Cellular Mechanisms of Thalamically Evoked Gamma Oscillations in Auditory Cortex

WILLIAM SUKOV AND DANIEL S. BARTH
Department of Psychology, University of Colorado, Boulder, Colorado 80309-0345

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Sukov, William and Daniel S. Barth. Cellular mechanisms of thalamically evoked gamma oscillations in auditory cortex. J Neurophysiol 85: 1235–1245, 2001. The purpose of this study was to clarify the neurogenesis of thalamically evoked gamma frequency (~40 Hz) oscillations in auditory cortex by comparing simultaneously recorded extracellular and intracellular responses elicited with electrical stimulation of the posterior intralaminar nucleus of the thalamus (PIL). The focus of evoked gamma activity was located between primary and secondary auditory cortex using a 64-channel epidural electrode array, and all subsequent intracellular recordings and single-electrode field potential recordings were made at this location. These data indicate that PIL stimulation evokes gamma oscillations in auditory cortex by tonically depolarizing pyramidal cells in the supra- and infragranular layers. No cells revealed endogenous membrane properties capable of producing activity in the gamma frequency band when depolarized individually with injected current, but all displayed both sub- and supra-threshold responses time-locked to extracellular fast oscillations when the population was depolarized by PIL stimulation. We propose that cortical gamma oscillations may be produced and propagated intracortically by network interactions among large groups of neurons when mutually excited by modulatory input from the intralaminar thalamus and that these oscillations do not require specialized pacemaker cells for their neurogenesis.

INTRODUCTION

Brain activation, as registered in the extracranial electroencephalogram (EEG), is typically regarded as a state in which the electrical activity of large neural networks is desynchronized compared with higher-amplitude rhythmic EEG patterns thought to characterize the cerebral cortex when not actively engaged in information processing. However, these conclusions have been recently challenged by results obtained from intracranial measurements, indicating the presence of locally synchronous oscillations in the gamma frequency band (~40 Hz) associated with vigilant states (Franken et al. 1994; Hamada et al. 1999; Jones and Barth 1997) and with sensory stimulation (Basar and Bullock 1992; Eckhorn et al. 1988; Franowicz and Barth 1995; Freeman 1978; Jones and Barth 1997; MacDonald and Barth 1995; Singer and Gray 1995). Fast rhythms appear as a substantial part of the background intracranial EEG and are synchronized in both intracortical and thalamocortical networks during activation induced by stimulation of the mesopontine cholinergic nuclei or by behavioral tasks in chronic experiments (Bouyer et al. 1981; Canu et al. 1994; Steriade et al. 1993b, 1996a,b).

While gamma oscillations appear to reflect transient synchronization of neural populations during information processing, their cellular mechanisms are still being elucidated. Several recent reports have implicated specific subtypes of cortical neurons with endogenous membrane properties that may produce sub- and suprathreshold activity in the gamma frequency band when sufficiently depolarized (Gray and McCormick 1996; Llinás et al. 1991; Silva et al. 1991; Steriade et al. 1998), suggesting that dedicated cells may serve as the neural generators or pacemakers of gamma oscillations in sensory cortex. However, both computer modeling (Lumer et al. 1997; Lytton and Sejnowski 1991; Traub et al. 1999) and in vitro physiological investigations (Buhl et al. 1998; Plenz and Kitai 1996; Whittington et al. 1995) have demonstrated that gamma oscillations may also appear as an emergent property within networks of mutually connected and tonically excited inhibitory and excitatory neurons without the requirement of specialized cells for gamma neurogenesis.

In vivo studies of the rodent reveal gamma oscillations in a focal region of ventrotemporal cortex, overlapping primary and secondary auditory zones, that occur spontaneously (Brett and Barth 1996; Franowicz and Barth 1995; MacDonald and Barth 1995; MacDonald et al. 1996) and in response to sensory stimulation (Franowicz and Barth 1995; MacDonald and Barth 1995). These oscillations may also be reliably evoked by electrical stimulation of the posterior intralaminar nucleus of the thalamus (PIL) (Barth and MacDonald 1996; Brett and Barth 1997; Sukov and Barth 1998). While these studies were conducted in ketamine anesthetized animals, where both spontaneous and evoked gamma oscillations may be enhanced, we have used PIL stimulation in this preparation to study the spatiotemporal organization of gamma oscillations in large neuronal networks at the extracellular level, recording from high spatial resolution multi-electrode arrays placed on the cortical surface (Barth and MacDonald 1996; Brett and Barth 1997) and linear electrode arrays spanning the cortical laminae (Sukov and Barth 1998). The present study used PIL stimulation to investigate the cellular mechanisms of thalamically evoked gamma oscillations in auditory cortex by relating measurements of extracellular field potentials to simultaneously recorded intracellular responses in the ketamine anesthetized state.
Our specific objectives were to identify cells in specific cortical laminae that participate in evoked gamma oscillations, to characterize participating cells using depolarizing current injection to determine if they define a specific cell class displaying endogenous membrane properties conducive to oscillations in the gamma frequency range, and to determine the influence PIL inputs have on cortical neurons that gives rise to the evoked gamma response.

METHODS

Surgical preparation

All procedures were performed in accordance with the guidelines of the Colorado Institutional Animal Care and Use Committee for the humane use of laboratory animals in biological research. Male Sprague-Dawley rats (250–350 g) were anesthetized to surgical levels with ketamine (100 mg/kg) and xylazine (25 mg/kg) and secured in a stereotaxic frame. A unilateral craniotomy extending from bregma to lambda and lateral to the midline, 6.4 mm ventral to the cortical surface was performed over the right hemisphere, exposing a wide region of parietotemporal cortex from which the dura was removed. The exposed cortical surface was regularly bathed with physiological saline and body temperature maintained using a regulated heating pad. Additional ketamine and xylazine were administered as required to maintain a level of anesthesia such that the corneal reflex could barely be elicited. Animals were killed without regaining consciousness by anesthesia overdose at the conclusion of the experiment.

Stimulation

Auditory click stimuli were presented using a high-frequency piezoelectric speaker placed ~15 cm lateral to the contralateral ear. Clicks were generated by computer-controlled monophasic square-wave pulses (0.3 ms), which were shown in previous studies to activate most of the auditory cortex in the rat. Subcortical electrical stimulation consisted of 500-ms trains of current pulses (10–15 μA; 0.5-ms pulse-width; 1,000 Hz) delivered with a stainless steel bipolar electrode positioned in the PIL (4.8 mm posterior to bregma, 3.0 mm lateral to midline, 6.4 mm ventral to the cortical surface).

Field potential recording

Epipial maps of the auditory evoked potential (AEP) complex were recorded using a flat multi-channel electrode array consisting of 64 silver wires arranged in a 8 × 8 grid (tip diameter: 100 μm; inter-electrode spacing: 500 μm) covering a 3.5 × 3.5 mm area of the cortical surface in a single placement (see Fig. 1A). Click stimulation resulted in a highly repeatable averaged surface response (n = 100) whose spatial distribution was used to consistently position the electrode array across animals. Epipial potentials were referenced to a silver ball electrode secured over the contralateral frontal bone and were simultaneously amplified (×1,000), analog filtered (band-pass cutoff = −6 dB at 0.001–5000 Hz, roll-off = 5 dB/octave) and digitized at 10 kHz. Following AEP mapping, the surface response during electrical stimulation of the PIL was recorded to determine the locus of maximum-evoked gamma oscillations. The electrode array was then removed, and a small Plexiglas stabilizing plate was brought in contact with the pia that contained a 0.5-mm access hole centered over the region of PIL-evoked gamma oscillations for subsequent intracellular recording. A single silver-wire electrode was mounted in the immediate periphery of the access hole to simultaneously monitor evoked responses at the cortical surface.

Intracellular recording

Intracellular recordings were obtained using glass micropipettes pulled from thin-walled (1.0 mm) aluminosilicate glass (tip diameter = 0.05 μm). In most penetrations, electrodes were filled with 2.0 M K+ -acetate so that both action potentials (APs) and postsynaptic potentials (PSPs) could be recorded. In some penetrations, electrodes were filled with 100 mM lidocaine and N-ethyl bromide quaternary salt (QX-314; Research Biochemicals, Natick, MA) in 2.0 M K+ -acetate that was iontophotically ejected into the cell by applying 100-ms depolarizing pulses of 2.0 nA at 2.0 Hz until all APs were blocked (Connors and Prince 1982). The in vivo impedance of electrodes ranged from 110 to 140 MΩ. Recording and current injection was performed using an Axoclamp 2-A amplifier (Axon Instruments) equipped with a 0.1 gain head-stage (Axon Instruments Model HS-2A). Micropipettes were advanced perpendicularly into the cortex in 0.5-μm increments (100 μm/s) using a piezo translator compensated with a motor drive (Märzhäuser PM-10) equipped with a micrometer which indicated the depth of the electrode tip. Criteria for an acceptable cell impalement were a resting membrane potential of at least −60 mV maintained for ≥30 min required to collect single trial data and obtain satisfactory characterization of the cell using depolarizing current pulses (McCormick et al. 1985) and action potential heights of ≥70 mV and half-amplitude action potential widths of no more than 2.0 ms. In cells receiving QX-314, characterization was performed immediately on penetration prior to AP blockade. In cells not receiving QX-314, characterization was performed after all other responses had been recorded. Hyperpolarizing currents up to −0.5 nA were applied on initial cell penetration and were maintained for ~5 min, until the membrane potential stabilized. All subsequent recordings were performed with no hyperpolarizing holding currents. When a stable cell impalement was obtained, 1-s trials (n = 50–100) were recorded during PIL stimulation (250-ms baseline followed by 500-ms stimulus train). Intracellular and surface records were low-pass filtered at 3 kHz (~6 dB at 3 kHz, roll-off = 5 dB/octave), digitized at 10 kHz, and stored for subsequent analysis.

RESULTS

Figure 1 depicts methods for mapping the AEP complex at the cortical surface to determine the locations of primary (area 41) and secondary (areas 36 and 20) (Krieg 1946) auditory cortex prior to intracellular recording (Fig. 1A). The AEP in all animals began with a positive/negative fast wave (Fig. 1C; P1/N1) that was of largest amplitude and shortest poststimulus latency over area 41 (Fig. 1C, solid trace; Fig. 1B), and smaller amplitude with a delay of ~5 ms over area 36 (Fig. 1C, dashed trace; Fig. 1B). This spatial and temporal pattern was similar to previous studies (Barth and Di 1990) and permitted consistent alignment of the electrode array across animals.

Similar to the AEP, electrical stimulation of the PIL also produced an initial biphasic response at the cortical surface. However, this was followed by large-amplitude (~0.5 mV) oscillations in the gamma frequency band [37.9 ± 0.16 (SE) Hz, n = 15] that lasted the duration of the 0.5-s stimulus train (Fig. 1, D and E). As reported in previous studies (Barth and MacDonald 1996; Brett and Barth 1997; Sukov and Barth 1998), gamma oscillations evoked by PIL stimulation were typically superimposed on a negative offset [−0.2 ± 0.08 (SE) mV, n = 15] compared with the prestimulus baseline (Fig. 1E). While evoked gamma oscillations were evident in all areas of auditory cortex, they were of largest amplitude in a region straddling the border of areas 41 and 20 (Fig. 1D, dashed box),
slightly caudal to the locus of the maximum amplitude of the AEP. Following surface mapping, the array was removed and the location of maximum evoked gamma oscillations stereotaxically targeted for subsequent single channel intra- and extracellular recording.

Stable intracellular recordings were obtained from 46 cells in 21 animals. Most of these cells \( n = 45 \) characterized as regular spiking (RS). A cell was classified as RS if in response to the injection of a depolarizing current pulse (0.5–1.0 nA; 500 ms), it fired a train of APs exhibiting strong spike frequency adaptation with half-amplitude AP width >0.5 ms [average half-amplitude spike width was 1.2 ± 0.2 (SD) ms; average spike height was 78 ± 12 (SD) mV] (Connors and Gutnick 1990; McCormick et al. 1985). One cell was classified as a fast spiking (FS) neuron. This classification was based on its distinctly narrow APs (<0.5-ms half-amplitude width) (Connors and Gutnick 1990; McCormick et al. 1985; Simons 1978; Simons and Woolsey 1979) and capacity for very high-frequency spontaneous and evoked AP discharge (>200 Hz) (Jones et al. 2000; Simons 1978; Simons and Woolsey 1979). No cells displayed firing patterns typical of intrinsic bursting (IB) cells during current injection. However, while all cells receiving QX-314 characterized as RS cells, their identity cannot be established with absolute certainty due to possible subtle effects of QX-314 immediately on penetration. RS cells were distributed uniformly throughout the supra (\( n = 21 \))- and infragranular (\( n = 24 \)) layers. The FS cell was located at a depth of 550 µm below the cortical surface.

All RS cells responded to PIL stimulation with a steady depolarization \([13.3 ± 1.8 \text{ (SE) mV}]\) from resting membrane potential (Fig. 2B). This began abruptly at stimulus onset, remained approximately flat for the 500-ms stimulus duration, and usually required 50–200 ms to return to baseline following the stimulus. Of the 26 RS cells not receiving QX-314, 22 displayed multiple APs \([6.2 ± 0.98 \text{ (SE) } \text{ per trial during PIL-evoked depolarization (the first 50 ms following stimulus onset, associated with the extracellular P1/N1 wave, were excluded from analysis)\text{. Visual examination of individual trials suggested that the APs of a given cell maintained a consistent phase relationship with the evoked gamma oscillations recorded at the cortical surface (Fig. 2A). This relationship was examined by computing cumulative time histograms (CTH) of AP latency time-locked to successive gamma waves in the surface record. The positive amplitude peaks of surface gamma waves were determined automatically using a peak-seeking algorithm (Barth and MacDonald 1996; Sukov and Barth 1998) and used as a reference for computing time-locked average

**FIG. 1.** Localization of the posterior intralaminar nucleus of the thalamus (PIL)-evoked gamma response within auditory cortex. A: an 8 × 8 electrode array covering 3.5 mm² of ventrotemporal cortex in the right hemisphere was used for epipial recording of auditory evoked potentials (AEP) from primary (area 41) and secondary (areas 36 and 20) auditory regions. B: averaged AEP responses to click stimuli produce a stereotyped pattern in auditory cortex, permitting consistent localization of the primary and secondary zones based on response latency and amplitude. C: enlargements from dashed boxes in B illustrate the initial biphasic positive/negative (P1/N1) component of the AEP in area 41 (solid trace) that precedes a similar smaller amplitude response in area 36 (dashed trace) by ~5 ms. D: electrical stimulation of the PIL nucleus of the thalamus with 500-ms trains of current pulses elicits a stereotypical extracellular response throughout auditory cortex with the highest amplitude response consistently focused over the border between areas 41 and 20 (dashed box). E: enlargement of the dashed box in D illustrates the typical response to PIL stimulation, consisting of an initial P1/N1 response, dominated by the negative component (truncated here), followed by large amplitude gamma frequency oscillations that last for the duration of the PIL stimulation (horizontal bar).
extracellular gamma oscillations and associated CTH. To limit noise, only gamma waves exceeding 1 SD from the mean, computed across all single trials for a given animal, were included (Fig. 2A; ↓). Figure 2C depicts a single cycle of time-locked gamma oscillation averaged in this way (n = 1,059 gamma waves) and superimposed on the associated CTH (n = 992 APs) for an RS cell located at a depth of 950 μm below the cortical surface. The averaged extracellular gamma oscillation is displayed as a solid trace peaking at 0.0 ms, and again as a dashed trace, shifted to align with peaks of the CTH for comparison. The CTH indicated that APs were tightly time-locked to the surface oscillations with a similar periodicity. The cross-correlation function between the averaged gamma oscillation and CTH (Fig. 2D) revealed a dominant period of 24 ms (42 Hz), APs delayed by 8 ms relative to the positive amplitude peak of the surface response, and a maximum cross-correlation coefficient (ρxy) of 0.96 (P < 0.01; based on the Fisher z transform of ρxy referred to a normal distribution) (Otnes and Enochson 1978). Figure 2, E–H, shows results for two additional cells at depths of 110 and 800 μm, respectively, indicating similar periodicity and phase-locking between the surface and intracellular records and suggesting no laminar specificity to this relationship. In all, 17 of the 22 RS cells that produced APs in response to stimulation of the PIL did so in a periodic fashion that was significantly correlated with extracellular gamma oscillations (ρxy = 0.51–0.96; P < 0.01; Table 1). The laminar distribution of correlated cells was uniform with no apparent predominance in either the supragranular or infragranular layers. Time lags between the CTH and averaged gamma oscillations were generally earlier in the supragranular (−4 ms) compared with the infragranular layers (+1.1 ms) layers, but this difference was not significant (P > 0.05).

Figure 3 depicts FS cell responses during PIL stimulation. Similar to RS cells, the FS cell responded with a steady depolarization but of lower amplitude (~6.0 mV) and building slowly throughout the stimulation period. Superimposed on the steady depolarization were trains of thin (<0.5 ms) APs that were far more numerous per trial than those observed in RS cells (Fig. 3B). While observation of raw records suggested that APs were equally probable at all phases of the averaged surface gamma oscillations (Fig. 3, A and B), the CTH indicated a distinct periodicity that was time-locked to the averaged surface gamma oscillation (Fig. 3C) with a significant correlation (ρxy = 0.64; P < 0.01) at a lag of −7 ms.

During most trials, depolarizing steady potentials in RS cells during PIL stimulation were also accompanied by smaller-amplitude (~5–10 mV) deflections, suggestive of synchronized postsynaptic potentials (PSP) recordable at the soma (Fig. 4A; dashed box). To examine the relationship between...
The previous analysis averaged true STPs with those derived using digitally smoothed (Fig. 4B) and appeared time-locked to the evoked gamma oscillations at the surface (Fig. 5G). Time-locking averaging (Fig. 5G) and cross-correlation (Fig. 5H) indicated a common periodicity and timing between STPs and extracellular gamma oscillations that was nearly identical to that of APs before their suppression. Analysis of the remaining 18 cells with QX-314 was performed after complete AP blockade. All responded to PIL stimulation with depolarizing shifts [14.2 ± 2.1 (SE) mV] and the similarity between temporal characteristics of these averages and previously computed CTHs in the same cells. However, two cells (Table 1; c70 and c460) produced no APs during PIL stimulation. Yet they still displayed averaged STPs that were significantly correlated with surface gamma oscillations, suggesting that STPs are produced by synchronized postsynaptic currents and are not dependent on AP discharge in a given cell. To further explore this possibility, 19 additional cells were injected with QX-314 to block APs during recording. Figure 5 shows a particularly interesting example because in this cell the QX-314 took effect gradually, making it possible to study both APs and subsequent STPs during evoked gamma oscillations in the same cell. Within ~30 s after cell penetration, APs were already of reduced amplitude (Fig. 5B). Their peaks appeared to be aligned with the troughs of gamma oscillations in the surface record, observable on individual trials (Fig. 5A), and in the CTH (Fig. 5C) and cross-correlation function (Fig. 5D). After ~10 min, the QX-314 had completely suppressed all APs. STPs were still clear in the raw record (Fig. 5F) and appeared time-locked to the evoked gamma oscillations at the surface (Fig. 5E). Time-locking averaging (Fig. 5G) and cross-correlation (Fig. 5H) indicated a common periodicity and timing between STPs and extracellular gamma oscillations that was nearly identical to that of APs before their suppression. Analysis of the remaining 18 cells with QX-314 was performed after complete AP blockade. All responded to PIL stimulation with depolarizing shifts [14.2 ± 2.1 (SE) mV] and

### Table 1. Maximum cross-correlation between intra- and extracellular responses in RS cells without QX-314

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<th>ρxy</th>
<th>Lag, ms</th>
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<td>5</td>
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<td>c70</td>
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CHT, cumulative time histogram. ** P < 0.01.

Three subthreshold potentials (STP) and extracellular gamma oscillations, each trace was median-filtered (Fig. 4B) and digitally smoothed (Fig. 4C). Median-filtering is a nonlinear digital technique that was used in the present context to truncate APs, leaving an approximation of the depolarizing potentials that trigger them (Gray and McCormick 1996). This method has little effect on the morphology of STPs and permits combined averaging without distortion due to APs. STPs, derived in this way, appeared phase-locked to surface gamma oscillations in the raw record (Fig. 4D). This was confirmed by computing averaged STPs, time-locked to the peaks of surface gamma waves (Fig. 4E). The data shown in Fig. 4 are from the same cell (c950) depicted in Fig. 2, A–D. The averaged STP (Fig. 4E; dark trace) maintained an 8-ms delay in relationship to the averaged gamma oscillation (Fig. 4E; light trace) with a cross-correlation function (Fig. 4F; solid trace) that was nearly identical to that computed for the CTH alone in this cell (Fig. 4F; dashed trace). The averaged STPs of 24 cells were significantly correlated with averaged extracellular gamma oscillations (ρxy = 0.51–0.96; P < 0.01; Table 1). Seventeen of these cells also had CTHs that were significantly correlated with extracellular gamma oscillations (Table 1). Similar to the CTH, time lags between the averaged STPs and averaged gamma oscillations were earlier in the supragranular (−1.27 ms) compared with the infragranular layers (+2.38 ms) layers; but, again, this difference was not significant (P > 0.05). However, phase lags of the CTH and averaged STPs in these cells were significantly correlated (rxy = 0.96; P < 0.01).

The previous analysis averaged true STPs with those derived from suprathreshold events by median-filtering. The necessary inclusion of derived STPs in the analysis no doubt influenced...
17 had averaged STPs significantly correlated with surface gamma oscillations ($r = 0.56$ – $0.95$; $P < 0.01$; Table 2).

While PIL stimulation consistently produced steady depolarizing intracellular potentials accompanied by both APs and STPs time-locked to extracellular gamma oscillations, the injection of intracellular current did not produce noticeable sub- or suprathreshold activity in the gamma frequency range. RS cells generated trains of APs with frequencies <100 Hz at large (1.0 nA) levels of current injection and with marked frequency adaptation occurring within the first 100 ms of current onset (Fig. 6A). AP frequency varied with the level of depolarizing current and showed no apparent preference for gamma frequencies, but this was not systematically studied. However, PIL stimulation during smaller current injections (0.75 nA) typically produced an increase in AP frequency with concurrent time-locking of the AP to surface-evoked gamma oscillations and little further depolarization beyond that pro-

![Figure 4](http://jn.physiology.org/)[1240](10.220.33.6 on October 29, 2016)

**FIG. 4.** Time-locked subthreshold potentials (STP) in RS cells derived from median-filtered data. **A:** RS cells typically displayed STPs (dashed box) interspersed with APs during PIL stimulation. **B:** median-filtering was used to truncate APs so that their underlying depolarizing potentials could be averaged along with the STPs. **C:** median-filtered data were digitally smoothed (10–100 Hz) prior to averaging. **D:** remaining membrane oscillations appeared time-locked to PIL-evoked gamma oscillations at the surface. **E:** averaged extracellular surface (light trace) and intracellular (dark trace) potentials were of similar periodicity, with the intracellular response shifted by 8 ms relative to the extracellular record. **F:** this example was from the same cell (c950) used for analysis of CTH in Fig. 2. Cross-correlation functions between the averaged surface gamma wave and STP (dark trace) were similar to those previously computed for the CTH in this cell (dashed trace).

![Figure 5](http://jn.physiology.org/)[1240](10.220.33.6 on October 29, 2016)

**FIG. 5.** Time-locked STPs in RS cells receiving N-ethyl bromide quaternary salt (QX-314) to block APs. **A–D:** following cell penetration but prior to full abolition on APs with QX-314, this cell produced APs in response to PIL stimulation (**B**) that remained time-locked with surface oscillations (**A**), confirmed in both the CTH (**C**) and cross-correlation function (**D**). **E–H:** when APs had been fully blocked, intracellular records (**F**) revealed that in response to PIL stimulation the cell exhibited a steady depolarization and associated STPs that appeared related to the evoked surface oscillations (**E**). Time-locked averaging (**G**) and cross-correlation (**H**) revealed a phase-relationship and similar periodicity between the two that was nearly identical with the CTH results prior to AP blockade (**C** and **D**).
duced by the injected current (Fig. 6B). When APs were blocked with QX-314, current injections produced equivalent depolarizations but without consistent STPs (Fig. 6C). The occurrence of STPs in these cells invariably coincided with spontaneous surface extracellular gamma oscillations or with PIL-evoked gamma oscillations, suggesting that they were not produced by endogenous oscillatory properties of the depolarized membrane but were instead exogenously derived PSPs, accentuated by movement of the membrane potential away from resting levels during current injection.

**DISCUSSION**

The PIL is part of a system of intralaminar and midline thalamic nuclei that provide what have been considered to be nonspecific projections to the cortex (Herkenham 1986; Jones 1985), a designation inspired by their widespread afferent input and cortical projection fields (Macchi and Bentivoglio 1986). However, improved tracing methods have revealed that the projection fields of certain intralaminar nuclei in the rodent, including the PIL, are far more specific than previously thought (Berendse and Groenewegen 1991; Linke 1999). The PIL may be considered to be a distinct part of the ascending auditory pathway in the rat, receiving its major afferent input from the inferior colliculus (Arnault and Roger 1987; Ledoux et al. 1987) and sharing reciprocal connections with the ventrotemporal cortex (Arnault and Roger 1987, 1990; Linke 1999). Maps of the epipial response to electrical stimulation of the PIL presented here replicate earlier findings (Barth and MacDonald 1996; Brett and Barth 1997; Sukov and Barth 1998) and provide functional evidence for a localized evoked response in register with these anatomically defined recipient zones for PIL projections to primary and secondary auditory cortex.

Despite the specificity of cortical projections from the PIL, its apparent influence on cortical excitability remains consistent with the earliest views of the intralaminar nuclei as modulatory (Lorente de Nö 1949), a view based on anatomical evidence for termination of nonspecific thalamocortical projections in the supragranular cortical laminae (Berendse and Groenewegen 1991; Cunningham and Levay 1986; Herkenham 1980; Linke 1999) and on functional evidence for their activating influence as measured in the electroencephalogram (Dempsey and Morison 1942). Prolonged stimulation of the PIL produces a steady and focal increase in the excitability of cells within auditory cortex. The increased excitability is apparent as a steady depolarization recorded intracellularly in all cells of the present study as well as a steady negative potential shift recorded extracellularly at the cortical surface. Extracellular recordings, performed with laminar electrodes, demonstrate that the PIL-evoked surface negative shift reverses polarity in the depth (Sukov and Barth 1998), a pattern expected...
from an equivalent current dipole oriented perpendicular to the cortical surface. This dipolar pattern indicates that the shift is produced intracortically, as opposed to reflecting volume conducted potentials from subcortical structures, and that it is produced by synchronized PSPs in the parallel apical dendrites of large populations of cortical pyramidal cells (Barth et al. 1989; Mitzdorf 1985; Sukov and Barth 1998). Combined with the present demonstration that PIL stimulation results in prolonged intracellular depolarization, it is likely that the extracellular surface negativity reflects summed excitatory PSPs imposed on the distal ends of apical dendrites, an observation that is consistent with the predominant synaptic termination of PIL fibers in layer I of temporal cortex (Linke 1999).

It is interesting that infragranular RS cells display PIL-evoked depolarization as consistently as those in the supragranular layers despite the fact that, at least in primary auditory cortex of the rat, the apical dendrites of RS cells are shorter than those of electrophysiologically identified IB cells that have extensive arborizations in layer I (Hefti and Smith 2000). There are perhaps three factors contributing to this result. First, infragranular apical dendrites can reach layer I even though their arborizations are far less extensive than IB cells. Second, it is notable that our focus of maximum PIL-evoked gamma activity is not directly within primary auditory cortex but is instead at the caudal and lateral border, a region that may contain infragranular RS cells with more extensive dendritic projections to layer I and a region that may receive distinct PIL projections to both layer I and layers III/IV (Linke 1999). Finally, as noted earlier, while all cells receiving injections of QX-314 characterized as putative RS cells during depolarizing current injection, their identity cannot be established with absolute certainty due to possible subtle effects of QX-314.

Accompanying the steady depolarization evoked in auditory cortex by PIL stimulation are oscillations of extracellular membrane potential in the gamma frequency band that suggest a role for the thalamus in their neurogenesis. There is now abundant evidence that gamma-band oscillatory rhythms are not a unique property of cortex and may be found in the lateral geniculate nucleus (Ghose and Freeman 1992; however, also see Gray et al. 1989), the ventroposterior and ventrolateral nuclei (Steriade et al. 1996b), the thalamic reticular nucleus (Pinault and Deschênes 1992; Steriade et al. 1996b), as well as the centrolateral nucleus of the intralaminar group (Steriade et al. 1993). More importantly, synchronization of gamma oscillations has been reported in thalamocortical networks during anesthesia, resting sleep, and during behavioral arousal (Steriade et al. 1996b). Yet while thalamocortical and reciprocal corticothalamic circuits can become synchronized during gamma oscillations, this synchronization is not required for gamma neurogenesis in cortex. Spontaneous gamma oscillations persist in auditory cortex after total destruction of the PIL (MacDonald et al. 1998) and following widespread lesioning of the acoustic thalamus capable of completely eliminating the AEP complex (Brett and Barth 1996). Similarly, gamma oscillations in rat auditory cortex have been observed in vitro following electrical stimulation of the superior thalamic radiation in thin slices (400 μm) with no intact thalamocortical link (Metherate and Cruikshank 1999), in slices of rat cortex with more limited portions of cortical circuits intact (Chagnac-Amitai and Connors 1989; Whittington et al. 1995), and in cortical cultures with no extrinsic inputs (Plenz and Kitai 1996). Thus it is not likely that the PIL serves as a pacemaker for cortical gamma oscillations but instead plays a modulatory role, providing a source of excitation that activates intrinsic cortical oscillatory mechanisms.

There have been several recent reports that have proposed putative cellular generators of gamma oscillations in the cortex (Gray and McCormick 1996; Silva et al. 1991; Steriade et al. 1998; for a review see Traub et al. 1999). Each has identified a unique pyramidal cell type that possesses intrinsic membrane properties producing AP bursts with periodicity in the gamma frequency band. A distinct class of cells in the supragranular layers of cat visual cortex, termed “chattering cells” (CH), intrinsically generate 20- to 70-Hz repetitive burst firing in response to depolarizing current injection and exhibit similar burst firing associated with membrane oscillations during visual stimulation (Gray and McCormick 1996). Fast-rhythmic bursting (FRB) cells, discovered in the supragranular layers of cat motor and association cortex, display dynamic responses to depolarizing current injection, ranging from rhythmic bursting (30–40 Hz) during moderate levels of stimulation to higher-frequency tonic firing with increased current levels (Steriade et al. 1998). FRB cells were identified as both pyramidal as well as sparsy spiny interneurons. A separate group of layer V pyramidal neurons have also been identified in rat sensorimotor cortex that display intrinsic rhythmicity near gamma frequencies when sufficiently depolarized (Silva et al. 1991). Putative inhibitory interneurons have also been identified with intrinsic oscillatory properties in the gamma frequency range (Llinás et al. 1991).

Yet while each of these distinct cell types clearly participates in cortical gamma oscillations, none have been shown to represent an essential intracortical generator across species or sensory modalities. CH and FRB cells have not been observed in vivo studies of rat sensory cortex (Jones et al. 2000; Zhu and Connors 1999). The present results indicate no cells in any of the cortical layers that respond to depolarizing current injection with phasic gamma frequency AP bursts suggestive of intrinsic oscillatory properties. This, despite the fact that these recordings were performed in a highly active generator of evoked and spontaneous gamma oscillations in the rodent cortex (Barth and MacDonald 1996; Brett and Barth 1996, 1997; Franowicz and Barth 1995; MacDonald and Barth 1995; MacDonald et al. 1996). Furthermore when APs in cells of the supragranular layers were blocked with QX-314, none revealed endogenous membrane oscillations during depolarizing current injection even at quite large (~2.0 nA) levels of excitation (Fig. 6C). However, CH cells require suprathreshold activation to exhibit intrinsic oscillations (Gray and McCormick 1996) and therefore would not be detected with QX-314.

It is possible that our failure to identify any cells with endogenous membrane properties producing AP bursts in the gamma frequency range can be explained by sampling error. However, this seems unlikely for three reasons. First, given the percentage of FRB (28%), CH (34%), and intrinsically bursting layer V cells (59% in deep layer V) reported previously, we should have encountered a similar percentage here that belonged to one or more of these classes, if they exist in rat auditory cortex in same ratio. Second, CH, FRB, and layer V...
 bursting cells have been described as morphologically similar to RS pyramidal cells with large soma that should present little challenge to penetration with sharp electrodes used here. Finally, in a previous study (Jones et al. 2000), we used similar methods to examine rodent vibrissa/barrel cortex, also a very active generator of stimulus-evoked and spontaneous gamma oscillations in the anesthetized (MacDonald and Barth 1995; MacDonald et al. 1996) and unanesthetized animal (Jones and Barth 1997). Stable in vivo recordings were obtained from RS ($n = 58$), FS ($n = 4$), and IB ($n = 5$) cells, none of which produced intrinsic gamma oscillations or AP bursts in response to a range of depolarizing currents. However, it should be noted that the subset of FRB cells that are sparsely spiny (Steriade et al. 1998), and thus probably interneurons, and intrinsically oscillatory interneurons (Llinares et al. 1991), may have been undersampled by our present recordings given that we were able to capture only a single FS cell, which has a similarly small soma.

While none of the cells in the present study produced independent gamma oscillations when depolarized artificially, nearly all displayed APs and subthreshold PSPs time-locked to episodic gamma oscillations when the entire population was tonically depolarized by stimulation of the PIL. The simplest explanation for this result is that gamma oscillations are an emergent property of circuit interactions between cortical cells when mutually excited and do not require an intrinsic pacemaker for their neurogenesis. This conclusion is consistent with computer models of large-scale neural systems that emphasize the importance of network interactions in the generation of fast oscillations (Lumer et al. 1997). Neural modeling demonstrates that while gamma oscillations may persist in the absence of cortico-thalamic projections, they are markedly suppressed by removing either back-projections from supra- to infragranular layers or forward projections from the infragranular layers to the granular and supragranular layers. These interlaminar loops provide a mechanism of high-gain amplification capable of driving local cortical networks into fast oscillations. The predicted influence of reciprocal excitatory connections between supra- and infragranular pyramidal cells in the genesis of gamma oscillations is in agreement with the present demonstration of APs and PSPs in both laminae that are tightly time-locked to the population response. It is also in agreement with previous laminar analysis of PIL-evoked gamma oscillations in auditory cortex, indicating fast rhythmic interactions between supra- and infragranular pyramidal cell groups. These appear to be a repetition of the biphasic P1/N1 wave that characterizes the sensory evoked potential complex, with an average 6 ms time lag between gamma waves in the supra- and infragranular layers, producing a 90° phase shift at 40 Hz that is optimal for resonance if the coupled circuits approximate a driven oscillator (Sukov and Barth 1998).

Our results also suggest that inhibitory cells participate in the generation of cortical gamma oscillations. The FS cell we were able to study during PIL stimulation displayed high-frequency APs, both spontaneously and during all phases of evoked surface gamma oscillations. However, CTH and cross-correlation analysis revealed a periodic fluctuation in AP probability that was time-locked to the averaged population gamma wave. FS cells in neocortex have been identified as sparsely-spiny or smooth GABAergic inhibitory interneurons (Kawaguchi 1993; McCormick et al. 1985). Since these cells receive input from both supra- and infragranular pyramidal cells, it is not surprising that their APs are time-locked to the evoked gamma response, a result that does not necessarily imply direct involvement in its neurogenesis. While our data include only a single FS cell and are therefore inconclusive on their own, they are in agreement with in vitro evidence obtained from slices of rat hippocampus (Whittington et al. 1995), guinea pig frontal cortex (Llinares 1992; Llinares et al. 1991), mouse somatosensory cortex (Buhl et al. 1998), and in cortical cultures (Plenz and Kitai 1996), indicating an important role for inhibitory postsynaptic potentials (IPSPs) in phasing the suprathreshold activity of pyramidal cells during gamma oscillations. Of particular interest are the results from somatosensory cortex, which demonstrate that GABAergic IPSPs phase, rather than evoke, APs in pyramidal cells (Buhl et al. 1998); tonic depolarization of the pyramidal cell population close to threshold is required before gamma oscillations can be produced. Whereas in vitro, tonic depolarization must be established artificially, using kainate or elevated $K^+$, in vivo, the PIL may serve as a key source of tonic depolarization, inducing reciprocal excitatory and inhibitory neural networks to rhythmic firing.

The putative cortical circuit responsible for gamma oscillations indicated by the present results, and the findings of our previous laminar analysis of PIL-evoked gamma oscillations, is shown in Fig. 7. Prolonged stimulation of the PIL results in tonic depolarization of the distal apical dendrites of both supra- and infragranular pyramidal cells (Fig. 7A). This produces an extracellular current sink near the cortical surface, recorded as a steady negative potential shift in the epipial population response, accompanied by steady depolarization of both popula-

![FIG. 7. Schematic diagram of neural components and connectivity which may give rise to PIL-evoked gamma oscillations, depicted as a coronal section through auditory cortex. A: afferent excitatory inputs to the cortex from the PIL synapse at the distal apical dendrites of supra- and infragranular pyramidal cells. Prolonged activation of PIL inputs results in tonic depolarization of the apical dendrites, producing an extracellular current sink near the cortical surface (recorded as a steady negative potential epipially) and an intracellular depolarization lasting the duration of the stimulus. Excitation of the pyramidal cells results in activation of 2 reciprocal circuits that serve to phase population discharges at the gamma frequency. The 1st circuit involves negative feedback between pyramidal and inhibitory interneurons (B). The other circuit employs reciprocal excitatory interactions between infra- and supragranular pyramidal cell populations (C). In addition, excitatory projections from pyramidal cells to cortical (D) and thalamic (E) sites allows distant recruitment and/or synchronization of gamma frequency oscillatory activity.](http://jn.physiology.org/content/1243/1/1230/F7.large.jpg)
tions of pyramidal cells. As the pyramidal cells begin to fire APs, two reciprocal circuits are engaged to phase these discharges at the gamma frequency. The first involves intralaminal negative feedback between the pyramidal cells and respective inhibitory interneurons (Fig. 7B), producing excitatory/inhibitory interactions that may serve as a pacemaker for gamma oscillations. The second involves reciprocal excitatory interlaminar interactions between the two pyramidal cell populations (Fig. 7C). This may serve as a pacemaker by itself but may also establish spatial and temporal patterns of oscillatory interactions along the vertical cortical axis with outputs capable of synchronizing more distal cell populations in cortex (Fig. 7D) and thalamus (Fig. 7E).

This model fits the present results using constituent circuit elements and connections that are not specific to auditory cortex or to the rodent. If the neural interactions responsible for cortical gamma oscillations are indeed a repetitive realization of interactions giving rise to middle latency components of the classic evoked potential complex (Basar et al. 1987; Sukov and Barth 1998), it may be assumed that they possess the same inter-areal and -species similarities as the archetypal transient evoked response (Steriade 1984). However, unlike the transient evoked response, gamma oscillations require a prolonged source of excitatory drive to the principle neurons. The source of mutual excitation may be regional, such as that provided by PIL stimulation, serving the function of selective arousal of auditory cortex and consistent with a view of the intralaminar thalamus as focal and probably modality specific modulator of cortical excitability. Mutual excitation may also be far more specific, such as that produced when the cells of functionally related cortical columns are concurrently excited by a common preferred stimulus, producing locally synchronized gamma oscillations (Singer and Gray 1995). This model does not rule out the participation of cells with specialized membrane properties favoring synaptic drive in the gamma frequency range. Cells with endogenous oscillatory characteristics may be seen not as essential generators of cortical gamma oscillations but as preferentially tuned recipients, responding to and perhaps enhancing patterned and transient synchronization of activity in sub-populations of cells within sensory cortex during information processing.

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REFERENCES


CELLULAR MECHANISMS OF GAMMA OSCILLATIONS


