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Gamma Oscillations in the Auditory Cortex of Awake Rats

Paulo Vianney-Rodrigues^{1,2,*}, Ovidiu D. Iancu³, and John P. Welsh⁴

¹Dept. of Physiology and Pharmacology Oregon Health Sciences University 3181 S.W. Sam Jackson Park Rd. Portland, Oregon 97239-3098

³Dept. of Biomedical Engineering Oregon Health Sciences University 3303 SW Bond Avenue Portland, OR 97239

⁴Seattle Children's Research Institute Center for Integrative Brain Research 1900 9th Ave Seattle, WA 98101

Abstract

Numerous reports of human electrophysiology have demonstrated gamma (30–150 Hz) frequency oscillations in the auditory cortex during listening. However, only a small number of studies in non-human animals have provided evidence for gamma oscillations during listening. In this report, multi-site recordings from primary auditory cortex (A1) were carried out using a 16-channel microelectrode array in awake rats as they passively listened to tones. We addressed two fundamental questions: 1) Is passive listening associated with an increase in gamma oscillation in A1?; if so, 2) Are A1 gamma oscillations during passive listening coherent within local networks and/or over long distances? All sites within A1 showed a short-latency burst of activity in the low-gamma (30–70 Hz) and high-gamma (90–150 Hz) bands in the local field potential (LFP). Additionally, 53% of sites within A1 also showed longer-latency bursts of gamma oscillation that occurred episodically for up to 350 ms after tone onset, but these varied both in latency and occurrence across trials. There was significant coherence in the low-gamma band between spike activity and the LFP recorded with the same electrode. However, neither LFPs nor the spike activity between sites spaced at least 300 μ m apart showed coherent activity in the gamma band. The experiments demonstrated that gamma oscillations are present, but not uniformly expressed, throughout A1 during passive listening and that there is strong local coherence in the spatiotemporal organization of gamma activity.

Keywords

oscillations; sensory processing; auditory system; *Rattus norvegicus*

Introduction

Gamma oscillations are induced in the temporal lobe of humans passively listening to sequences of clicks played at 40 Hz (Galambos, 1981). Gamma oscillations are also induced in humans listening to transient tones and phonemes (Edwards *et al.*, 2005), discriminating pure tones and phonemes (Crone *et al.*, 2001), during focused attention (Tiitinen *et al.*, 1993), temporal binding of successive sensory events (Joliot *et al.*, 1994), and phantom perception of tinnitus (Weisz *et al.*, 2007). Clinical observations in patients with schizophrenia, psychosis, and autism have led to the hypothesis that gamma oscillations may

*Corresponding author phone: 971-221-4805 // Fax: 215 898 7504 paulor@mail.med.upenn.edu.

²Current address: University of Pennsylvania Medical School Department of Otorhinolaryngology 3400 Spruce - 5 Ravdin Philadelphia, PA 19104

be compromised in psychiatric disorders (Welsh *et al.*, 2005; Uhlhaas & Singer, 2006; Welsh *et al.*, 2010).

Despite the large number of reports showing that sounds induce low-gamma (30–80 Hz) and high-gamma (90–150 Hz) frequency oscillations in the auditory cortex of humans, there are only limited studies exploring such oscillations in the auditory cortex of experimental animals. In a conflicting literature, some experiments were unable to demonstrate that tones trigger gamma oscillations in awake rats (Cotillon-Williams & Edeline, 2003) or bats (Horikawa *et al.*, 1994). Yet, other experiments showed that social calls or tones triggered prominent gamma oscillation in the A1 of awake bats (Medvedev & Kanwal, 2008) and awake monkeys (Steinschneider *et al.*, 2008).

The fundamental reasons for the different outcomes have not been immediately obvious. Studies in gerbils and cats (Lakatos *et al.*, 2004; Jeschke *et al.*, 2008) suggested that gamma oscillations within auditory cortex may be modulated by attention and arousal. Other studies have suggested that neuronal synchrony and oscillation is more readily detected in LFPs and in multi-unit activity than in single-unit recordings (DeCharms & Merzenich, 1996; Fries *et al.*, 2001, 2008; Wommesdorf *et al.*, 2006). Thus, if gamma oscillations are not spatially widespread within A1 but are restricted to local networks, as has been suggested by *in vitro* and theoretical studies (Oswald *et al.*, 2009), they could be missed in single-unit recordings and especially in subjects that are not aroused, alert, or attentive.

The purpose of the experiments was to address two fundamental questions in awake and alert rats: 1) Is passive listening associated with an increase in gamma oscillation in auditory responses from A1?; if so, 2) Are gamma oscillations during passive listening coherent within local networks and/or over long distances within A1? We employed a multiple-microelectrode approach that allowed simultaneous recordings of local field potentials (LFPs) and multiunit spike clusters (MSCs) from up to 16 sites within A1. Power spectrum analysis was used to determine whether gamma oscillations are induced by a tone during passive listening (Question 1) and coherence analysis was used to determine the spatial extent of gamma oscillation synchrony during passive listening (Question 2). Our findings demonstrated that passive listening is associated with an increase in gamma oscillation within A1 that is highly variable in prevalence and timing and that the synchrony of gamma oscillations during passive listening within A1 is largely restricted to local networks.

Methods

The basic paradigm was to obtain unilateral multi-site recordings from A1 of awake rats using acutely implanted electrodes as they listened to a free-field pure tone.

Animal preparation

The subjects were 9 Sprague-Dawley albino rats (Taconic Farms, Germantown, NY). Protocols were approved by the Oregon Health Science University Institutional Animal Care and Use Committee. Three days before the recordings, rats were anesthetized with ketamine (66 mg/kg) and xylazine (13 mg/kg) and a 1.5 mm diameter craniotomy was made at the stereotaxic coordinates of A1 (A/P: −4.5 mm; M/L: 7.5 mm; D/V: 3.0–4.0 mm from Bregma) and sealed with silicone. A stainless steel electrode inserted into the frontal cortex was used for a reference. An acrylic cement head cap allowed the skull to be fixed in a stereotaxic frame without earbars (Kopf Inc., Model 880). Two days post-surgery, the awake rats were adapted to the apparatus. A plastic restrainer was used to reduce body movement. Recordings were carried out after the rats learned to sit calmly in the apparatus. The rats were monitored visually through a Plexiglas window throughout the experiment to ensure that they were alert during the recordings. Alertness was confirmed by examining the

reactivity of the rat to gentle stimulation of the tail as well as the depth EEG from the temporal lobe in real-time to ensure that it did not demonstrate slow delta waves characteristic of sleep and drowsiness.

Stimulus generation and setup

Tones were generated by a computer-controlled function generator (Hewlett-Packard, model 32120A) attached to an attenuator (Hewlett-Packard, model 350D) that was coupled to a tweeter speaker (P. Audio, model PHT409). Sound intensity was calibrated with a 6.4 mm diameter condenser microphone (Bruel & Kjaer, model 4135) placed 25 cm in front of the speaker and coupled to a sound level calibrator. Recordings were conducted in a sound-attenuated chamber inside a Faraday cage. Sound attenuation (-30 dB) was produced by 133-mm thick, Sonex-lined Plexiglas.

Neural recordings

Neural activity within A1 was recorded with an array of 16 insulated microelectrodes fabricated from 100- μ m diameter tungsten rod (AM Systems, Inc., No. 7190), electrochemically etched to a point (2–8 M Ω , Schwarz & Welsh, 2001; Johnson & Welsh, 2003). The microelectrodes were configured into a 4 $\times$ 4 array having an inter-electrode distance of 300 μ m to enable recording from 16 sites in approximately 1 mm². Each microelectrode was moved independently in 10 μ m steps (Alpha-Omega EPS system, Nazareth, Illit, Israel) until action potentials were isolated. Recordings were performed by a MultiNeuron Acquisition Processor (Plexon, Inc., Dallas, TX, USA). Action potentials were amplified 5,000 to 15,000 times, filtered between 0.4 and 8 kHz, and sampled at 40 kHz. The LFP signals were filtered between 3–170 Hz and 12 bit digitized (PCI-6071E, National Instruments) at 1 kHz in register with the spikes.

A 2-Hz stream of pure tone bursts (50 ms, varied between 1–30 kHz) was used to search for and isolate multi-unit activity as the 16 microelectrodes were individually inserted into A1. After all of the microelectrodes in the array were positioned to record as many short-latency (< 20 ms) MSCs as possible, the experiment was carried out by delivering 10-kHz pure tone bursts (20 ms, 75 dB) semi-randomly every 30 (± 5) sec. That the recordings were from A1 was confirmed in the initial recording sessions by identifying prominent frequency tuning. The stimuli used to measure frequency tuning consisted of 10 iterations each of 1 to 30 kHz pure tones (50 ms) ranging from 20 to 75 dB. Off-line sorting was used to separate spikes from background and to isolate single-units and MSC (Off-Line Sorter software, Plexon). Previous reports demonstrated that single-units are less optimal than MSCs for detecting synchrony among A1 neurons (DeCharms & Merzenich, 1996; Fries *et al.*, 2001, 2008 Womesdorf *et al.*, 2006). Therefore, we restricted the spike analysis to MSCs consisting of 2 to 4 neurons.

Data set

The analysis was restricted to 63 LFP sites from 9 rats that showed the characteristic middle latency auditory evoked potential (MLAEP) and 63 MSC sites from 7 rats that met the criterion of having short response latencies (< 20 ms) to pure tones between 5–20 kHz. Successful simultaneous recordings of a LFP showing the MLAEP and a short-latency MSC response occurred in 51 sites. For technical reasons, 12 LFP sites showing a prominent MLAEP were recorded without an MSC and 12 MSC sites with short-latency responses were recorded without LFPs.

Data epochs of 1600 ms duration (500 ms of baseline and 1100 ms post tone onset) were analyzed. Data selection and pre-processing was performed with Neuroexplorer software (Nex Technologies, Littleton, MA, USA) and Matlab (The MathWorks Inc., Natick MA,

USA). All of the LFP trials were visually inspected off-line and individual trials contaminated by movement artifacts identified by activity bursts that saturated the amplifiers were identified and discarded. Power spectrum analysis was used to discard channels that showed 60-Hz line noise in the LFP. Frequency bandwidths of interest were defined as low-frequency (1–30 Hz), low-gamma (40–70 Hz), and high-gamma (90–150 Hz).

Power spectrum density (PSD) analysis

PSD analysis was used to test the hypothesis that gamma activity would be evoked in A1 during passive listening in the awake rat. Spectral analysis was applied to both LFPs and MSCs.

Spectral analyses were performed with the Chronux Matlab toolbox (www.chronux.org) which applied the multitaper method to perform PSD analysis (Precht *et al.*, 1997; Mitra & Pesaran, 1999; Halliday & Rosenberg, 1999; Jarvis & Mitra, 2001; Pesaran *et al.*, 2002, Mitra & Bokil, 2008). The multitaper method utilized an optimal set of orthogonal tapers (Slepian functions) that have optimal variance and bias properties before applying the fast Fourier transfer algorithm. The Slepian functions were concentrated in a specific time duration (T) and frequency bandwidth (W). For each value of T and W, a maximum of $K=2TW-1$ tapers was used. Each data epoch was multiplied for each of the orthogonal tapers and then Fourier transformed. The PSD is the magnitude-square of the Fourier transform with the units of volts^2 for the LFP and spikes/s^2 for MSC.

To compute the power spectra of LFPs, a time bandwidth product of 3 and 5 Slepian taper functions was used in 200 ms data epochs, which concentrated the spectral estimate in approximately 16 Hz. Changes in the LFP power evoked by the tone were calculated by normalizing to a pre-tone (100–430 ms) baseline period, according to the following equation: $(\text{evoked power}-\text{baseline power})/(\text{evoked power}+\text{baseline power})$. The normalization procedure created a modulation index in which a positive value indicated an increase in power relative to the pre-tone period, while a negative value indicated a decrease in power relative to the pre-tone period.

Changes in the temporal patterns of MSC were also examined by PSD analysis and supplemented by autocorrelation. Autocorrelograms (1 ms bins) were calculated from the spike trains, normalized by the number of spikes in the train, and smoothed with a 5 ms Gaussian window. Spike spectrograms were computed using 200 ms overlapping windows in 5 ms steps and normalized by the firing rate. The normalized PSD of MSC was used to compare the temporal pattern of the spike trains between experimental groups. The MSC PSDs were normalized using the same equation for normalizing the PSDs of LFPs. Because the sparse firing of A1 neurons during the pre-tone period precluded normalizing to a baseline, the PSD of each MSC was normalized to an artificial neuron with a matching firing rate but whose spike activity had a Poisson distribution.

Coherence analysis

Coherence analysis was used to test the hypothesis that passive listening would be associated with an increase in correlated gamma band activity in A1. Coherence analysis between the different recording configurations was used to contrast changes in gamma band synchronization at long-range with those occurring within local networks.

Coherence is the cross-spectrum of two signals normalized by the product of the spectra of each signal and was used to measure the correlation between two signals as a function of frequency. Coherence values provided a normalized linear association of phase and amplitude between two signals ranging from 0 (independence) to 1 (constant phase and

amplitude co-variation; Halliday & Rosenberg, 1999; Mitra & Bokil, 2008). Coherence analysis of LFP recordings (LFP-LFP coherence) and MSC recordings (MSCMSC coherence) was used to determine changes in long-range coherence during passive listening. Coherence functions of LFPs and MSCs recorded by the same microelectrode (LFP-MSC coherence) were used to determine changes in locally-correlated activity during passive listening.

Statistics

Significant differences in values of power were determined using a 2-way permutation analysis of variance (PERMANOVA), which calculates an F statistic without assuming a normal distribution (Anderson, 2001; Anderson, 2005). The factors were experimental Group (2 levels) and Frequency (3 levels). In calculating the PERMANOVA F statistic, 1000 random permutations of the data were employed. *Post-hoc* pairwise PERMANOVA tests were used following a significant main effect of Group or a Group $\times$ Frequency interaction using the Dunn-Sidak correction to a criterion alpha level of 0.05, lowering it to 0.0034. Differences between pre- and post-tone values of power and coherence were determined with a permutation t-test, employing 1000 permutations. To evaluate differences in coherence, significance levels were calculated by estimating the variance using the jackknife error bar method over tapers and trials (Thomson & Chave, 1991; Efron & Tibshirani, 1993; Pesaran *et al.*, 2002). Differences in coherence spectra between experimental groups were determined with the Aversen's jackknife U statistic. Matlab libraries from the Chronux and Fathom toolboxes (www.rsmas.miami.edu/personal/djones/matlab) were used. Significance was set $P < 0.05$. Data were plotted as the mean $\pm$ 1 standard error of the mean (SEM).

Results

General characteristics of A1 recordings

Sixty-three LFP recordings showed the MLAEP response at 14–20 ms post tone onset, which is a defining characteristic of A1 (Fig. 1A). MSCs obtained from 63 microelectrodes showed short latency (< 20 ms) responses to the tone (Fig. 1B). Across the 63 MSC sites, average peak firing occurred 16.2 ms after tone onset. A representative microelectrode within A1 recorded an LFP with peak MLAEP negativity at 15 ms (Fig. 1C), an MSC having a 14.7 ms response latency to a 10 kHz tone (Fig. 1E), and V-shaped frequency tuning to 11 kHz (Fig. 1E). The response characteristics confirmed that the recordings were from A1 (Barth & Di, 1990, 1991; Eggermont & Ponton, 2001; Talwar *et al.*, 2001).

Gamma oscillation in A1 LFPs during passive listening

PSD analysis of LFPs and MSCs was used to test the first hypothesis that passive listening is associated with an increase in gamma oscillation in A1. Band-pass filtering the LFPs revealed that all of the recording sites within A1 showed a burst of gamma activity that was evoked at short latency by the tone in both the low-gamma and high-gamma bands (Fig. 2). The evoked bursts of gamma activity were time-locked to tone onset and were the high-frequency component of the MLAEP (Fig. 2A–C), and therefore termed “gamma activity” bursts. Evoked bursts of gamma activity were always a rapidly damped oscillation. Because the bursts of gamma activity were present at all sites within A1 and were highly stereotypic in onset and phase, they were observed after averaging (Fig. 2B,C).

Fifty-two percent of the LFP sites ($n=33$) showed additional gamma bursts that occurred after the peak negativity of the MLAEP but were not invariantly time-locked to tone onset (Fig. 2D–F). These gamma bursts were distinguishable from the evoked gamma activity burst by being much smaller in amplitude and not always rapidly damped, often showing

similar amplitude over two or more periods and not associated with a fast component of the LFP. Based on those characteristics, we termed these events as “gamma oscillations.” Figure 2D shows a single unfiltered evoked LFP in which the MLAEP was followed by intermittent high frequency oscillations throughout the 350 ms after tone onset (arrows in Fig. 2D). Filtering the wide-band evoked response revealed that the tone induced oscillation bursts in both the low (Fig. 2E) and high (Fig. 2F) gamma bands. The variable onset and amplitude of the induced bursts prevented them from being observed in average records.

Sites that showed induced bursts of gamma oscillation by visual inspection were defined as “oscillatory,” while the remainder was defined as “non-oscillatory.” There were significant differences in the normalized PSD between oscillatory and non-oscillatory sites ($F_{1,188}=16.15$, $P < 0.001$) and across frequencies ($F_{5,188}=6.73$, $P < 0.001$; Fig. 2G). *Post-hoc* tests revealed significantly greater power at the high-gamma and low-frequency bands for oscillatory as compared to non-oscillatory sites (both $P < 0.01$; Fig. 2H). While non-oscillatory sites only showed an increase in power at the low-frequency band, oscillatory sites showed an increase in both the low-frequency and high-gamma bands. Thus, the increase in high-gamma power at oscillatory sites was independent of the increase in power at the low-frequency band.

The prevalence of the induced bursts of high gamma oscillation was variable across sites and trials within A1. Across LFP recordings, the percentage of trials in which tones induced bursts of gamma oscillation ranged from 10–80%. To further quantify the nature of the induced oscillations, 132 tone trials were subdivided into ones that did ($n=66$) and did not ($n=66$) induce oscillations in the LFPs of oscillatory sites. Fig. 2I shows the mean normalized PSD of the LFPs on the two trial-types, indicating that induced oscillations were specifically due to an increase in power at the high-gamma band. PERMANOVA of mean power values indicated a significant effect of trial-type ($F_{1,209}= 8.23$; $P = 0.004$) and *post-hoc* tests indicated that the greatest difference in power between oscillatory and non-oscillatory trials was in the high gamma band ($P < 0.05$; Fig. 2J). Thus, although tones did not induce oscillations on all trials, when oscillations were induced the greatest increase was in the high-gamma band.

Oscillations in A1 MSCs during passive listening

Tones induced oscillatory spiking in MSCs over a much wider range of frequencies than was seen in the simultaneously recorded LFPs. Autocorrelation analysis indicated that the tone-induced spiking of 32% of the MSCs ($n=20$) could be defined as oscillatory on the basis of at least the occurrence of spike doublets at a characteristic interval, and many MSC showed three peaks in the autocorrelogram. The remainder of the MSC ($n=43$) did not show a flanking peak, and was defined as non-oscillatory.

The PSTH of oscillatory and non-oscillatory MSCs showed identical changes in firing rate evoked by the tone (Fig. 3A). However, there were significant differences in the temporal structure of the tone-induced firing between oscillatory and non-oscillatory MSCs as indicated by PSD analysis (Fig. 3B). Statistical comparison of normalized power indicated a significant interaction of group and frequency ($F_{5,188} = 11.13$, $P < 0.001$). *Post-hoc* tests indicated a significant increase in normalized power at all frequency bands for oscillatory as compared to non-oscillatory MSCs (all pairwise comparisons $P < 0.05$; Fig. 3B). Moreover, only oscillatory MSCs showed an increase in gamma power evoked by the tone. Figures 3C and D show examples an oscillatory and a non-oscillatory MSC, respectively, and their comparison to rate-matched neurons having a Poisson spike distribution. As compared to the Poisson PSD, only oscillatory MSCs showed an increase in power at the low-frequency range (asterisk, Fig. 3C).

Examination of individual oscillatory MSCs reinforced the finding that MSC oscillations occurred over a wide range of frequencies. Figure 4 (A–F) shows the MSC of two representative A1 sites. The first site showed a short-latency response at 15 ms post tone onset (asterisk, Fig. 4A), a highly regular 60 ms interspike interval in the 300 ms post tone-onset (arrows, Fig. 4B), and oscillatory activity at 20 Hz in the low-frequency (beta) band, as indicated by the MSC spectrogram (box in Fig. 4C). The second site showed a similar short-latency response (16 ms, asterisk Fig. 4D), a dominant interspike interval of 11 ms (arrowheads, Fig. 4E), and a MSC spectrogram showing increases in power at 30 (beta) and 135 (high gamma) Hz after tone onset (boxes in Fig. 4F). Figure 4G shows the dominant oscillation frequencies for every oscillatory MSC recorded, demonstrating a high degree of variability among the sites. The oscillatory MSCs were equally distributed into 3 groups on the basis of their highest oscillation frequency induced by the tone. The majority of the MSCs (65%) showed an increase in power in the low- and high-gamma bands, and nearly all of those showed a secondary peak of power in the beta frequency band (10–30 Hz).

Local, but not long-range, gamma coherence during passive listening

Coherence analysis was used to test the second hypothesis that long-range and/or local coherence of gamma-band activity is associated with passive listening. Long-range coherence was calculated for pairs of LFPs and for pairs of MSCs recorded from two microelectrodes separated by 0.3 to 1 mm. Local coherence was calculated for LFPs and MSCs recorded with the same microelectrode. It has been estimated that extracellular microelectrodes can record single neurons within a maximum radius of 140 μm (Henze *et al.*, 2000), and that the LFP is composed of synaptic activity over a longer distance, perhaps as great as 350 μm (Berens *et al.*, 2008; Katzner *et al.*, 2009). While the precise radii of recording sensitivity varies as a function of electrode impedance and the geometry of the active site, it is generally understood that action potentials recorded by an extracellular microelectrode represent local activity near the electrode tip while LFPs represent the summed activity over a larger volume.

Long-range coherence was analyzed between 57 recording sites that provided 178 pairwise combinations of LFPs and 116 pairwise combinations of MSCs. Coherence from 20 to 350 ms post tone-onset was compared to a pre-tone baseline. Long-range coherence between LFPs was significantly increased only in the low-frequency band (Fig. 5A, $P < 0.01$). Oscillatory LFP sites (62 combinations) showed a small but significant increase in coherence in the low-frequency band over non-oscillatory LFP sites (Fig. 5B; $P < 0.05$), but neither group showed significant coherence in the gamma band relative to the pre-tone baseline (both $P > 0.05$). In addition, no significant long-range coherence between MSCs was induced by the tone at any bandwidth (Fig. 5C; $P > 0.05$) and oscillatory MSCs were no more coherent with each other than were non-oscillatory MSCs (Fig. 5D, $P > 0.05$).

The lack of significant long-range coherence in the high-gamma band indicated that although high-gamma bursts were induced in A1 by the tone during passive listening, there was no fixed timing between them. The sometimes asynchronous nature of the gamma bursting was observed in individual records. Figures 5E and 5F show 4 simultaneously recorded LFPs on one trial for two different rats, showing the wideband response and after filtering for the high-gamma band. Although the wide-band responses were nearly identical across sites, the high-gamma records showed offset bursts of oscillation that occurred intermittently during the 100–350 ms after tone onset whose timing relative to one another was at times coincident (arrows in Fig. 5F), but at other times asynchronous (arrowheads in Fig. 5F).

In contrast, there was a robust increase in local coherence between 51 MSCs and their simultaneously recorded LFPs in the beta (10–30 Hz) and low-gamma bands in response to

the tone. The average coherence spectrogram of the 51 recording sites revealed a narrow band of local coherence at 18–22 Hz prior to the tone, which broadened to cover the frequency range of 15–50 Hz after tone onset (Fig. 6A). The coherence spectrum was quantified for the period of 20–350 ms post tone onset to avoid artifactual increases in coherence due to the sharp onset responses of the MSC and LFP triggered by the tone. Coherence between LFPs and MSCs was only increased significantly for the low gamma band (20–60 Hz; $P < 0.01$) following tone onset (Fig. 6B). Perispikes triggering of the low-gamma filtered LFP to occurrences of MSC spikes revealed a robust spike-triggered oscillation in the LFP having a period of 18.6 ms, corresponding to a 54 Hz oscillation (Fig. 6C). Onset of the tone increased the magnitude of the MSC-triggered gamma oscillation by 263%, as well as its duration (Fig. 6C), indicating that passive listening was associated with a greater recruitment of neurons firing at the gamma frequency in the local network. The occurrence of MSC spikes preceded the peak negativity of the LFP gamma oscillation by 4.1 ms, indicating that MSC spikes were part of a circuitry mechanism that drove local network activity within A1 at the gamma frequency. Overall, the data demonstrated that passive listening was not associated with long-range coherence, but rather with robust local coherence within small neuron networks in A1.

Discussion

Main findings

The experiments tested the hypotheses that passive listening in awake rats is associated with an increase in gamma oscillation and coherence in A1. Our results demonstrated that bursts of high-gamma oscillation in LFPs occur intermittently for 100–350 ms after onset of a pure tone during passive listening. Under the conditions of this study, passive listening to a tone was not associated with a significant increase in coherence of gamma oscillation over distances greater than 300 μm but was associated with an increase in local gamma coherence. This was demonstrated by a significant increase in coherence at the gamma frequency band between LFPs and MSC action potentials recorded simultaneously by the same microelectrode during passive listening. The results suggest that gamma oscillations are brought about by the activity of local neuronal networks that are not coherent across A1, at least during passive listening.

Gamma oscillations reflect local synchrony

It is well established that the LFP amplitude reflects the synchrony of the synaptic drive within the local area since the LFP is the aggregate of post-synaptic potentials of the neuronal population within a 250–500 μm radius of the electrode (Mizdorf, 1985; Engel *et al.*, 1990; Berens *et al.*, 2008; Katzner *et al.*, 2009). Moreover, extracellular microelectrodes record action potentials of neurons within a much smaller radius, perhaps up to 140 μm for a low-impedance electrode (Henze *et al.*, 2000). Therefore, coherence between an MSC and the simultaneously-recorded LFP in our study reflected the temporal relationship between the spiking of A1 neurons and the local synaptic drive impinging on the network in which they are embedded. The coherence analysis indicated that neurons within A1 integrate their input and output specifically at the low-gamma range. We conclude that the increase in gamma power is generated locally during passive listening within neighboring, small neuronal networks that act independently, because significant local coherence was measured in the absence of long-range coherence between MSCs or LFPs. The finding of local coherence at sites within A1 was consistent with the finding of Metherate & Cruikshank (1999) that demonstrated correlated activity at 10–60 Hz between intracellular and LFP recordings in the A1 maintained alive *in vitro*. The findings are also consistent with results suggesting that high frequency oscillations within the gamma and high-gamma bands reflect

cortical micro-column processing in the visual cortex (Liu & Newsome, 2006; Berens *et al.*, 2008).

Comparison with other studies

We found that multiunit neuronal clusters in the A1 of awake rats passively listening to tones showed oscillations over frequencies ranging from the beta to high gamma bands. A prior study did not find any evidence for oscillations in A1 neurons of awake rats (Cottillon-Williams & Edeline, 2003). It is possible that the differences in outcome were due to a different experimental paradigm and analysis method. For instance, previous studies quantified oscillations by computing the autocorrelogram or the PSTH of single-units (Horikawa *et al.*, 1994; Cottillon-Williams & Edeline, 2003). The present study compared the MSC spectra after tone onset to the spectra of Poisson spike trains having matched firing rates. The analyses highlight different aspects of the data, for instance the time domain for autocorrelograms and the frequency domain for power spectra. Moreover, the variability in the prevalence of the oscillations encountered in our data suggests that the internal state of the animal may be an important variable that determines the presence of gamma oscillations in A1. Although we monitored the rats through the experiment to ensure they were awake and verified that analyses were only performed on records that lacked delta activity reflecting sleep and drowsiness, we did not explicitly control for constancy of internal state. A combination of different experimental settings, data analysis methods, and uncontrolled internal states may underlie the different outcomes reported between this and previous studies.

That gamma activity in A1 is sensitive to variation in internal state is emphasized by the significant differences during wakefulness and anesthesia. For instance, clicks induced gamma oscillations in anesthetized rats at latencies much longer than we observed, beginning no sooner than 300 ms after stimulus onset (Franowicz & Barth, 1995). Our recordings from awake rats indicate that bursts of gamma oscillations can begin much earlier, as soon as 100 ms after tone onset. A short latency for induced gamma bursts was also reported in awake monkeys (Steinschneider *et al.*, 2008). Moreover, our observations in awake rats indicated a response in the high gamma band that was comparable to the high gamma response in the A1 of awake monkeys. The similarity of latency and frequency of induced gamma oscillations in awake animals suggests that wakefulness is associated with a faster onset of induced gamma bursts and reinforces the importance of using awake preparations to study gamma oscillations evoked by sensory stimuli.

Is long-range coherence of gamma oscillation affected by receptive field properties?

We observed that a pure tone increased local neuronal synchrony in A1 without inducing synchrony between neurons separated by 300 μm . It is possible that the inability to record long-range coherence in gamma oscillation was due to the organization of A1 and our particular recording paradigm. Neuronal synchrony may be correlated with receptive field properties of the neurons in A1 (DeCharms & Merzenich, 1996; Brosch & Schreiner, 1999; Steinschneider *et al.*, 2008). The manner in which receptive field properties correlate with coherence between sites displaying gamma oscillations in A1, however, is unclear. Because our experimental paradigm used simultaneous MSC recordings, we were unable to match the sensory stimuli to the respective properties of single neurons and therefore explain a lack of long-range synchrony. Thus, further experiments are needed to clarify if there is any correlation between receptive field characteristics and neuronal synchrony in the gamma range.

What is the significance of gamma oscillations in A1?

Gamma oscillations have been implicated in the coding of complex acoustic features. For instance, gamma oscillations were induced in the A1 of the awake mustached bat by species-specific vocalizations that were presented in the forward, but not backward direction (Medvedev & Kanwal, 2008). However, our results demonstrated that gamma oscillations within A1 may not only be involved in coding complex acoustic features, because they were induced by a pure tone. On the other hand, it is possible that the tone bursts that we employed represented some feature in rat vocalizations, such as a formant, as they can for mice (Ehret & Riecke, 2002). Rats have vocalizations with flat frequencies across 16–30 kHz over a wide duration (20–3000 ms; Brudzynski *et al.*, 1993; Bruszyński, 2001). Thus, to the extent that a tone burst may represent a vocalization formant, tone bursts having higher frequencies than the one we employed may more effectively induce gamma oscillation in A1. Moreover, it is also possible that changes in the magnitude and coherence of gamma oscillations within A1 may be modulated by attention-like processes. For instance, electrically stimulating arousal centers such as the basal forebrain, the posterior intralaminar nucleus, and the thalamic reticular nucleus modulated gamma oscillations in A1 (Metherate *et al.*, 1992; Barth & MacDonald, 1996; MacDonald *et al.*, 1998; Sukov & Barth, 1998, 2001) and arousal modulated low-gamma oscillations in the auditory cortex of cats and ferrets (Lakatos *et al.*, 2004; Jeschke *et al.*, 2008). Although the rats in the present study were generally in an aroused and alert state, further experiments are needed to investigate whether attention modulates long-range and local neuronal oscillations in A1.

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Abbreviations

LFP	local field potential
A1	primary auditory cortex
MSC	multiunit spike clusters
PSD	power spectrum density
PSTH	peri-stimulus time histogram
MLAEP	middle latency auditory evoked potential

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Fig. 1. Electrophysiological characteristics of A1

(A) Mean tone-evoked LFP of 63 recording sites in A1 showing the MLAEP with peak negativity at 19.1 ms and a prominent P100 wave. Time 0 is tone onset. (B) Mean PSTH of 63 A1 MSC showing peak firing 16.2 ms after tone onset. (C) A representative evoked LFP from a single site in A1 showing peak negativity at 15 ms and the P100. (D) PSTH of the MSC recorded simultaneously with the LFP shown in C. Peak firing occurred 14.7 ms after tone onset. (E) Frequency tuning of the MSC shown in D, showing peak sensitivity at 11 kHz.

Fig. 2. Gamma oscillations in A1 LFPs

(A–C) Mean wideband LFP before (A) and after band-pass filtering for the low-gamma (B) and high-gamma (C) bands of 63 A1 LFPs. Time 0 is tone onset. (D–F) A representative A1 LFP (D) on a single trial showed induced bursts of gamma oscillation (arrows) that were more easily seen after filtering to pass only the low- (E) and high-gamma (F) bands. (G) Mean normalized PSD functions for oscillatory (black line) and non-oscillatory (gray line) LFPs calculated for 50–350 ms post tone onset. (H) Mean values of normalized power for three bandwidths of interest for oscillatory and non-oscillatory LFP sites. (I) Mean normalized PSD functions for oscillatory LFP sites on trials in which gamma oscillations were (black line) and were not (gray line) induced by the tone. (J) Mean values of normalized power for three bandwidths of interest for oscillatory LFPs on oscillatory and non-oscillatory trials. In H asterisks indicate significant ($P < 0.05$) difference from 0 and in J, asterisks indicate $P < 0.05$ by pairwise comparison.

Fig. 3. Induced oscillations in A1 MSC

(A) Mean PSTHs of spikes from MSC sites defined as oscillatory (black line) and non-oscillatory (gray line). (B) Mean values of normalized power for three bandwidths of interest for oscillatory and non-oscillatory MSC. Oscillatory MSC showed significantly more power at all bandwidths (asterisks indicate $P < 0.05$). (C, D) Mean PSD functions averaged over trials for an oscillatory MSC (C, black line) and a non-oscillatory MSC (D, black line) and compared to rate-matched artificial neurons having a Poisson spike distribution (gray lines). Asterisk in C indicates a significant difference between the cells ($P < 0.05$).

Fig. 4. MSC spike oscillations at the beta and gamma frequencies

(A–C) Example of a 20-Hz oscillatory MSC showing its PSTH (A), autocorrelation histogram of its activity 20–350 ms post tone (B), and peri-stimulus time spectrogram (C). (D–F) Identical analyses for a 135-Hz oscillatory MSC. Boxes in C and D indicate regions of significantly enhanced oscillation following tone onset. (G) Peak oscillation frequencies for 20 MSC arranged in ascending order of maximum oscillation frequency. The MSCs are equally distributed into 3 groups having maximum oscillation frequencies in the beta, low-gamma, and high-gamma bandwidths (colored boxes).

Fig. 5. Weak long-range coherence within A1 during passive listening

(A) Coherence spectra calculated for all possible LFP pairs before (gray line) and after (black line) tone onset. (B) Coherence spectra post-tone onset for the same LFPs divided into oscillatory (black line) and non-oscillatory (gray line) LFP sites. (C, D) Same analyses as in A and B but for 63 MSCs. (E, F) Wide band (1–170 Hz; E) and high-gamma (90–150 Hz; F) filtered LFP records taken from a single trial from 4 simultaneously-recorded sites in two different experiments. Arrows and arrowheads indicate times of coincident and asynchronous induced gamma bursts, respectively. Asterisks in A and B indicate $P < 0.05$.

Fig. 6. Robust local coherence in gamma oscillations during passive listening

(A) Mean peri-event coherogram of 51 recording sites indicated that the tone significantly increased coherence in the low-gamma band between simultaneously recorded LFPs and MSCs. (B) Mean coherence spectra calculated before (gray line) and 20–350 ms after (black line) tone onset. Asterisk indicates $P < 0.05$ for the low-gamma band. (C) Spike-triggered averaging of the wide-band LFP indicated that a low-gamma oscillation was time-locked to action potentials in A1 and that the magnitude of the spike-locked gamma oscillation was increased by the tone.