Beta and Gamma Oscillations in the Olfactory System of the Urethane-Anesthetized Rat

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Beta and Gamma Oscillations under Urethane Anesthesia

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ABSTRACT
Fast oscillations in the beta (15-40 Hz in awake rats) and gamma (50-100 Hz) frequency ranges are prominent in field potentials induced by odorants in the mammalian olfactory bulb (OB) and piriform cortex (PC). Whereas the gamma oscillation has been studied for over 50 years, the beta oscillation has attracted attention only recently, and its origin, mechanism, and relationship to gamma are unknown. To address these questions, we have examined responses induced by odorants in the urethane-anesthetized rat -- a preparation well suited for the analysis of mechanisms. We found that both oscillations could be induced by odorants in a concentration-dependent manner. Analysis with a concentration series and spectral methods revealed that the beta and gamma oscillations were distinct and not harmonically related, indicating generation by independent mechanisms. The beta oscillation was synchronous at sites up to 4 mm apart in the OB, the greatest distance tested. In contrast, the gamma oscillation was synchronous in some experiments and asynchronous in others (frequency differed slightly at different sites, resulting in progressive phase shifts). Current source-density (CSD) analysis indicated that, for both oscillations, the field potentials in the OB were generated by synaptic currents in granule cells. The two oscillations were differently affected by surgical interruption of the lateral olfactory tract (LOT). This lesion abolished the beta oscillation, whereas the gamma oscillation was still induced in the OB. Our results confirm previous reports that the gamma oscillation is generated within the OB, but indicate that the beta oscillation requires the participation of PC.

KEYWORDS
olfactory bulb, olfactory cortex, piriform cortex, olfaction, odorant
INTRODUCTION

Neural activity in the mammalian olfactory system is modulated by a pair of fast oscillations, defined by their frequencies as gamma (50-100 Hz in awake rats) and beta (15-40 Hz). The firing of mitral and tufted cells of the olfactory bulb (OB) and pyramidal cells of piriform cortex (PC) is constrained by these oscillations to occur within narrow time windows (Eeckman and Freeman 1990; Kashiwadani et al. 1999). Consequently, a distinct sequence of synaptic events is expected to occur within each cycle of a fast oscillation (Ketchum and Haberly 1993). Both dendritic integration and synaptic plasticity can be highly dependent on the precise temporal relationships between two synaptic inputs, or between an input and a postsynaptic action potential (Sjöström and Nelson 2002). Therefore, an understanding of the functional role of the oscillatory patterning of activity may be required for a complete understanding of the processing of olfactory information.

The gamma oscillation has been described in field potential recordings from the OB and PC of awake animals (Freeman 1959, 1978; Bressler 1984; Boeijinga and Lopes da Silva 1988; Kay and Freeman 1998) and animals anesthetized with urethane (Adrian 1950; Mori et al. 1992; Kashiwadani et al. 1999). It is coherent both within and between the two structures (Freeman 1978; Bressler 1984, 1987; Boeijinga and Lopes da Silva 1988; Kay and Freeman 1998). The amplitude and frequency of this oscillation may reflect previous olfactory experience and the behavior of the animal (Freeman 1960; Freeman and Schneider 1982; Freeman and Viana di Prisco 1986; Bressler 1988; Boeijinga and Lopes da Silva 1989; Kay and Freeman 1998; Chabaud et al. 2000; Ravel et al. 2003). The gamma oscillation appears to originate in the OB, as it is abolished in PC by removal of the OB (Becker and Freeman 1968), and preserved in the OB when conduction through the olfactory peduncle is blocked by cooling (Gray and Skinner 1988). The negative feedback loop between excitatory mitral and tufted cells and inhibitory granule cells has been proposed to be the underlying generator (Rall and Shepherd 1968; Freeman 1975). The phase relationship between unit activity and the field potential in the OB is consistent with this hypothesis (Eeckman and Freeman 1990; Kashiwadani et al. 1999).

Early reports refer to an oscillation, at about half the frequency of the gamma oscillation, which is more prominent in PC than in the OB (Freeman 1959; Becker and Freeman 1968; Bressler 1984). More recently, a 15 - 35 Hz beta oscillation has been described in the OB, PC, entorhinal cortex, and dentate gyrus, which is induced by olfactory stimulation with certain
organic solvents or components of predator secretions (Vanderwolf 1992; Zibrowski and Vanderwolf 1997; Chapman et al. 1998). Beta frequency activity increases following repeated exposure to odorants (Vanderwolf and Zibrowski 2001) and during odor sampling in a learned odor-discrimination task (Ravel et al. 2003; Neville and Haberly in preparation). A clear distinction between the beta and gamma frequency ranges has not always been made, however. The origin and direction of propagation of beta waves have also been controversial (Bressler 1984; Boeijinga and Lopes da Silva 1989; Chapman et al. 1998; Kay and Freeman 1998).

A fuller understanding of the mechanisms of generation, the patterns of propagation, and the relationship between these two fast oscillations is required. This goal would be significantly advanced by the development of an anesthetized preparation in which both oscillations could be induced by controlled odorant stimulation of the olfactory mucosa.

We have developed the urethane-anesthetized rat as a model system for the investigation of beta and gamma oscillations in the mammalian olfactory system. Here, we describe conditions under which either oscillation can be elicited by controlled olfactory stimulation, we present an initial description of their organization within the OB, and we begin to address their mechanisms of generation.

METHODS

Experiments were performed on adult male Long Evans rats (200-400 g) anesthetized with urethane (i.p. 1.5-2.0 g/kg). In general, the smaller rats required the higher dosages to achieve the desired level of anesthesia. Anesthesia was judged by toe-pinck, with either no reflexive withdrawal or a weak reflex restricted to the limb receiving the pinch. Body temperature was maintained at 35-38 °C. The trachea was surgically opened and two tubes were inserted, one directed caudally through which the rat breathed freely and the other directed rostrally to the back of the nasal cavity. The application of suction to this tube allowed air to be drawn through the nose. The rat was immobilized in a custom head holder, the left eye and zygomatic arch were removed, and the orbital socket and ventrolateral surface of the skull were exposed. Holes were drilled over the OB, the lateral olfactory tract (LOT), and the PC according to surface landmarks. For experiments using the multisite silicon probes, a small hole was cut in the dura. In some cases agarose was applied to reduce heartbeat artifacts. For experiments in which the LOT was lesioned, the lesion was made near the border of the anterior olfactory cortex (also
called the anterior olfactory nucleus) and the anterior PC, using a hooked needle under direct visualization with an operating microscope. Interruption of the LOT without disruption of the underlying centrifugal fibers was verified physiologically as described in the text. In most cases, the lesion was also verified histologically following the experiment. The animal was perfused with 4% formaldehyde, and the brain was cut in 120 µm coronal sections on a freezing microtome. All procedures were approved by the IACUC of the University of Wisconsin-Madison.

A glass and Teflon olfactometer delivered clean humidified air at a rate of 1000 ml/minute to a cone over the rat’s nose. Odorants were introduced through an additional air stream of 100 ml/minute that passed over a strip of filter paper saturated with an odorant at a specified dilution. Odorants were diluted in mineral oil, water, or for some food extracts a water/ethanol mixture which matched the composition of the original extract. When the suction solenoid was activated, air was drawn through the nose at a rate of 300-600 ml/minute for 1.5 seconds, repeated at 6 second intervals. Typically, four odorant conditions and a control (clean air) condition were alternated in a pseudo-random order, with three clean air presentations between each experimental trial. Thus, an odorant or control trial occurred every 24 seconds, and a particular odorant was presented once in 140 seconds on average. Some degree of response adaptation may therefore have occurred, especially in experiments where a single odorant was used at different concentrations as the four odorant conditions (Wilson 1998). However, the collection of long blocks of trials should have allowed a steady state to be achieved.

Field potential recordings were made with tungsten microelectrodes (5 MΩ, A-M systems) or multichannel silicon recording probes (University of Michigan Center for Neural Communication Technology). All recordings were monopolar, referenced to a Ag+/AgCl ground wire placed between the skin and the skull caudal to the exposure. All penetrations were approximately perpendicular to the brain surface. Signals were passed through a unity-gain preamplifier, then amplified x1000, band pass filtered (3-3000 Hz or 0.08-3000 Hz; -3 dB/octave), and digitized at 1 kHz or faster. Aliasing of frequencies greater than 500 Hz is not a problem because very little power is present at frequencies greater than 200 Hz. Electrical stimulation used constant-current square wave pulses of 100-200 µs delivered through tungsten microelectrodes. All experiments were controlled with software written in LabVIEW.
Spectral analysis was performed on epochs of data (800 ms long beginning 200 ms after stimulus onset, unless otherwise noted) after application of a Hanning window. For visual clarity, data are displayed as amplitude spectra, $\sqrt{2} \frac{|\text{DFT(signal)}|}{N}$. The more conventional power spectrum is the square of the amplitude spectrum and thus contains the same information. In addition, amplitude spectra may be plotted on a linear scale in units of volts, facilitating comparison to time-domain data. The square root of the cross power spectrum is the analogous display for bivariate data and is presented in Fig. 5Bi. For current source-density (CSD) analysis, field potentials were recorded at 16 locations spaced at 100 µm increments over depth, or at 22 locations spaced at 40 µm increments superficially and 100 µm increments deeply (1200 µm total probe length). The voltage vs. depth function was smoothed by application of a three-point median filter and/or a spatial low pass filter at 200 µm, and the unscaled second derivative was determined at each recording location by fitting with a five-point third order polynomial (Freeman and Nicholson 1975).

RESULTS

Odor-induced beta and gamma oscillations

Under urethane anesthesia, we recorded odor-induced oscillations in the field potential in the OB (n = 30 rats) or simultaneously in the OB and the PC (n = 26 rats). Typical OB responses to olfactory stimulation with controlled concentrations of amyl acetate are shown in Fig. 1. When air containing a relatively high concentration of odorant was drawn through the nose, we observed a prominent gamma frequency oscillation (45-70 Hz; ~60 Hz in Fig. 1A). Stimulation with a lower odorant concentration induced a beta oscillation (12-30 Hz; ~15 Hz in Fig. 1B). An intermediate concentration elicited a response consisting of a mixture of the two oscillations. Typically for such mixed-frequency responses, as in Fig. 1C, the gamma oscillation was stronger early in the response, whereas the beta oscillation was stronger later. The threshold concentration required to induce gamma varied from rat to rat, and in some rats gamma could not be induced even at the highest concentrations tested (n = 8/56). In a small number of rats, beta could not be induced (n = 5/56). The source of this variation is unknown, but may be due to variations in anesthetic depth or airflow through the nose. Within a rat, responses were highly reproducible and generally stable for several hours. Some odorants, including amyl acetate and cherry and mint extracts, were more effective than others at inducing the gamma oscillation.
Stimulation with concentrated toluene or trimethylthiazoline (TMT) usually induced a beta oscillation (toluene: n = 17/30; TMT: n = 8/10), as has been shown in awake rats (Zibrowski and Vanderwolf 1997), but in some experiments elicited a mixed gamma/beta response (toluene: n = 9/30; TMT: n = 1/10), and was ineffective in a few experiments (toluene: n = 4/30; TMT: n = 1/10).

Several observations suggest that the beta and gamma oscillations are distinct, rather than a single process that can oscillate at a wide range of frequencies. Importantly, mixed-frequency responses, such as the one illustrated in Fig. 1C, were composed of a mixture of the two oscillations rather than a single oscillation of intermediate frequency. This was also apparent in the amplitude spectra, in which peaks were observed in the beta frequency range, the gamma range, or both, but never at intermediate frequencies (30-45 Hz) (Fig. 2A). Likewise, when an initial gamma oscillation was replaced by a beta oscillation during the course of stimulus presentation, this was generally accomplished by a decrease in the amplitude of the gamma oscillation without a change in its frequency and a concurrent increase in the amplitude of the beta oscillation, as illustrated in Fig. 2B. A smooth transition through intermediate frequencies was not observed. These observations indicate that the gamma and beta oscillations are distinct, rather than simply the two extremes of a single oscillation.

It has been proposed that the beta oscillation is a subharmonic of the gamma oscillation (Boeijinga and Lopes da Silva 1989). We tested this hypothesis by plotting the peak frequency of the gamma oscillation against the peak frequency of the beta oscillation for each rat that expressed both oscillations (Fig. 2C; n = 43). If the two oscillations were harmonically related, these points would be expected to cluster around the lines of integral slope. No such clustering was seen. Furthermore, linear regression produced a slope not significantly different from zero (p > 0.5). We therefore conclude that beta and gamma oscillations are not harmonically related.

Spatial organization within the OB and mechanisms of generation

To analyze the extent to which these oscillations are synchronous at different locations within the OB, we recorded from three sites in the lateral OB, separated by 2-4 mm. We never observed a gamma oscillation at one site and a beta oscillation at another site simultaneously. In all such recordings, the beta oscillation was synchronous at all sites (Fig. 3A; n = 12/12 rats). In contrast, the gamma oscillation was more heterogeneous in its synchronization. In some
experiments, the gamma oscillation was synchronous at all sites (Fig. 3B; n = 5/9 rats with consistent gamma to at least one odorant). However, in other experiments the gamma oscillation did not have the same dominant frequency at all sites (n = 4/9). An example is shown in Fig. 3Ci, in which the gamma oscillation at one site had a slightly lower frequency than at the other two sites, which led to a progressive phase shift between the signals. Such differences in frequency were typically very repeatable from trial to trial. The averaged amplitude spectra for these rats contained two peaks in the gamma band, the relative heights of the two peaks being different for different sites (Fig. 3Cii).

Field potentials are an indirect reflection of the activity of neurons; they are generated by current flows through the extracellular space linking inward and outward membrane currents. We therefore sought to identify the synaptic currents underlying the oscillatory field potentials using the CSD method. With silicon probes, we recorded field potentials at 16 or 22 locations spaced over depth at a single site in the lateral OB (n = 24 rats). From these potentials, we performed one-dimensional CSD analysis to determine the location and time course of the membrane currents underlying the observed field potentials. The resulting plots of net membrane current as a function of time and depth were interpreted with reference to the net membrane currents evoked by electrical stimulation of specific sets of fibers (Aroniadou-Anderjaska et al. 1999; Nakashima et al. 1978). An example is shown in Fig. 4. Following shock stimulation of the LOT (Fig. 4Ai), a sink (warm colors, net inward current) occurred with a latency of 3.3 ± 0.1 ms (mean ± SEM, n = 24 rats), which reversed after 11.6 ± 0.4 ms. This was accompanied by a source (cool colors, net outward current) at greater depth. The sink was attributed to inward synaptic currents into granule cell dendrites in the external plexiform layer, following glutamate release from the secondary dendrites of antidromically activated mitral cells. The accompanying source was attributed to passive “return” currents, principally charging of the membrane capacitance of granule cell somata and deep dendrites in the granule cell layer. Shock stimulation of olfactory nerve bundles (Fig. 4Aii; n = 10 rats) resulted in a more superficial sink attributed to inward synaptic current into the primary dendrites of mitral and tufted cells in the glomerular layer, with source current briefly visible in the external plexiform layer. This was followed by a sink-source dipole attributed to mitral and tufted cell synapses onto granule cells (this dipole presumably masked the continuing source current for the glomerular sink). Granule cells also receive a centrifugal projection from cortical pyramidal cells onto their deep dendrites
in the granule cell layer (Luskin and Price 1983). Activation of this fiber system by shock stimulation deep in anterior PC (Fig. 4Aiii) resulted in a deep sink accompanied by more superficial source current -- the mirror image of the dipole which resulted from mitral cell activation (compare Figs. 4Ai and 4Aiii). However, the currents following centrifugal fiber stimulation had a longer latency (6.1 ± 0.3 ms, n = 17 rats) and slower time course (38.4 ± 1.5 ms to reversal) than those following LOT stimulation, presumably due to the slower conduction velocities of centrifugal axons.

When examined with the CSD method, both the gamma (n = 22 rats) and beta oscillations (n = 24 rats) consisted of alternating dipoles in the external plexiform layer and granule cell layer (Fig. 4B,C). These dipoles resembled the dipoles due to synaptic currents into granule cells, as revealed by electrical stimulation (Fig. 4Ai,Aiii). We therefore conclude that the field potentials of both oscillations are predominantly generated by granule cell synaptic currents. It is not possible to unambiguously determine whether the mitral/tufted cell inputs, the centrifugal inputs, or both are active during each oscillation. However, the similarity in the time courses of the mitral cell input to granule cells (Fig. 4Ai) and each cycle of the gamma oscillation (Fig. 4B) suggests that rhythmic synchronous activation of these synapses may be responsible for the observed field potentials during the gamma oscillation. The time course of each cycle of the beta oscillation resembled that of the centrifugal input to granule cells (compare Figs. 4Aiii and 4C), which suggests that rhythmic activation of this fiber system may underlie the field potentials during the beta oscillation.

**Oscillations in piriform cortex**

In 20 rats, both beta and gamma oscillations were simultaneously recorded in both the OB and PC. The amplitude of the beta oscillation was typically comparable in the two areas, whereas the gamma oscillation always had a smaller amplitude in anterior PC than in the OB and could not be detected in the posterior PC (data not shown). In general, both oscillations were nearly synchronous between the two locations. The form of gamma asynchrony observed within the OB in some experiments (e.g. Fig. 3C) was not observed between the OB and PC in these recordings. However, small time lags from the OB to PC were usually observed, which were greater on average for the beta than the gamma oscillation. Two methods were used to quantify these time lags. First, each oscillation was isolated by filtering and the time lags from OB to PC
were estimated for each rat from the averaged cross-correlograms (Fig. 5A). The beta oscillation occurred with a mean lag of $3.0 \pm 0.4$ ms, significantly longer than the mean gamma oscillation lag of $0.92 \pm 0.15$ ms ($p < 0.001$, paired T-test). Second, the time lags from OB to PC were estimated from the values of the averaged phase spectrum at the peak beta and gamma frequencies, as determined from the cross spectrum (Fig. 5B). By this measure, the mean beta oscillation lag was $3.0 \pm 0.7$ ms, significantly greater than the mean gamma oscillation lag of $0.96 \pm 0.25$ ms ($p < 0.01$, paired T-test). The two methods produced highly correlated estimates ($r^2 = 0.83$ for gamma lags, $r^2 = 0.82$ for beta lags).

To investigate the role of the olfactory cortex in the generation of these oscillations, we surgically interrupted the LOT (Fig. 6; $n = 6$ rats). This manipulation prevented odor-induced activity from reaching PC, while sparing the return projection and central modulatory inputs to the OB. Prior to the lesion, odorants could induce beta ($n = 5/6$) and gamma oscillations ($n = 5/6$), in both the OB (Fig. 6Ai) and anterior PC (Fig. 6Bi). Following the lesion, the gamma oscillation was preserved in the OB ($n = 5/5$ rats expressing gamma before the lesion) whereas the beta oscillation was completely eliminated (Fig. 6Aii; $n = 5/5$). This indicates that the gamma oscillation is generated within the OB, while the beta oscillation requires an intact pathway from the OB to PC. As expected, all oscillatory activity was abolished in the PC following the lesion (Fig. 6Bii; $n = 6/6$). We verified that the LOT was interrupted by the lesion, while the centrifugal projection was spared, as follows. The antidromically evoked EPSP in OB following shock stimulation of the LOT overlying anterior PC was taken as a measure of conduction through the LOT past the lesion site. This EPSP was reduced by $94 \pm 3\%$ following the lesion (mean $\pm$ SEM, $n = 6$ rats) (Fig. 6C). The orthodromically evoked EPSP in OB following shock stimulation deep in anterior PC was taken as a measure of conduction by the centrifugal fiber system. This EPSP was reduced by only $10 \pm 6\%$ following the lesion (Fig. 6D). Histological examination ($n = 4/6$) also confirmed the near-complete destruction of the LOT rostral to the anterior PC with minimal damage to the underlying tissue.

**DISCUSSION**

Early descriptions of the electrical activity in the mammalian olfactory system focused on the gamma oscillation (Freeman 1959, 1960, 1975, 1978; Bressler 1984, 1987, 1988; Boeijinga and Lopes da Silva 1988, 1989). While some of these reports described a spectral component in the
beta frequency range, especially in the PC (Freeman 1959; Becker and Freeman 1968; Bressler 1984; Boeijinga and Lopes da Silva 1989), greater attention was focused on the higher-frequency gamma oscillation. Starting in 1992, Vanderwolf and colleagues began to investigate a strong beta oscillation elicited by certain odorants, including some organic solvents and components of predator secretions (Vanderwolf 1992; Zibrowski and Vanderwolf 1997; Zibrowski et al. 1998; Vanderwolf and Zibrowski 2001). This oscillation is coherent between the OB, PC, entorhinal cortex, and dentate gyrus (Chapman et al. 1998). There has been some confusion regarding the nature of these two oscillations and the relationship between them, however. Some authors have not distinguished between them, and the nomenclature and frequency ranges described here have not been universally adhered to. Some of this confusion may be due to species differences -- the beta range extends up to ~40 Hz in the rat, which overlaps with the typical frequency range of the gamma oscillation in cats (Bressler and Freeman, 1980). Thus, we believe that the 38 Hz oscillation described in the cat by Boeijinga and Lopes da Silva (1988, 1989) and termed ‘beta’ by those authors in fact corresponds to the gamma oscillation, whereas the spectral peak that they describe at half that frequency corresponds to the beta oscillation. Recent studies in the rat have defined the beta frequency range as 12-35 Hz (Kay and Freeman 1998) or 15-40 Hz (Chabaud et al. 2003).

Fast oscillations have also been observed in the olfactory system of urethane-anesthetized animals at both beta frequencies (Heale and Vanderwolf 1994) and gamma frequencies (Adrian 1950; Mori 1992; Kashiwadani et al. 1999). However, the two oscillations have not previously been described in the same preparation.

We have described stimulus conditions under which two fast oscillations can be elicited in the olfactory system of the urethane-anesthetized rat. These oscillations are distinct (Fig. 2A,B) and not harmonically related (Fig. 2C). For several reasons, we believe that these oscillations correspond to the beta and gamma oscillations observed in awake animals. 1) The oscillations in our urethane-anesthetized rats are only slightly slower than the corresponding oscillations in awake animals (beta: 12-30 Hz under urethane vs. 15-40 Hz in awake animals; gamma: 45-70 Hz under urethane vs. 50-100 Hz in awake animals). 2) The mechanism of urethane has recently been described to consist of a modest potentiation of GABAergic and glycynergic transmission combined with a modest depression of AMPAR- and NMDAR-mediated glutamatergic transmission (Hara and Harris 2002). This pharmacological profile results in physiological
responses and synaptic time courses similar to those in unanesthetized animals (Scholfield 1980; Maggi and Meli 1986), unlike sodium pentobarbital, which prolongs GABA_A currents and has been shown to abolish the gamma oscillation in the OB (Freeman 1978), or ketamine, which blocks NMDA currents and results in a very fast (110-130 Hz) oscillation in the OB of unknown origin (Neville and Haberly, unpublished observations). 3) In our urethane-anesthetized animals, the gamma oscillation was consistently observed to be weaker in PC than in the OB, while the beta oscillation was at least as strong in PC as it was in the OB (Fig. 6Ai, 6Bi). Similar observations have been made in awake animals (Bressler 1984; Chabaud et al. 2000). 4) The results of the LOT lesion experiment, in which the gamma oscillation was not disrupted in the OB (Fig. 6Aii), parallel the results of a similar experiment in which transmission through the olfactory peduncle was cryogenically blocked in awake rats (Gray and Skinner 1988). These authors did not examine the beta oscillation, however, which was disrupted by our lesion. For these reasons, we conclude that the oscillations elicited by olfactory stimulation under urethane anesthesia do in fact correspond to the beta and gamma oscillations described in awake rats. The anesthetized preparation offers several practical advantages for the investigation of the mechanisms of generation and the spatial and temporal organization of these oscillations, including stability for intracellular recordings and accessibility for optical recordings, the absence of behavioral modulation and variable respiratory effects, and good control of the timing and concentration of odorant delivery to the olfactory mucosa.

The gamma oscillation has been hypothesized to be generated by the negative feedback loop between mitral/tufted cells and granule cells in the olfactory bulb (Fig. 7A) (Rall and Shepherd 1968; Freeman 1975; Gray and Skinner 1988; Eeckman and Freeman 1990). Our results are consistent with this hypothesis. In particular, the time course of mitral cell input to granule cells resembles the time course of one half cycle of the gamma oscillation (Figs. 4Ai and 4C), and the gamma oscillation is preserved in the OB following destruction of the LOT (Fig. 6Aii).

The mechanism of generation of the beta oscillation has received little study. Boeijinga and Lopes da Silva (1989) consider beta to be a subharmonic of the gamma oscillation. Their recordings were made in cats, in which the gamma oscillation is substantially slower (mean 38 Hz; Bressler and Freeman 1980; Boeijinga and Lopes da Silva 1988) than in the rat, and the beta oscillation occurs at approximately half this frequency. Our analysis indicates that the frequency of the gamma oscillation is not an integral multiple of the frequency of the beta oscillation (Fig.
2C). Disruption of the beta oscillation by interruption of the LOT (Fig. 6) provides further evidence for a dissociation of mechanisms.

Two possible mechanisms for the generation of the beta oscillation are illustrated in Fig. 7B,C. One possibility (Fig. 7B) is that the oscillation is generated by the loop formed by mitral cells of the OB, pyramidal cells in PC, and granule cells in the OB. The axonal and synaptic delays of the LOT and the centrifugal pathway are consistent with this hypothesis (Fig. 4). Alternatively, the beta oscillation may be generated by a feedback loop or intrinsic mechanism within PC or another cortical area (Fig. 7C). Under this hypothesis, the oscillatory currents observed in the OB are the result of rhythmic volleys of action potentials in the centrifugal fibers as in Fig. 7B, but these are not an essential component of the generating mechanism. This possibility is supported by the observation that the beta oscillation may be of low amplitude or absent in the OB while it is strong in PC (Bressler 1984). The available data are insufficient to distinguish between these two possibilities, nor are they mutually exclusive. The time lag between the OB and PC signals was greater during the beta oscillation as compared to the lag during the gamma oscillation (Fig. 5). This may be a consequence of an increased contribution of centrifugal fibers to the OB field potentials during the beta oscillation.

In light of these proposed mechanisms, it is interesting to consider why strong odorant stimulation is required to induce gamma in our anesthetized animals while less intense stimulation suffices to induce beta. Mitral cells have been shown to sustain intrinsic subthreshold oscillations in vitro, which range in frequency from 10 to 50 Hz as a function of membrane depolarization and which can control the timing of action potential initiation (Desmaisons et al. 1999). While this intrinsic mechanism may help to support both oscillations, it cannot easily account for the discontinuous shift in frequency (Fig. 2A,B) or the apparent dissociation of mechanisms revealed by the LOT lesion experiment (Fig. 6). Two cellular mechanisms have been described which might underlie the switch from the beta mode of oscillation to the gamma mode with increasing odorant strength. First, although action potentials are actively propagated in mitral cell secondary dendrites (Lowe 2002; Xiong and Chen 2002), their amplitude is attenuated by A-type K⁺ channels (Christie and Westbrook 2003). It may be that the greater tonic depolarization generated in mitral cells by a strong odorant serves to inactivate these channels. This would enable larger amplitude action potentials in the secondary dendrites and thus greater dendrodendritic release of glutamate onto granule cell dendrites.
Second, the fast AMPA receptor-mediated component of the mitral cell to granule cell synapse is also under the control of A-type K⁺ channels in granule cell dendrites (Schoppa and Westbrook 1999). Tonic depolarization of granule cells during strong odorant stimulation may serve to inactivate these Kₐ channels, allowing the granule cells to respond rapidly to mitral cell inputs. Together, these two mechanisms could serve to increase the effectiveness of the fast feedback loop between mitral and granule cells during strong odorant stimulation and thus support the generation of the gamma oscillation. A weak odorant stimulus might be sufficient to cause an increase in mitral cell firing rates, relaying excitation to the cortex and recruiting the beta-generating mechanism, even if it was not strong enough to overcome the decremental propagation in mitral cell secondary dendrites or the Kₐ-mediated shunting of AMPA currents in granule cells.

The functional role of these oscillations remains unknown. Most generally, we hypothesize that they serve to constrain the firing times of neurons to narrow time windows, with defined temporal relationships between the firing times of different populations of cells. This would determine the relative timing of convergent synaptic inputs (Ketchum and Haberly 1993), as well as the timing relationships between synaptic inputs and the generation of a postsynaptic spike. These temporal relationships would affect how the postsynaptic cell integrates its multiple inputs and adjusts the weights of its synapses (Kanter and Haberly 1993; Sjöström and Nelson 2002). Different oscillations would thus support different cellular computations. The development of more specific hypotheses will require a determination of the firing times of various populations of cells relative to the field potentials as well as an examination of the correspondence between the oscillations and the behavior of awake animals.

Within this conceptual framework, our observation of gamma oscillations with slightly different frequencies at different sites in the OB, resulting in a progressive phase shift of the two signals (Fig. 3C), takes on particular significance. Previous studies in which gamma was recorded at multiple locations within the OB concluded that it had a common frequency at all sites, with variations in the amplitude and phase (Freeman 1978; Bressler 1984, 1987). These studies employed spectral analysis on relatively short epochs of data (75-200 ms), however, which resulted in broad spectral peaks (5-13 Hz) and poor resolution of small frequency differences. The difference may also be due to our method of stimulus delivery, with relatively long pulses of odorant delivered to the olfactory mucosa, as compared to the natural respiratory
rhythm of the rat, or to differences between the urethane-anesthetized and awake states. Finally, we did not observe such asynchrony in our simultaneous recordings from OB and PC. Two factors may have contributed to this. First, it may be that the cortex only follows the gamma oscillation when a large portion of the OB becomes synchronous. Second, these recordings were made from deep within the bulb (in contrast to the recordings at multiple sites within the OB, which were made from superficial layers). In deeper layer, dipoles from larger regions, including both the medial and lateral aspects of the bulb, would be pooled to generate the field potential. The OB signal would therefore more nearly reflect the average activity of the entire bulb, and local regions of asynchrony would be more difficult to detect. A full understanding of the extent and significance of gamma synchrony in the olfactory system will require additional experiments.
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FIG. 1. Odorants evoke both gamma and beta oscillations in the olfactory bulb (OB) of the urethane-anesthetized rat. The relative strength of each oscillation depends on odorant concentration. A: Field potential in response to stimulation with a high concentration of amyl acetate. Note the strong 60 Hz gamma oscillation. In this and subsequent figures, air was drawn through the nose beginning at time 0. B: A 15 Hz beta oscillation induced by a dilute concentration of the same odorant. C: A mixed-frequency response to an intermediate concentration of the same odorant, with a strong gamma component early and a strong beta component later.

FIG. 2. Beta and gamma oscillations in the OB are distinct and not harmonically related. A: Average amplitude spectra for the experiment illustrated in Fig. 1 (n = 6 presentations of each odorant concentration). Spectra were computed for an 800 ms window beginning 200 ms after stimulus onset. Note that increasing odorant concentration increased power in the gamma band and decreased it in the beta band, without a shift in the frequency of either oscillation or the appearance of a spectral peak at intermediate frequencies. B: Spectral amplitude as a function of time and frequency for the intermediate concentration. Spectral amplitude is indicated in grayscale, with white being high. Note that the transition from gamma to beta was accomplished by a decrease in gamma amplitude and increase in beta amplitude rather than a smooth shift through intermediate frequencies. At no time was there substantial amplitude in the 25-45 Hz range. Data were filtered at 10-80 Hz, then average spectra (n = 6) were computed for a 256 ms sliding window at 64 ms steps. C: Scatter plot of peak gamma frequency vs. peak beta frequency (each point is one rat; n = 43). There is no relationship between the frequencies (linear regression, p > 0.5), and points do not cluster around lines of integral slope (dotted lines), as would be predicted if the oscillations were harmonically related.

FIG. 3. The beta oscillation is synchronous at nearby sites in the OB; the gamma oscillation can differ in frequency at different sites. Field potentials were recorded simultaneously at three sites separated by 2-4 mm in the lateral OB. A: Beta oscillation with identical frequency at all sites and phase differences near zero. Such synchrony was observed in all experiments (n = 12/12 rats). B: Experiment in which the gamma oscillation had an identical frequency at all sites and phase differences near zero. Such synchrony was observed in 5/9 rats. C: Experiment in which
the gamma oscillation differed slightly in frequency at one of the sites. Such asynchrony was observed in 4/9 rats. No systematic relationship was apparent across rats between recording location and gamma frequency. 

\textit{Ci}: Sample trace. Note the progressive phase-shift: oscillations were in phase at \textit{first arrow}, but out of phase at \textit{second arrow}. 

\textit{Cii}: Average amplitude spectra for the three sites, computed for a 400 ms window beginning 150 ms after stimulus onset (n = 6 presentations of strong amyl acetate). Note that all three spectra have peaks at \~{}59 Hz, but the spectrum for one site (asterisk) has a larger peak at \~{}50 Hz.

**FIG. 4.** Current source density analysis reveals that oscillatory field potentials are generated by granule cells. 

\textit{Ai}: Net membrane currents as a function of time and depth in the OB following current pulse stimulation of the lateral olfactory tract (LOT). A sink (\textit{warm colors}, net inward current) in the external plexiform layer was accompanied by a source (\textit{cool colors}, net outward current) in the internal plexiform layer. The sink is attributed to inward synaptic current into granule cell dendrites following glutamate release from secondary dendrites of antidromically activated mitral cells. Approximate cellular layers are indicated: \textit{GL}, glomerular layer; \textit{EPL}, external plexiform layer; \textit{MCL}, mitral cell layer; \textit{IPL}, internal plexiform layer; \textit{GCL}, granule cell layer. 

\textit{Aii}: Same as \textit{Ai} for orthodromic activation by shock stimulation of olfactory nerves. A superficial sink is attributed to afferent synaptic inputs to the glomerular tufts of primary dendrites from mitral and tufted cells. This was followed by a sink/source dipole attributed to synaptic excitation of granule cells by mitral and tufted cells. 

\textit{Aiii}: Net membrane currents in OB following current pulse stimulation in deep anterior piriform cortex (PC). A sink in the internal plexiform layer was accompanied by source current in the external plexiform layer. The sink is attributed to activation of the centrifugal projection from cortical pyramidal cells onto basal dendrites of granule cells. Note the slower conduction velocity and greater dispersion of this pathway compared to the LOT. 

\textit{B}: Net membrane currents in OB during a gamma oscillation. Data were filtered at 30-150 Hz. Sink-source pairs in the external and internal plexiform layers alternated polarity with a time course that resembled that of the mitral cell input to granule cells (compare to \textit{Ai}). 

\textit{C}: Net membrane currents in OB during a beta oscillation. Data were filtered at 8-30 Hz. Sink-source pairs in the external and internal plexiform layers alternated polarity, but with a slower time course that resembled that of the centrifugal input to granule cells (compare to \textit{Aiii}).
Fig. 5. Timing relationships between OB and PC. Both beta and gamma were nearly synchronous in the OB and PC, but small time lags were observed that were greater for beta than for gamma. Ai: Cross correlogram computed from the 800 ms epoch beginning 200 ms after stimulus onset, after bandpass filtering 12-30 Hz to isolate the beta oscillation. Average of 30 trials in one rat. Peak correlation at positive Δt indicates the PC signal lags the OB signal. Aii: Average cross correlogram after bandpass filtering 40-75 Hz to isolate the gamma oscillation. Aiii: Scatter plot of OB to PC lags as estimated from the cross correlograms after filtering to isolate each oscillation (each point is one rat, n = 20; filled symbol corresponds to the rat in Ai,ii). Note that in most rats, the time lag from OB to PC was greater during the beta oscillation than during the gamma oscillation. Bi: Square root of the average cross power spectrum for the rat in Ai,ii. Raw data (dotted line) were smoothed by convolution with a 3 Hz Gaussian (solid line) and the peaks detected in the beta and gamma frequency ranges. Bii: Average cross phase spectrum. Negative phase indicates that PC lags OB at that frequency. The OB to PC lag was estimated from the phase spectrum at the frequencies corresponding to the peaks in the smoothed cross power spectrum. Biii: Scatter plot (n = 20 rats) of OB to PC lags as estimated from the phase spectra.

Fig. 6. Olfactory cortex is required for generation of the beta oscillation, but not the gamma oscillation. A,B: Amplitude spectra of responses to four odorants in OB and anterior PC, respectively, before and after surgical interruption of the LOT. Similar results were observed in all experiments. Ai: Before the lesion, both beta and gamma could be elicited in the OB. Aii: After the lesion, beta was abolished while gamma was at least as strong. Bi: In the anterior PC, both oscillations were observed before the lesion. The gamma oscillation was weaker in PC than in the OB. Bii: After the lesion, all oscillatory activity was abolished in PC. C,D: Physiological verification of the completeness and specificity of the lesion. Recordings were made in the OB. Ci: Before the lesion, stimulation of the lateral olfactory tract overlying anterior piriform cortex evoked an antidromic response in the olfactory bulb (surface-negative wave at short latency). Cii: This response was abolished after the lesion. Di: Stimulation in deep layers of anterior piriform cortex activated the centrifugal fiber system, which evoked a deep-negative response in the olfactory bulb. Dii: This pathway was not disrupted by the lesion.
FIG. 7. Possible mechanisms for generation of fast oscillations. A: Gamma generation by negative feedback (+, excitation; -, inhibition) between mitral/tufted cells (M) and granule cells (G) (Rall and Shepherd 1968; Freeman 1975; Gray and Skinner 1988; Eeckman and Freeman 1990). B: Mechanism for generation of beta oscillation by the loop encompassing mitral cells, cortical pyramidal cells (P), and granule cells. Axonal and synaptic delays are consistent with this mechanism. C: Generation of beta by intrinsic mechanisms within PC. In this scenario, the beta oscillation would be transmitted to the OB by the centrifugal projection, but this fiber system is not required for generation. The mechanisms illustrated in B and C are not mutually exclusive.
Figure 1

A

Voltage (mV) vs. Time (ms)

B

Voltage (mV) vs. Time (ms)

C

Voltage (mV) vs. Time (ms)
Figure 2

A

B

C

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strong amyl acetate
intermediate amyl acetate
dilute amyl acetate
clean air

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frequency (Hz)
time (ms)

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peak gamma frequency (Hz)
peak beta frequency (Hz)
Figure 4

Ai  LOT stim

Aii  ON stim

Aiii  centref. stim

B

C
Figure 5

Ai

Aii

Aiii

Bi

Bii

Biii