

Research paper

Response to broadband repetitive stimuli in auditory cortex of the unanesthetized rat

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Abstract

This study examines the ability of multi-unit clusters (MUCs) in layer IV/V of primary auditory cortex of the awake rat to respond to a series of broadband click trains. The data from 113 multi-unit clusters were analyzed for synchronous and nonsynchronous responses using several methods. Synchronous responses were measured using window analysis, circular statistics and spectral analysis. Nonsynchronous responses were measured during different time intervals during the click train (first 50 ms, 50–450 ms, and the entire click train). The results demonstrate that multi-unit clusters are capable of synchronizing to clicks at rates up to 166 Hz. The mean synchronization boundary (limiting rate) for the group was found to be 72 Hz. Mean peak response rate, mean response duration, and mean time-to-peak response decreased as the stimulus presentation rate (SPR) increased, resulting in a temporal sharpening of the population response. For fast SPRs (>50 Hz), 50% of MUCs exhibited nonsynchronous responses in which the firing rate increased with SPR, although this activity was most prevalent during the first 50 ms of the response. Sustained increases in firing rate with SPR were seen in 8% of the MUCs, while another 38% of MUCs exhibited sustained decreases during the click train.

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1. Introduction

A more complete description of information processing in the central auditory system is essential to understanding audition in general and speech processing in particular. Neurons at different levels of the auditory pathway exhibit differential tuning for both spectral and temporal properties of sound. For example, the minimum amount of time required for auditory neurons to recover from each sound onset generally increases with each synapse above the cochlea. As a result, the typical maximum repetition rate at which action potentials are significantly

synchronized to stimulus trains decreases from above 500 Hz in the periphery to less than 100 Hz in primary auditory cortex.

Previous results demonstrate that adjacent cortical neurons exhibit similar temporal tuning, but no consistent topographic arrangement of synchronization boundaries across auditory cortex has been reported (De Ribaupierre et al., 1972; Eggermont, 1991; Kilgard and Merzenich, 1999). Nor have any previous studies identified a correlation between synchronization boundaries and spectral tuning (De Ribaupierre et al., 1972; Lu et al., 2001; Lu and Wang, 2004; Phillips et al., 1989). Considerable variability in the synchronization boundary (limiting rate) has been observed across studies and published results range from 10 to 100 Hz. The cause of this variability is unclear, but is likely due to differences in stimulus acoustics, anesthesia, analysis techniques and species.

Abbreviations: MUCs, multi-unit clusters; PSTH, peri-stimulus time histogram; RRTF, repetition rate transfer function; SB, synchronization boundary; SPR, stimulus presentation rate

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A recent study identified a subpopulation of auditory cortex neurons in awake marmoset monkeys that respond to increases in the stimulus presentation rate by increasing their mean tonic firing rate (Lu et al., 2001). Their results demonstrate that slow (<40 Hz) stimulus trains elicit responses synchronized to the stimulus. Faster rates (>50 Hz) result in non-synchronized responses proportional to the stimulus presentation rate (SPR). This two stage mechanism has not been reported in other species.

The purpose of this study is to describe temporal coding of multi-unit clusters in the awake rat. Specifically this study will: (1) use multiple measures to quantify the synchronization boundary of multi-unit clusters in auditory cortex of awake rats to broadband clicks presented from 5 to 250 Hz; (2) report population changes in the mean peak response rate, mean response duration, mean response latency and mean time-to-peak response as a function of stimulus presentation rate; and (3) determine if multi-unit clusters display non-synchronous activity in the awake rat.

2. Materials and methods

2.1. Animal preparation

Fourteen-channel micro-wire electrodes were implanted to a depth of 550 μm in the right primary auditory cortex of 10 female Sprague–Dawley rats. The electrodes consisted of polyimide-insulated 50 μm diameter tungsten wires with an average impedance of 60 k Ω at 1 kHz in saline. Two tungsten references (Teflon insulation) were imbedded in the array and stripped of their insulation prior to implantation. Surgery was conducted using standard sterile procedures. Animals were anesthetized using ketamine, xylazine, and acepromazine (50, 20, 5 mg/kg, respectively). Atropine, and dexamethazone were administered subcutaneously prior to and following surgery. Surgical procedures were described in detail in previous publications, as were the multi-channel electrodes used to record data (Rennaker et al., 2005a). A custom built mechanical insertion device was used to implant the electrodes in layer IV/V (depth 550–600 μm) (Rennaker et al., 2005b). Animals were individually housed and exposed to a 12:12 h light-to-dark schedule. Recordings were taken during the light part of the cycle from unanesthetized animals.

2.2. Acoustic stimuli

Broadband clicks were presented at rates ranging from 4 to 250 Hz for 1 s at suprathreshold intensity (65 dB SPL). A maximum of 100 clicks were played at each stimulus presentation rate (SPR) on a given trial. The click trains were randomly interleaved and repeated 20 times with inter-train-intervals of 1 s. Broadband clicks were 80 μs in duration with 3 dB points at 1.6 and 31.6 kHz. The spectral properties were confirmed using a 1/4" condenser microphone (ACO Pacific) and analyzed for spectral content

using digital oscilloscope. An iso-intensity tuning curve (25 repetitions of a set of 45 pure-tone stimuli, of 35 ms duration and intensity 55 dB SPL, ranging from 1.3 to 32 kHz) was presented prior to the click train stimuli to evaluate frequency receptive fields for each multi-unit cluster (MUC).

2.3. Recording procedure

Acoustic stimuli were presented to awake restrained animals in a calibrated, free-field speaker in a double-walled acoustic chamber. The chamber consists of two boxes made with 3/4" high density sound board. The inside chamber is 25" \times 25" \times 19" and is covered with 3" thick Sonex acoustic foam. The attenuation was measured by playing a pure tone at 80 dB outside the box with frequencies at 2, 10, 20, and 30 kHz with the door open and closed. A minimum of 80 dB attenuation was obtained at all frequencies tested. The reverberation time was measured by playing a broadband click at 65 dB and measuring the time between the first and second peak. The reverberation time was determined to be 550 μs .

Awake animals were suspended above the booth floor in a restraint hammock and placed in the center of the acoustic chamber. A head-stage amplifier (Tucker Davis Technology) was directly attached to the electrode connector. Neural data were digitized at 25 kHz and bandpass filtered from 400 to 4000 Hz, 6 dB/oct. Neural responses that crossed a threshold set two standard deviations above the mean signal level were recorded using Brainware software. Multi-unit clusters (MUCs) that exhibited statistically significant response rates ($p < 0.05$) compared to the spontaneous rate were classified as driven. Only driven MUCs were used in this study.

2.4. Data analysis

The data were analyzed for synchronized responses using three methods: window analysis, circular statistics, and spectral analysis. Spontaneous rates were determined by taking the average spike rates during the 35-ms prior to the start of each click train.

Window analysis counted the number of spikes occurring in a 25-ms window starting at 8 ms following the onset of each click for all stimuli in the train. The average spike rate in each window was compared to spontaneous rate using a paired t -test ($\alpha = 0.01$). Repetition rate transfer functions were plotted and normalized by the onset response to the first stimuli in the click train.

Circular statistics were also used to determine the synchronous cutoff rate using Eq. (1). Each spike time was converted to a vector direction ' θ ' with unit length by dividing the spike time by the repetition rate in milliseconds and multiplying by $2 * \pi$ (Eq. 1A). The mean x and y components of this directional vector were calculated by summing the cosine and sine of each measure of theta and dividing by the number of spike times (Eq. 1B and 1C). The mean

vector length was determined by taking the square root of the sum of the squares (Eq. 1D). Statistical significance was determined by calculating the Rayleigh statistic (Eq. 1E). Rayleigh values greater than 13.8 correspond to a p -value less than 0.001 (Mardia and Jupp, 2000). The synchronous cutoff rate was defined as the fastest stimulus presentation rate to which the channel exhibited a statistically significant response ($p < 0.001$).

$$\varphi = (T/t) \times 2\pi; \quad (1A)$$

$$\bar{X} = \frac{1}{n} \sum_{i=1}^n \cos \varphi; \quad (1B)$$

$$\bar{Y} = \frac{1}{n} \sum_{i=1}^n \sin \varphi; \quad (1C)$$

$$\bar{R} = \sqrt{\bar{X}^2 + \bar{Y}^2}; \quad (1D)$$

$$\chi_2^2 \approx 2n\bar{R}^2. \quad (1E)$$

A third method, spectral decomposition, was also used to calculate the synchronization boundary. The data from each repetition was converted to an array of ones and zeros with ones corresponding to a spike in that bin. These bins were added together for each repetition and a discrete fast Fourier transform was performed on the data. The amplitude at the SPR frequency was compared to 30 measures of

peak values outside the SPR range. The spectral amplitude was calculated by taking twenty times the log of the ratio of the mean of the thirty values divided by the peak amplitude at the SPR. Statistical significance was determined by calculating the 99% confidence interval for the mean of the 30 non-SPR points. If the mean of the peak at the SPR fell outside the confidence interval it was classified as significant. Custom software was used to detect the synchronization boundary (SB). The SB value was defined as the SPR prior to the slowest SPR with a p -value less than 0.001 as was used in the circular analysis (the SPR prior to the first non-significant response).

For both the spectral analysis and circular statistics methods two measures of SB were made. Responses to the first and second halves of the train were analyzed separately. The response to the first 50 ms was excluded from the analysis of the first half of the click train. The minimum SB from these two measures was chosen for this study.

The temporal response properties were measured for each channel at each repetition rate below 100 Hz. The mean latency and mean end-latency were measured by finding the first point and last point in the period-PSTH (100 μ s bins), respectively, that were greater than the 95% confidence interval for the spontaneous rate. The end-latency

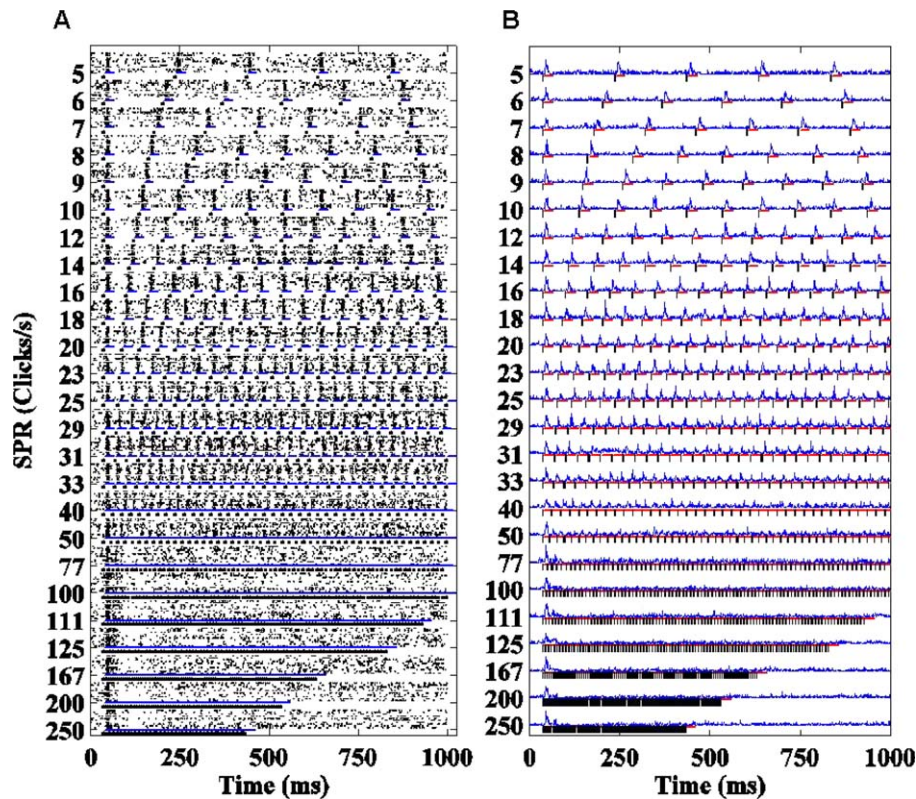


Fig. 1. (A) Representative raster plot from one MUC. For each stimulus presentation rate (SPR) a set of two markers are used to identify the tone and the fixed analysis window. The short marker denotes the click and the long marker denotes the analysis window. Each dot represents a spike occurrence. Neural responses to each of the click trains at a given SPR were placed vertically above the previous click train at that SPR. Twenty repetitions of each rate were presented. (B) The PSTH from the MUC. Obvious peaks can be seen for rates up to 50 Hz. The response to the first click is protracted for rates above 50 Hz.

is defined as the end of the onset response. Custom software was written to automatically detect the latency, peak value, peak time and end latency. In cases (<1% of the channels) where these values were incorrectly identified by the software, manual selection of the correct values could override the software values. The response duration was defined as the end-latency minus the latency.

Nonsynchronized rates were determined by calculating the spike rate for three different time periods: (1) during the entire click train duration, (2) during the first 50 ms of the stimulus train, and (3) the period 50–450 ms following the start of the click train. Paired *t*-tests were performed between the nonsynchronized rates and the spontaneous rates ($\alpha = 0.01$) to determine statistical significance. All animal testing was conducted in accordance with the University of Oklahoma's Institutional Animal Care and Use Committee regulations.

3. Results

3.1. Example result

A typical raster plot from one MUC is displayed in Fig. 1A. Periods of inhibition can be seen following the initial onset response of the cells for rates above ~ 16 Hz.

Below 16 Hz, considerable spontaneous firing occurs between clicks. The raster plot in Fig. 1A was converted to a peri-stimulus time histogram (PSTH) in Fig. 1B. The PSTH subjectively demonstrates that the MUC is synchronized to stimulus presentation rates of at least 40 Hz. Irregularly spaced peaks can be seen at higher SPRs.

Several analysis techniques have been used to determine the fastest SPR at which cortical neurons can synchronize, referred to as the limiting rate or synchronization boundary (SB). Fig. 2 displays the repetition rate transfer functions (RRTF) using four different analysis methods for the example MUC. The RRTF describes the strength of the synchronous responses as a function of SPR. Fig. 2A displays the results of the window analysis on the example MUC. Window analysis (see Section 2) measures a SB of approximately 40 Hz and exhibits a lowpass function. The mean number of spikes per stimulus drops off significantly ($p < 0.05$) at SPRs greater than 9 Hz. Circular statistics (2D), and spectral analysis (2B) measured the SB at 50 Hz. The RRTF using the circular (2D), vector strength (2C) and spectral analysis (2B) methods appear to be bandpass in nature with peaks at ~ 20 Hz.

Spectral analyses of the data from Fig. 1 for SPRs of 5 Hz (3A), 10 Hz (3B), 25 Hz (3C), 50 Hz (3D), 77 Hz (3E) and 100 Hz (3F) are displayed. The peaks to the right

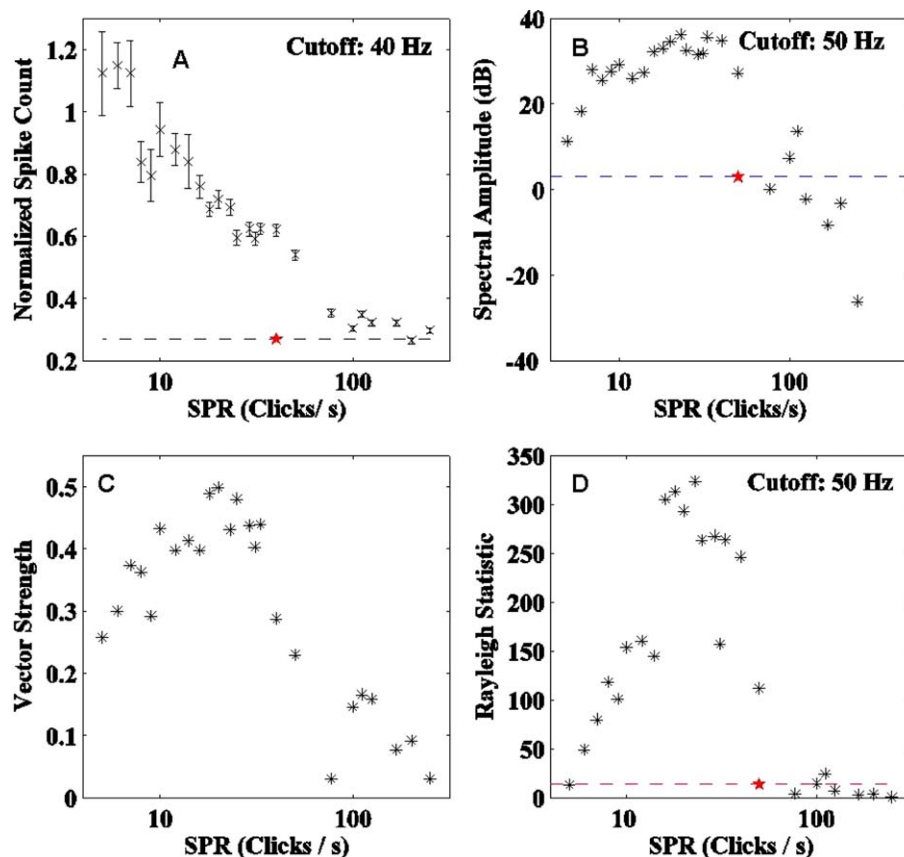


Fig. 2. RRTF for each of the three methods on the example MUC in Fig. 1. The stars denote the synchronization boundary for each of the methods. The MUC appears to have a lowpass response using the window analysis (A). The error bars in A are standard error of the mean. The circular, spectral and vector strength analyses have bandpass responses with peaks at ~ 20 Hz (B–D). Vector strength is provided for comparison with previous studies.

of the SPR frequency are harmonics that result due to the binary nature of the spike data (binned ones and zeros). The maximum spectral amplitude occurs at an SPR of 25 Hz and drops off with higher SPRs (see Fig. 3).

3.2. Group results

Multi-unit recordings were obtained from 140 channels in 10 Sprague–Dawley female rats in response to click trains. Of the 140 channels, 113 were defined as driven (see Section 2). Fig. 4 displays the mean RRTFs for the group using the three analysis techniques. The RRTF for the window analysis (4A) and spectral analysis (4B) are lowpass, while the circular statistic analysis (4C) is band-pass with a peak around 20 Hz. Window analysis (4A) shows a rapid drop-off in spike rate for SPRs above 10 Hz while the spectral analysis and circular begin to drop off above 20 Hz. The mean SB for the group using the window analysis (4A), spectral analysis (4B), and circular statistics (4C) were 51, 72 and 71 Hz, respectively.

An ANOVA performed on the measured SB using the three methods resulted in a p -value $\ll 0.001$. Paired t -test on the data revealed that the mean SB calculated by the window analysis was significantly lower than with the window and circular analyses ($p \ll 0.01$). Circular and spectral analyses were not significantly different from one another. Fig. 5 displays a histogram of the SB for all 113 channels for all three methods. Window analysis provides the most conservative estimate of the SB, which measured 75% of MUCs as having SBs at 50 Hz or less. Over 50% of the MUCs had SBs equal to or greater than 50 Hz using circular statistics and spectral analysis. Over 35% of MUCs had SBs of 100 Hz or greater using the circular and spectral analyses, while only 6% were 100 Hz or greater using the window analysis.

Fig. 6 displays the combined PSTH for all of the MUCs. The spike times for all MUCs were combined for each SPR. Stimulus locking (peaks following the click) can be seen for SPRs up to 125 Hz. SPRs greater than 125 Hz appear to have small peaks at irregular intervals. For SPRs

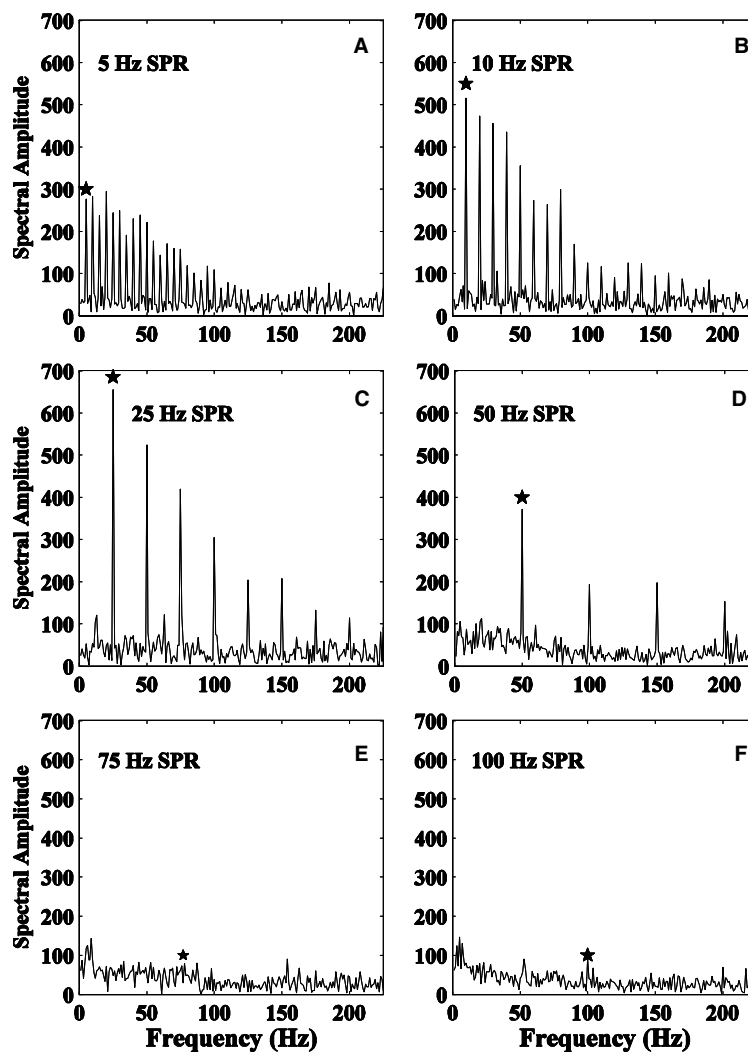


Fig. 3. Spectral analyses of all 10 repetitions on the example MUC for SPRs of 5, 10, 25, 50, 76, 100 Hz, respectively. The peaks to the right of the SPR frequency are harmonics that result due to the binary nature of the input data. The stars denote the SPR.

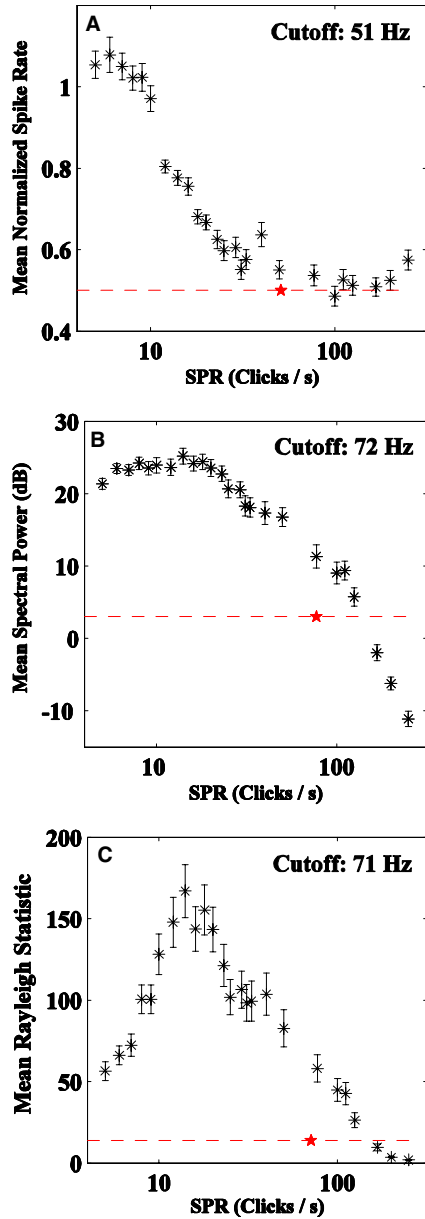


Fig. 4. An analysis of all 113 driven units using window, circular and spectral analyses is shown in A, B and C, respectively. Error bars are standard error of the mean. The stars denote the SB calculated from the entire population. These results are very similar to those seen with the MUC in Fig. 3. The SBs were determined to be 51, 72 and 71 Hz using the three techniques.

above 77 Hz, the onset response appears to be prolonged typically lasting 50–60 ms.

Fig. 7 displays a spectral analysis performed on the Group PSTH. The SB for the summed response of the entire population is 166 Hz compared to 72 Hz for the mean of the individual responses. This result is not surprising given that 35% of the MUCs have SBs of 100 Hz or greater using spectral analysis.

Based on an examination of the group PSTH in Fig. 6 it appears that the response to each tone becomes longer as the SPR increases. Period PSTHs were created for 6 SPRs

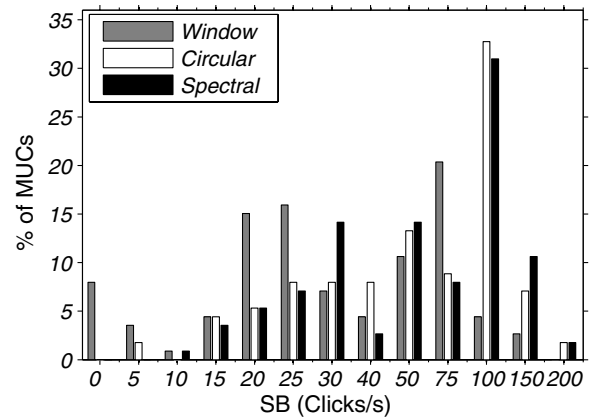


Fig. 5. Distribution of synchronization boundaries using all three techniques. The mean increases when comparing the window analysis to the circular and spectral. The data were placed in the following bins (0, 1–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–40, 41–50, 51–75, 76–100, 101–150, and 151–200 ms).

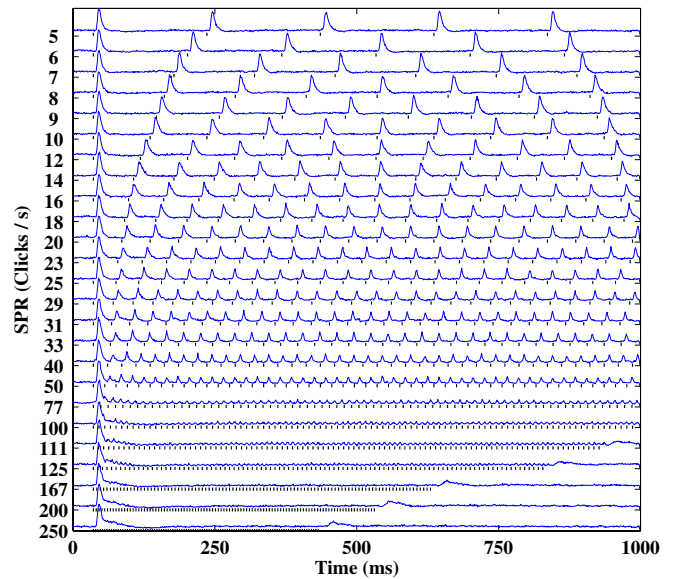


Fig. 6. The PSTH from all 113 driven channels. The dashed lines represent the click presentation. Clear peaks following each tone can be seen for rates up to 167 Hz. A protracted response to the first click appears at 50 Hz and becomes larger up to the highest SPR used in this study.

by combining responses from all of the MUCs to provide a graphical representation of the temporal changes. The period PSTH is shown for 0.5, 5, 10, 20, 50, and 76 Hz (Fig. 8). Both the average response strength and average response duration appear to decrease as the SPR increases.

Fig. 9 displays the mean temporal response properties for the group as a function of repetition rate including the mean peak rate, mean duration, mean latency, mean time-to-peak. Measures of the temporal properties could not be made above 77 Hz because the responses began to overlap, making accurate identification of the temporal features difficult. However, this SPR range appears to provide a meaningful description of the changes. It should be noted that these responses are for the entire population and

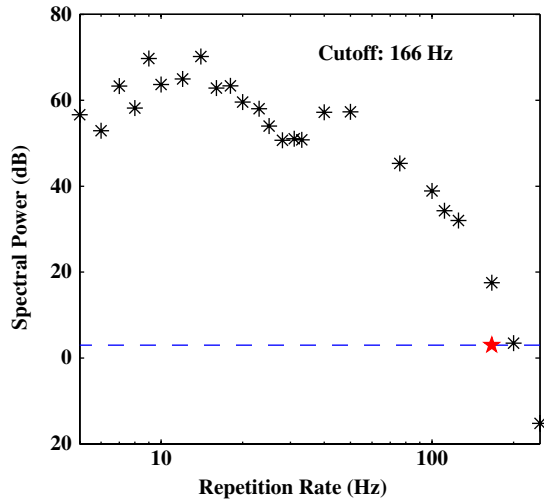


Fig. 7. Spectral analysis performed on the group data. This analysis used the summation of all spike times binned in 500 ms bins. The spectral analysis determined the SB at 166 Hz for the group. The dashed line represents the mean spectral amplitude outside the SPR range with the star denoting the SB.

consist of heterogeneous response properties. These results do not fully describe the changes of individual neurons.

Fig. 9A displays the change in the mean peak response rate as a function of SPR. The mean peak response rate

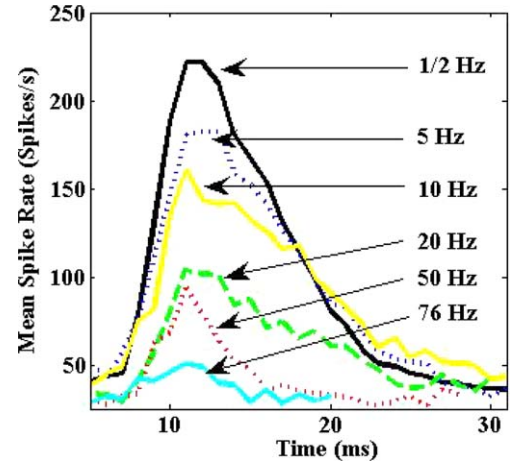


Fig. 8. The period PSTH for clicks 2–5 for six repetition rates from all 113 MUCs. The figure displays a shift in onset latency, peak time, a decrease in response strength and a decrease in response duration as the SPR increases.

is stable for SPRs up to 10 Hz. Above 10 Hz, the mean peak response rate decreases logarithmically. As the stimulus presentation rate increases above 14 Hz, the mean response duration decreases from 15 to 4 ms (Fig. 9B). The mean latency is stable for SPRs below 20 Hz (ANOVA: $p > 0.89$) (Fig. 9C). Above 20 Hz, the mean

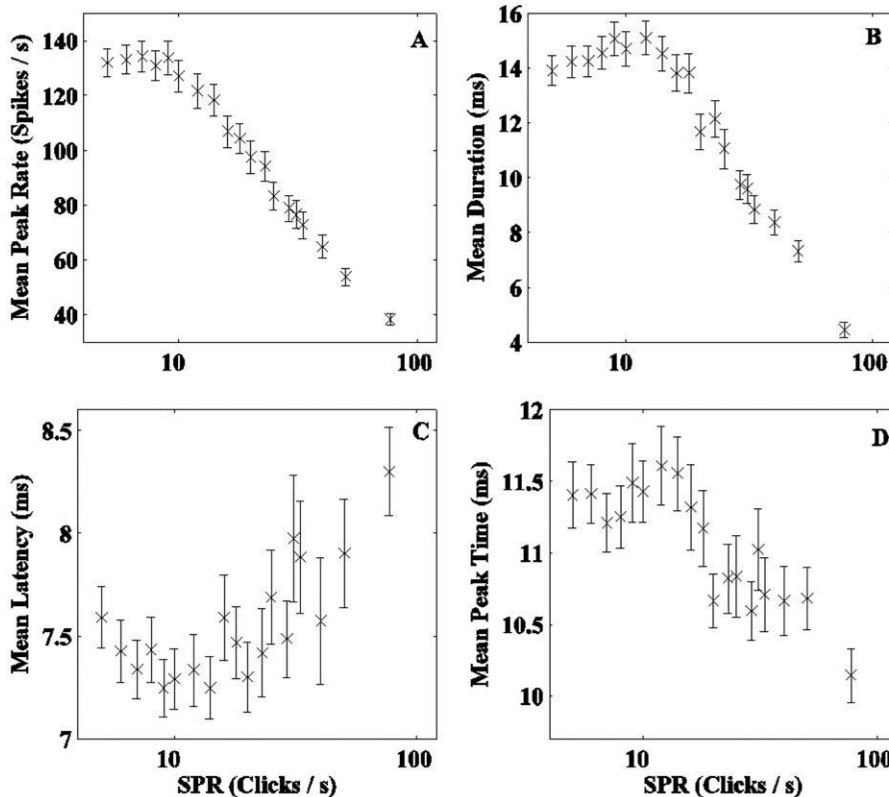


Fig. 9. Changes in the mean temporal PSTH measures for all 113 driven MUCs (error bars are standard error of the mean). (A) Mean peak spike rate decreases logarithmically for SPRs above 10 Hz. (B) Mean duration decreases from ~14 to 5 ms (ANOVA: p -value < 0.001). (C) The mean latency increases from 7.5 to 8.25 ms at 100 Hz. The change in latency was not significant (ANOVA: $p = 0.05$). (D) Mean time to the peak decreases from ~11.5 to 10.5 ms. The change in peak time was significant (ANOVA: p -value < 0.01).

latency increased from approximately 7.5 to 8.25 ms; although, the change in mean latency is not statistically significant (ANOVA: $p > 0.23$). The mean time-to-peak is lowpass with a significant decrease occurring for SPRs above 18 Hz (Fig. 9D). The mean time-to-peak decreased from 11.5 to 10.2 ms (ANOVA: $p < 0.01$). A significant change in the mean time-to-peak was not observed below 18 Hz SPR (ANOVA: $p > 0.95$).

Fig. 10 shows a scatter plot of the SB as a function of the mean peak spike rate for the 5-Hz SPR. A significant positive correlation exists between spike rate and the synchronization boundary using all three analysis methods (ANOVA: $p \ll 0.01$). This demonstrates that those MUCs that respond strongly to the clicks typically have the highest SB as previously reported. Significant negative correlations are seen between mean latency and mean time-to-peak ($p < 0.01$ and $p < 0.001$, respectively) with the SB. However there is no clear correlation between the characteristic frequency or response duration and the SB.

Previous studies established that a subpopulation of neurons in auditory cortex encode temporal information nonsynchronously by increasing tonic firing rates with SPR. Fig. 11 displays three nonsynchronous measures for the population. The mean spike rate for the group significantly increases during the first 50 ms following the first click in the stimulus train. The spike rate remains stable for SPRs below 20 Hz. Above 20 Hz, the spike rate increases significantly from 180 spikes per second up to 260 spikes per second. Analysis of the entire train duration did reveal an increase with SPR for rates above 100 Hz. Further analysis of the nonsynchronous response from 50 to 450 ms following the start of the click train reveals that there is not an increase in the population spike rate with SPR (Fig. 11 “400 ms”). Therefore it appears that the increase with SPR for the population is due to a decrease in the total train duration (time to present 100 clicks) with SPR and the inclusion of the nonsynchronous response

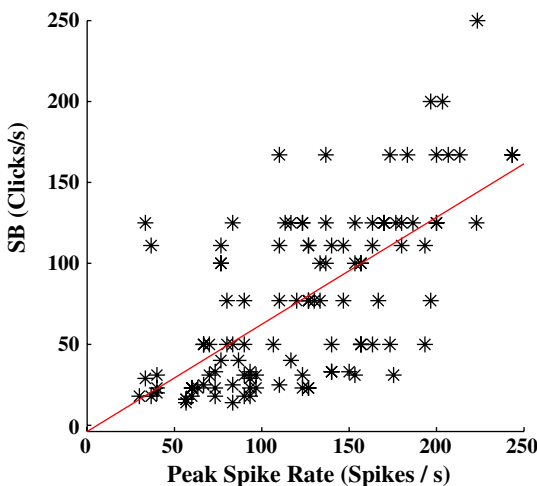


Fig. 10. A scatter plot of the SB as a function of the mean peak rate to the slowest rate for all 113 driven channels. A significant correlation exists between the peak rate and the cutoff rate (ANOVA: p -value $\ll 0.001$).

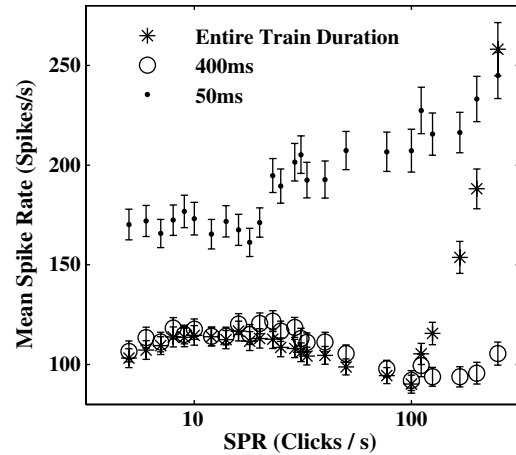


Fig. 11. Nonsynchronous responses for three different time periods. Spike rate significantly increases with SPR during the first 50 ms of the tone train. Additionally, spike rate increases with SPR during the entire tone train duration. However, analysis of the time from 50 to 450 ms does not exhibit a significant increase in spike rate with SPR.

during the first 50 ms. Caution must be taken when interpreting these results. The data are from 113 multi-unit clusters and not single units. The heterogeneous nature of the MUCs does not allow for conclusive findings on the lack of nonsynchronous coding. Only 8% (9/113) of MUCs exhibited significant increases in the sustained firing rate during the period 50–450 ms following the start of the click train. Cells with nonsynchronous responses could have been masked by other cells on the same channel. In fact, 34% of MUCs exhibited significant decreases in the tonic firing rate in the period from 50 to 450 ms as the SPR increased. The interaction of these two responses could be responsible for the lack of excitatory nonsynchronous responses seen in this study.

4. Discussion

This study examines the ability of multi-unit neurons in primary auditory cortex of the awake rat to respond to a series of broadband click trains. First, the results demonstrate that multi-unit clusters in layer IV/V of rat primary auditory cortex are capable of synchronizing to clicks at rates up to 166 Hz. The mean synchronization boundary (SB) was found to be between 51 and 72 Hz depending upon the analysis technique used. Second, the temporal response properties of the multi-unit clusters (MUCs) change with stimulus presentation rate (SPR). Specifically, mean peak response rate, mean response duration, and mean time-to-peak response decreased as the SPRs increased above 50 Hz resulting in temporal sharpening of the population response. Third, for SPRs greater than 50 Hz, the MUCs exhibited increases in firing rate during the first 50 ms. During the first 50 ms the response rate increased with SPRs above 20 Hz for 50% of MUCs (ANOVA: $p < 0.01$). MUCs exhibited increases (8%) and decreases (38%) in tonic firing rates (ANOVA: $p < 0.01$)

during the period from 50 to 450 ms from the start of the click train. The heterogeneous nature of multi-unit clusters makes it impossible to determine if a larger portion of the cells were exhibiting excitatory nonsynchronous responses. It is possible that tonic inhibitory responses masked tonic excitatory responses on the same channel. However, the group data do demonstrate that a third class of inhibitory nonsynchronous responses was present in layer IV of the rat auditory cortex. This finding has not been previously reported.

4.1. Comparison with previous studies

While it has been well demonstrated that the spectral content is coded by the tonotopic organization of the auditory system (Eggermont, 1991; Kilgard and Merzenich, 1999; Sally and Kelly, 1988; Schreiner, 1992), the ability of the auditory system to code temporal properties is less well understood. Humans are capable of distinguishing gaps in noise bursts with intervals as low as 2 ms (Plomp, 1964; Ronken, 1970). It has been shown that rats are also capable of detecting gaps as short as 2 ms and that primary auditory cortex is required to perform at this resolution (Ison et al., 1991; Syka et al., 2002). Synchronization boundaries in the cortex are typically reported at 100 Hz. If cortical cells are responsible for gap detection as demonstrated, rats should only be able to detect a gap 10 ms or greater in duration (Ison et al., 1991; Syka et al., 2002). Given the behavioral results, there must be some mechanism in the cortex for encoding temporal features on the order of a few milliseconds.

Previous studies on temporal processing have reported SBs in primary auditory cortex ranging from 10 to 300 Hz in various species and preparations (De Ribaupierre et al., 1972; Eggermont, 1991; Gaese and Ostwald, 1995, 2003; Kilgard and Merzenich, 1999; Liu et al., 2003; Lu and Wang, 2004; Phillips et al., 1989; Schreiner et al., 1997). It has been shown that synchronization boundaries in anesthetized subjects are lower than awake subjects (De Ribaupierre et al., 1972). The maximum SBs reported in this study are comparable with the values published on unanesthetized subjects (166 Hz) (De Ribaupierre et al., 1972; Lu et al., 2001; Steinschneider et al., 1980).

A previous study in pentobarbital anesthetized rats found that with an SPR of 13 Hz the average response rate dropped to 50% of the response rate to the tone in isolation (Kilgard and Merzenich, 1999). The result of the current study found that the response rates were 50% of the response in isolation at ~25 Hz, demonstrating a two-fold increase in the cutoff (using this measure) compared to the anesthetized rat. While this difference could be attributed to the auditory stimuli, (tones vs. clicks) it is likely that the effect is a result of the anesthesia.

The perception of repetitive stimuli changes with SPR. At low SPRs, the stimuli are perceived as discrete events, but as the SPR increases above 10–15 Hz, the stimuli begin

to fuse. At rates between 40 and 250 Hz the perception of a pitch associated with SPR arises (Miller and Taylor, 1948). A similar transition is reported with amplitude modulated stimuli (Joris et al., 2004). A goal of temporal coding studies is to examine the neural correlates underlying changes in perceptual quality with SPR.

While the SB provides a measure of the maximum following rate of cells in the cortex, it does not lend insight into gradual changes in the response dynamics that lead to those perceptual changes. Based on previous results (Lu and Wang, 2004), it is possible that the gradual transition from synchronous to nonsynchronous firing accounts for the perceptual changes. At slow stimulus presentation rates, cells respond in phase to the stimuli, but as the stimulus presentation rate increases, more cells begin to respond nonsynchronously while fewer cells are synchronized.

In order to provide more insight into the neural basis of perceptual changes, this study measured the effects of increasing the SPR on several features of the population peri-stimulus time histogram. The mean PSTH response properties of the MUCs are stable for SPRs below 15–20 Hz. As the SPR increases above 15 Hz, the mean population response strength decreases, the mean response duration decreases sharply from 15 to 4 ms, and the mean time-to-the-peak response shifts 1 ms closer to the onset response. This change in the temporal response properties could lead to a more complete explanation of the perceptual changes resulting from increased stimulus presentation rate. The population of cells responding at high SPRs responded with fewer spikes, but in a much shorter time frame (4 ms vs. 15 ms). Down stream feature detectors may see this input as more temporally precise and could provide a mechanism for distributed synchronous coding. Each cell may respond with a few spikes, but input from thousands of cells in a 4-ms window could sum temporally to encode high SPRs. It may be this temporal shift that results in the perceptual transition from discrete events, through roughness, to continuous stimuli. It is interesting to note that the temporal changes begin to occur at the same stimulus presentation rate that perceptual fusion occurs.

Another question that is motivated by these results is what mechanism is responsible for the temporal sharpening. It is possible that the temporal sharpening is the result of intrinsic properties of the cells, or network interactions, or some combination of the two. A recent study in ketamine anesthetized rats using a paired pulse paradigm found that decreases in the following rate for ISI greater than 100 ms resulted from synaptic depression rather than persistent changes in membrane conductance (Wehr and Zador, 2005). They reported that the response to the second stimulus was completely inhibited by GABA currents if the inter-stimulus-interval is less than ~100 ms. Given these results, it would be expected that cortical cells should significantly decrease their response strength to stimuli presented faster than 10 Hz.

Bandrowski et al. demonstrated that AMPA/Kinate receptors are necessary for neurons to entrain to stimuli presented faster than 10–20 Hz. Ketamine is known to reduce slow excitatory glutamatergic *N*-methyl-D-aspartate (NMDA) receptor currents, but also reduces the fast excitatory post-synaptic potential component mediated by non-NMDA receptors (Ampa/Kinate) (Leong et al., 2004). It is therefore likely that the ketamine decreases the ability of neurons to entrain to stimuli presented at rates faster than 10–20 Hz.

It has been shown that EPSP from neurons in auditory cortical slices were able to entrain to a series of electrical stimuli at rates up to 100 Hz (highest rate tested) (Bandrowski et al., 2002). Blockage of metabotropic Glutamate (mGLUR) receptors with MCPG prevented the decrease in amplitude of the EPSP for successive stimuli. This result suggests that presynaptic mGLUR are in part responsible for the decreased following rate with increases in SPR.

4.2. Nonsynchronous coding

The nonsynchronous response measures shown in Fig. 11 demonstrate that while there is an increase in firing rate with SPR during the entire duration of the tone train, this effect is due to increases in the response during the initial 50 ms of the train and a decrease in the total duration of the train (maximum of 100 clicks). By looking at the period from 50 to 450 ms, these artifacts can be removed. The mean spike rate was measured from 50 to 450 ms following the start of each click train. The plot of mean response rate during 50–450 ms following the start of the tone train does not exhibit an increase in firing rate with SPR. Only 8% of MUCs exhibited significant increases in firing rate during this period.

Lu and Wang proposed a two-stage processing model for coding repetition rates (Lu et al., 2001). Two major classes of responses to click trains were described: synchronized and nonsynchronized. The nonsynchronized class has a high-pass function which increased their firing rate with the stimulus presentation rate. These cells use increases in tonic spike rates to encode stimulus rates above 20 Hz (Wang et al., 2003). The nonsynchronous responses in this study were seen for SPRs above ~50 Hz and increased with SPR. Lu et al. found that single units were either synchronous or nonsynchronous; the single units did not exhibit both coding schemes [Lu;Wang]. The MUCs in this study exhibited both synchronous and nonsynchronous responses. This difference is likely due to the summation from the two populations on the same electrode.

Over 50% of the MUCs exhibited significant increases in nonsynchronous activity, but only during the first 50 ms of the tone train. Nine of the of the 113 (8%) MUCs exhibited sustained excitatory nonsynchronous responses lasting the entire duration of the tone train versus 55% of single units seen in the previous study (Lu et al., 2001). Another 33% of single units exhibited significant decreases in the tonic firing rate with SPR. These two responses on the same electrode

would compete and conceal significant nonsynchronous responses. Given the large percentage of sustained inhibitory responses it is highly likely that this is the case in some percentage of MUCs in this study.

Lu et al. recorded from superficial layers, whereas recordings in this study were from layers IV/V. It is possible that some of the disparity is the result of columnar processing differences similar to that reported in visual cortex. Cells in visual cortex have on-center and off-center receptive fields in layer 4C. Cells in superficial or deeper layers of the same cortical column display simple and complex receptive fields that detect orientations or movement. It is possible that processing in the cortical columns of auditory cortex are transforming the data from synchronous to nonsynchronous responses.

4.3. Analysis methods

Three statistical analyses were performed on the data in this study to allow a comparison with other studies. These measures were compared using ANOVA and paired *t*-tests. The results of this analysis demonstrated that the mean SB were 51, 71, and 72 clicks per second using the window, circular statistics and spectral analyses, respectively. The window analysis generated statistically significantly lower mean SB values than the circular and spectral analysis methods. The measured mean SB values are not different between the circular and spectral analysis. The variation in SBs using different analysis techniques is consistent with previous findings (Eggermont, 1991).

Subjectively, a comparison of the three analysis methods created one main concern. Circular statistics regularly generated Rayleigh statistics greater than 1000 as reported by other researchers (Lu et al., 2001). Typical SB values using circular statistics are two or three orders of magnitude greater than the statistically significant boundary of 13.8 ($p = 0.001$). One possible explanation for this finding is the large number of spikes from awake subjects. To calculate the Rayleigh statistic, the square of the vector strength is multiplied by twice the number of spikes. Given the high spontaneous rates in the awake rat, the Rayleigh statistic could be overestimating the significance of the data (Eq. (1)).

Another method for determining the synchronization boundary is a window technique (Kilgard and Merzenich, 1999). The number of spikes occurring in a window of fixed size and time following each stimulus are counted and compared to spontaneous spike rates for determining the synchronization boundary. Our results demonstrate that the window analysis technique is appropriate for the slow repetition rates used in that study. However, at faster rates, the window analysis does not provide an accurate measure of the SB. First, as SPR increases, windows overlap. Second, the temporal response properties of the MUCs change with SPR. As the SPR increases, the mean response duration decreases from 14 ms to less than 5 ms, and therefore, because the window is averaging over a 25-ms period, the significance of the response will decrease as SPR increases.

Development of a window analysis that adjusts window duration with rate could be implemented to minimize this effect. It should also be noted that the window analysis would generate spurious results with sustained nonsynchronous responses and should not be used in those cases.

Previous studies have demonstrated notched, bandpass and lowpass response profiles (Eggermont, 1991; Kilgard and Merzenich, 1999; Lu et al., 2001; Schreiner et al., 1997). In this study, circular statistics generated a bandpass RRTF while the window analysis and spectral analysis are lowpass. The drop at low SPRs with circular statistics is likely an artifact resulting from this method (Eggermont, 1991). Spike counts do not drop for SPRs below 10 Hz using the window analysis. Therefore the bandpass response is a function of a change in the temporal properties of the MUCs and not changes in spike rates below 20 Hz. An examination of the raster plots provides some insight into this effect. Circular statistics are based upon the vector strength, where each spike represents a vector of length one around a unit circle. One revolution is equal to the inter-click-interval (ICI). A visual inspection of the raster plots reveal an inhibitory period following the response to the first click that lasts for approximately 60–100 ms (Fig. 2). Following the inhibitory period the MUCs return to their spontaneous rates. For SPRs with ICIs greater than the inhibitory period, the return of spontaneous activity reduces the mean vector strength (Fig. 5D) (Eggermont, 1991).

In summary, the findings of this study suggest that the rat may also use a two stage mechanism for coding sequences of broadband stimuli. Stimuli presented at rates below 20–50 Hz are coded by responses synchronized to the stimulus. Rates presented above ~50 Hz are encoded by either transient increases in firing rate during the first 50 ms, or by sustained changes in the firing rate (increases or decreases) during the stimulus train. Changes in the population response duration, peak response and time-to-peak also suggest a temporal sharpening of the population with increases in SPR. It is possible that this temporal sharpening could be used by populations of cells to synchronize downstream inputs to code fast SPRs. The multi-unit responses provide a view of population coding and not single units. The data presented represent the mean response for the population. Heterogeneous responses from single units are hidden by this method and therefore this study does not provide a complete picture of individual unit coding of SPRs.

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