200 Hz</td>
<td>5-120 Hz</td>
<td>5-120 Hz</td>
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<tr>
<td>Duration</td>
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<td>1 sec</td>
<td>10 sec</td>
<td>0.4 sec</td>
<td>0.4 sec</td>
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<tr>
<td>Onset Removal</td>
<td>100 ms</td>
<td>100 ms</td>
<td>None</td>
<td>70 ms</td>
<td>70 ms</td>
</tr>
<tr>
<td>Sound Level</td>
<td>Best Level</td>
<td>Best Level</td>
<td>Best Level</td>
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<td>65 dB SPL</td>
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<tr>
<td>Species</td>
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<td>Marmoset</td>
<td>Macaque</td>
<td>Macaque</td>
<td>Macaque</td>
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<tr>
<td>Cortical Depth</td>
<td>Superficial</td>
<td>Superficial</td>
<td>All</td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td>Search Stimuli</td>
<td>Spontaneous</td>
<td>Short Tones</td>
<td>Short Tones</td>
<td>Large Battery</td>
<td>Large Battery</td>
</tr>
</tbody>
</table>

Major parameters for three previous studies investigating AM in monkey cortex are compared with parameters in current study.
A

Bandwidth in Octaves

Number of Cells

B

BMF Classification

Spike Count (n = 160, μ = 29.0)

Vector Strength (n = 126, μ = 17.3)

C

Spike Count BMF

Vector Strength BMF

Number of Cells
Spike Count, SD above/below
Spontaneous, largest >= 60 Hz

Phase Projected VS, best <= 30 Hz

Rayleigh > 17.7
Rayleigh ns
Modulation Frequency

<table>
<thead>
<tr>
<th>Firing Rate/Spontaneous</th>
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<tbody>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.15</td>
</tr>
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<tr>
<td>0.25</td>
</tr>
<tr>
<td>0.3</td>
</tr>
<tr>
<td>0.35</td>
</tr>
<tr>
<td>0.4</td>
</tr>
<tr>
<td>0.45</td>
</tr>
</tbody>
</table>

VSpp

rMTF (Synchronized)
rMTF (Non-synchronized)
tMTF (Synchronized)
**A**

VS$_{pp}$ vs BF

- 60 Hz, Synchronizing points only

**B**

Slope (VS$_{pp}$/octave) vs Modulation Frequency

- Black line: All cells
- Grey line: Only significant MFs

Correlation Coefficient