How Global Are Olfactory Bulb Oscillations?

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Kay LM, Lazzara P. How global are olfactory bulb oscillations? J Neurophysiol 104: 1768–1773, 2010. First published July 21, 2010; doi:10.1152/jn.00478.2010. Previous studies in waking animals have shown that the frequency structure of olfactory bulb (OB) local field potential oscillations is very similar across the OB, but large low-impedance surface electrodes may have favored highly coherent events, averaging out local inhomogeneities. We tested the hypothesis that OB oscillations represent spatially homogeneous phenomena at all scales. We used pairs of concentric electrodes (200 μm outer shaft surrounding an inner 2–3 μm recording site) beginning on the dorsal OB at anterior and medial locations in urethane-anesthetized rats and measured local field potential responses at successive 200 μm depths before and during odor stimulation. Within locations (outer vs. inner lead on a single probe), on the time scale of 0.5 s, coherence in all frequency bands was significant, but on larger time scales (10 s), only respiratory (1–4 Hz) and beta (15–30 Hz) oscillations showed prominent peaks. Across locations, coherence in all frequency bands was significantly lower for both sizes of electrodes at all depths but the most superficial 600 μm. Near the pial surface, coherence across outer (larger) electrodes at different sites was equal to coherence across outer and inner (small) electrodes within a single site and larger than coherence across inner electrodes at different sites. Overall, the beta band showed the largest coherence across bulbar sites and electrodes. Therefore larger electrodes at the surface of the OB favor globally coherent events, and at all depths, coherence depends on the type of oscillation (beta or gamma) and duration of the analysis window.

INTRODUCTION

One of the most salient features of mammalian olfactory bulb (OB) electrophysiology is the oscillatory nature of the local field potential (LFP). Regular oscillations in several frequency bands have been associated with specific sensory, motor, and behavioral events (Kay et al. 2009). Respiratory oscillations overlapping the theta frequency band (1–12 Hz) track the inhalation cycle driving sensory stimulation. Coherence between sniffing behavior or respiratory oscillations and the hippocampal theta rhythm has been associated with some types of odor learning and discrimination performance (Kay 2005; Macrides et al. 1982). Beta oscillations (15–30 Hz) have been observed in many olfactory and limbic areas accompanying associative learning and odor sensitization (Hermé-Vazquez et al. 2007; Lowry and Kay 2007; Martin et al. 2004, 2007). These oscillations are highly coherent within olfactory-limbic networks and depend on reciprocal connections between the OB and higher cortical areas (Martin et al. 2006). Gamma bursts represent cooperative activity among networks of mitral cells (Eeckman and Freeman 1990) and have been proposed to be a common waveform engaged coherently within the entire OB network, occupying a wide band in the gamma frequency range (Bressler 1988; Bressler and Freeman 1980; Freeman and Viana Di Prisco 1986; Kay 2003), and they show modest to almost no coherence with higher cortical areas (Beshel et al. 2007; Kay et al. 2009).

The LFP can be measured using many types of electrodes, with recording surfaces generally ranging from 1 to 200 μm and impedances in the range of 100 KΩ to 10 MΩ. Much of the data that support global similarity in the OB oscillatory signal are from large electrodes (200 μm diam) placed in a grid array on the surface of the OB. Analysis of simultaneous recordings from these arrays showed that the two to three Fourier frequencies or principle components of the waveform with the largest coefficients over the array of recording electrodes comprise most of the power in the EEG signal measured at the pial surface of the cortex (Freeman 1978; Freeman and Viana Di Prisco 1986). However, several studies have characterized differences in frequency structure or power dependent on cortical depth or bulbar location (Bressler 1984; Martin et al. 2004). It is possible that the electrodes themselves favor highly coherent phenomena, because large conductive surfaces average over large populations, especially at the cortical surface (Mitzdorf 1985; Neville and Haberly 2003).

We tested whether the OB oscillatory LFP is globally coherent at smaller scales as well. It was assumed so from these earlier studies, but smaller, high-impedance electrodes may not produce the same picture as large, low-impedance electrodes (Pesaran 2009; but see Nelson and Pouget 2010). We used simultaneous recordings from concentric electrode pairs (outer/large vs. inner/small recording leads) in two different locations at successive depths as measures of signals averaged over different sized neural populations in the neighborhood of the electrodes. We found that there is a high degree of coherence in all frequency bands across the OB for both electrode sizes but that coherence is significantly lower when the electrodes are in different locations than at the same location. In the superficial layers, cross-site coherence on the outer/larger electrodes was the same as within-site (outer-inner) coherence values and significantly greater than cross-site coherence for the inner/smaller electrodes. Furthermore, gamma band coherence was restricted to small time windows (0.5 s), on the order of a sniff cycle, and did not persist in long time windows (10 s), whereas beta and respiratory oscillations showed coherence over long time windows.

METHODS

Animals and surgical procedure

Experiments were performed on 14 male Sprague-Dawley HSD rats (330–450 g; purchased from Harlan, Indianapolis, IN). Rats were...
anesthetized with a presurgical cocktail (35 mg/kg ketamine, 5 mg/kg xylazine, 0.75 mg/kg acepromazine), followed by urethane (0.8–1.5 g/kg). The bone over the left olfactory bulb was removed with a hand drill (centered at 8.5 mm anterior to bregma and 1.5 mm lateral from the midsagittal line), and the dura was removed, with care taken to preserve the integrity of the dorsal bulbar surface. Reference and ground electrodes were connected to skull screws located on the posterior dorsal skull on either side of the midline.

All procedures were carried out with approval from and oversight by the University of Chicago Institutional Animal Care and Use Committee, according to guidelines set by Association for Assessment and Accreditation of Laboratory Animal Care.

Electrodes

One bipolar stainless steel electrode (100 μm diam formvar-coated stainless steel wire, California Fine Wire; ~1.5 mm vertical tip separation) was first placed in the posterior portion of the OB (~7 mm anterior to bregma) at depths ranging from 400 to 2,200 μm. This electrode was used to record OB LFP signals to obtain a clear and consistent theta band respiratory signal throughout the procedure and was positioned in each rat for optimal visualization of this oscillation. Two concentric electrodes were positioned at the anterior (~10 mm anterior to bregma) and medial (~8.5 mm anterior to bregma and 1.5 mm lateral to midsagittal line) dorsal surface of the OB (Micro Probe, Gaithersburg, MD). The outer recording site was a stainless steel ring with a diameter of 200 μm (~100 kΩ at 1 kHz); the inner recording site was a high-impedance tungsten electrode with a diameter of 2–3 μm (1–4 MΩ) and extended ~200 μm from the end of the larger electrode to avoid coupling between the two in situ. Both concentric probes were initially placed so that the smaller/inner lead was 200 μm deep to the pial surface and the larger/outter lead was on the pial surface (Fig. 1A).

Recordings

Data were recorded from all six leads simultaneously (CED Spike-2 software, power 1401 16-channel interface, AM systems 3600 amplifier and headstage), with a sampling rate of 2 kHz, analog filters set at 0.3–300 Hz, and a gain of 2,000. (For 1 rat, a Neurolynx Cheetah32 recording system was used, with 0.1–475 Hz analog filters, 2,016 Hz sampling rate, and gain of 4,000.) One of the skull screws was used as reference. In some cases, we used a 60 Hz notch filter, because under anesthesia, the gamma oscillation frequencies with our protocol were typically ~60 Hz. Two different odorants (nonanol and amyl acetate) were administered through a nose cone for stimulation periods of ~15 s, followed by ~40 s of no odor between trials. Three stimulations of each odor were made at each depth. After the six odor periods for a given depth, the concentric electrodes were lowered 200 μm, and the odor stimulation was repeated. Sampling was done from 10 to 11 depths, with final recordings made in the granule cell layer (~2,000 μm). Because the OB is shallower at the anterior end than it is medially, at the deeper locations, the two probes were in different layers or in different parts of the granule cell layer (Fig. 1A, dashed outlines).

Analysis

Data were examined for presence of gamma activity on the electrodes (example in Fig. 1B), and those with no visible gamma were excluded (4 rats). Subsequent analyses were performed on the remaining 10 rats. Data were analyzed using Igor Pro 6.1.2.1 (Lake Oswego, OR) and the Chronux toolbox (http://www.chronux.org/; Mitra and Bokil 2008) in MATLAB (MathWorks, Natick, MA).

Six 10-s intervals during odor stimulation (3 for each odorant) and matched 10-s segments before odor stimulation were analyzed for each recording depth across all leads. To allow comparisons of power across subjects and electrode depths, we normalized the amplitude of our signals by the SD of the first 10-s segment for each rat. Power and coherence were estimated using multitaper settings in the Chronux toolbox for MATLAB [for 10-s windows, T*W time-bandwidth product was 9, and 17 tapers (2TW-1) were used; for 0.5-s windows, T*W = 2, and 3 tapers were used.] All analysis windows were padded with zeros to the next power of two for FFT analysis. We used the jackknife method for estimating the 95% confidence bands for all spectral measures.

Coherence estimates across leads were calculated for the two concentric electrodes on a single probe (outer vs. inner anterior and outer vs. inner medial) and electrodes of the same type across locations (anterior vs. medial outer electrodes and anterior vs. medial inner electrodes) for each 10 s window in two ways. The first method used tapers and coherence estimates applied to the entire 10 s window, and these estimates were averaged across all the 10 s windows for a given depth. The second method used 20 individual nonoverlapping 0.5 s windows to estimate coherence within each 10 s window. The first method favors oscillatory events that have a consistent phase relationship throughout a 10 s window, and the second favors events that may have only brief periods with consistent phase. We used coherence to estimate the extent of the respiratory wave from an electrode that is placed in the granule cell layer in the deep posterior OB (low-pass filter at 4 Hz). Markers above the theta trace note automatically detected peaks.

![Fig. 1](http://jnp.jn.org/fig1.png)

**Fig. 1.** Electrodes, recording sites, and sample data. **A:** left: concentric electrode—an outer insulated stainless steel cannula with the end ring exposed (200 μm diam, ~100 kΩ impedance) surrounds an inner tungsten microelectrode with a 1–3 μm tip (~1–4 MΩ impedance). **Right:** electrode positions relative to the olfactory bulb (OB) layers; both electrodes begin with the outer cannula on the surface of the OB and the inner probe at a depth of 200 μm. The probes are advanced in 200 μm increments to a depth of 2 mm. After the probes cross the mitral cell layer, the different depths of the OB at the anterior and medial locations causes the 2 probes to be in different parts of the cortex. Dashed lines indicate an example of the extent of travel for the probes. **B:** sample signals from 2 concentric electrodes (ant, anterior; med, medial; O, outer; I, inner; 5 s of data are shown). Top 4 traces are raw local field potentials (LFPs; 0.3–300 Hz). Note that the 2 anterior and 2 medial leads are more similar to each other than either is with the other location. Below the raw data are the gamma band–filtered (30–75 Hz) traces from anterior and medial inner electrodes. The trace labeled deep θ is the surrogate of the respiratory wave from an electrode that is placed in the granule cell layer in the deep posterior OB (low-pass filter at 4 Hz). Markers above the theta trace note automatically detected peaks.
Fisher’s Z transform of the coherence [Z-coherence = \( \tanh^{-1}(\text{coherence}) \)] to distribute the values from zero to infinity instead of zero to one, as we and others have previously (Boeijinca and Lopes da Silva 1988; Kay and Beshel 2010; Kay and Freeman 1998). The baseline for significant coherence was determined using coherence estimates from signals within rats across different depths.

RESULTS

To address the similarity of oscillatory activity across sites, depths, and electrode sizes, we analyzed data from concentric electrode pairs (Fig. 1A) in 10 rats at 10 successive 200\( \umu \text{m} \) depths per rat with and without odor stimulation. We used the power spectrum to determine whether the frequency structure changed with location, electrode size, or depth. We used coherence to estimate the relative magnitude of signal similarity across pairs of electrodes, either within a location (large vs. small/outer vs. inner lead) or across locations (outer or inner leads in anterior and medial locations). Because of the difference in bulbar depth for the anterior versus medial OB, we assume that the superficial locations (depths 1–4) represent the best comparisons across electrode sites within the same cortical layer (dashed outlines in Fig. 1A). Deeper sites were compared as a measure of frequency similarity across different cortical layers or different parts of the granule cell layer. Simultaneous data samples from the two concentric leads at both locations in the superficial dorsal OB are shown in Fig. 1B. The respiratory frequency calculated from the low-frequency rhythm on the static deep electrode was stable throughout sessions and similar across rats [1.13 \( \pm 0.12 \) (SD) Hz].

Power

We used the power spectrum from 10 s odor stimulation windows to estimate the frequency structure of LFP activity from each electrode. We found prominent activity in theta, beta, and gamma bands, but the relative power of these three oscillatory phenomena varied with depth (Fig. 2A). The respiratory rhythm was strong at every depth, increasing in power with depth. Beta oscillations (~20 Hz) were most prominent at superficial locations (200–600 \( \umu \text{m} \)) across all leads. At deeper locations, a peak at ~30–35 Hz was evident, which could be either high-frequency beta or low-frequency gamma. Gamma oscillations could be seen at every depth on all electrodes but were weakest at the two most superficial locations, particularly from the anterior leads, and strongest in the deep layers. Analysis of matched preodor segments showed only small differences in anterior leads, and strongest in the deep layers. Power analysis of matched prestimulus periods showed similar profiles, but the gamma peaks were less robust without odors (Fig. 3B).

DISCUSSION

Recent and older studies have addressed the role of electrode size and shape in local signal averaging (Berens et al. 2010; Freeman 1978; Katzner et al. 2009; Pesaran 2009; Xing et al. 2009). The LFP measured from a point in cortex includes components primarily within a 100 \( \umu \text{m} \) radius but also contains the influence from neurons \( \pm 250 \umu \text{m} \) away from a given recording site, on the scale of a cortical column. The LFP around a small high-impedance electrode tip (~1 \( \umu \text{m} \); 1–4 \( \text{MO} \)) thus may sum only over the column in which it resides, whereas larger electrodes with larger recording surfaces and lower impedance (~100 \( \text{KO} \)) likely sum over much larger areas. With a 200 \( \umu \text{m} \) diameter, the electrode may include the activity of several cortical columns, \( \pm 700 \umu \text{m} \) or more in diameter. This corresponds to an area \( \approx 2\times7 \) times larger than that covered by the smaller electrodes, assuming that the spatial activity of olfactory bulb oscillations across the cortical surface estimated from large high-impedance electrodes would translate to the finer scale provided by small high-impedance electrodes.

We used multitaper coherence methods to examine the degree to which activity in all LFP frequency bands is shared between electrodes of different sizes within a single location (outer and inner electrodes on a single probe) or between electrodes of the same size across locations in the anterior and medial dorsal OB. We found that coherence across the outer (200 \( \umu \text{m} \)) and inner (3
leads on a single probe within a cortical location was significantly higher than across areas between either outer or inner electrodes in the deep layers of the OB using 10 s windows to estimate coherence (Fig. 2B). In the superficial layers, coherence across sites for outer leads was not different from coherence within a site and was greater than for inner leads across sites. These results suggest a modest influence of electrode size in estimating waveform similarity at the cortical surface; larger low impedance electrodes are biased toward globally coherent activity and smaller high-impedance electrodes are biased toward locally coherent activity. In the deeper layers, we found no differences between the two types of electrodes.

We did find differences in coherence frequency structure depending on the size of the window used to produce the coherence estimates (Figs. 2B and 3A). Using long time windows (10 s), we found coherence peaks primarily in the beta and respiratory bands. Using short time windows (0.5 s), we found significant coherence peaks in the 40–50 Hz range.
within sites that varied by depth and electrode site and a second gamma or high beta peak at 30–35 Hz. We also found modest gamma peaks in deep cross-site coherence measures, but the beta band predominated in this cross-site coherence at the short time scale. Differences between odor and prestimulus periods could be seen in the power of gamma band coherence within sites. This is consistent with the idea that gamma oscillations represent local processing of odor-specific information (Kay et al. 2009; Rojas-Líbano and Kay 2008).

Our results suggest that gamma frequency events in the rat OB are locally coherent in small time windows on the order of an inhalation period (0.5 s), but that beta band events are globally coherent in time windows as long as 10 s. These data support the hypothesis that beta and gamma oscillations represent different processes and neural circuits within the mammalian olfactory system, as we and others have proposed elsewhere (Beshel et al. 2007; Kay and Beshel 2010; Kay and Freeman 1998; Kay et al. 2009; Lowry and Kay 2007; Martin et al. 2004, 2007).

Gamma oscillations have been proposed as one mechanism for binding assemblies of neurons in response to sensory stimuli (Buzsaki and Chrobak 1995; Singer 1993). Experimental and theoretical results suggest that networks of oscillatory cells activated by different inputs and directed at different outputs may segregate within a heterogeneous gamma rhythm and that gamma band oscillations are particularly good at forming assemblies that are stable in the face of distracters (Borgers et al. 2005; Colgin et al. 2009). We found that there was no consistent phase relation-
ship in the gamma band even within local regions; coherence across outer and inner electrodes showed no peak when using 10 s segments but did show a peak for 0.5 s segments. This suggests that, within local areas, gamma oscillations do not have a consistent phase relationship across inhalation cycles and that there is inhomogeneity even on this very fine spatial scale. This leaves open the possibility of multiple oscillatory assemblies competing within a small group of cortical areas or columns. There is precedent for multiple local gamma-coupled assemblies in the OB. Recordings with small glass pipette electrodes in the rabbit olfactory bulb under urethane anesthesia have shown that mitral cells can couple with local gamma oscillations and with each other dependent on the odorant used for stimulation and that this effect is limited to neurons within a relatively local area (~300 μm) in which cells tend to have overlapping inputs (Kashiwadani et al. 1999). If OB local assemblies are formed from neurons associated with neighboring glomeruli, and gamma oscillatory assemblies can separate in frequency space, the LFP would show gamma activity spread over a relatively wide band as the electrode sums over many assemblies.

As a final comment, we note that the differences in coherence between outer and inner electrodes in the superficial layers are relatively small, with high background coherence in most bands across all positions recorded simultaneously. Our results suggest that, although larger low-impedance electrodes show more globally coherent activity than smaller high-impedance electrodes, the frequency structure from both types of electrodes is very similar, and activity is strongly coherent across different locations in the OB.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

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