How global are olfactory bulb oscillations?

Leslie M. Kay\(^1\,^2\) and Philip Lazzara\(^2\,^3\)

\(^1\) Department of Psychology, The University of Chicago, Chicago IL, USA
\(^2\) Institute for Mind and Biology, The University of Chicago, Chicago IL, USA
\(^3\) St. John’s College, Annapolis, MD, USA

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Corresponding Author:

Leslie M. Kay
Institute for Mind and Biology
940 E 57\(^{th}\) St.
Chicago, IL 60637
773-702-6174
fax: 773-702-6898
e-mail: L.Kay@uchicago.edu

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Abstract

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 prior to and during odor stimulation. Within locations (outer vs. inner lead on a single probe), on the timescale of 0.5s, coherence in all frequency bands was significant, but on larger timescales (10s) 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 (Hermer-Vazquez et al. 2007; Lowry and Kay 2007; Martin et al. 2007; Martin et al. 2004). 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 1-200 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
diameter) placed in a grid array on the surface of the OB. Analysis of simultaneous recordings from these arrays showed that the 2-3 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; 1992; 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 (0.5s), on the order of a sniff cycle,
and did not persist in long time windows (10s), while 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 grams; 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 diameter 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 - 2200 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 approximately 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/outer lead was on the pial surface (Fig. 1A).

Recordings

Data were recorded from all 6 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 2000. (For one rat a Neuralynx Cheetah32 recording system was used, with 0.1-475 Hz analog filters, 2016 Hz sampling rate and gain of 4000.) One of the skull screws was used as reference. In some cases we used a 60 Hz notch filter, since under anesthesia the gamma oscillation frequencies with our protocol were typically lower than 60 Hz. Two different odorants (nonanone and amyl acetate) were administered through a nose cone for stimulation periods of ~15 seconds, followed by ~40 seconds 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 10-11 depths with final recordings made in granule cell layer (~ 2000 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-second intervals during odor stimulation (3 for each odorant) and matched 10-second
segments prior to 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 standard deviation of the first 10s 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-bandwith 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 10s window, and these estimates were averaged across all the 10s windows for a given depth. The second method used 20 individual non-overlapping 0.5s windows to estimate coherence within each 10s window. The first method favors oscillatory events that have a consistent phase relationship throughout a 10s window, and the second favors events that may have only brief periods with consistent phase. We used Fisher’s Z transform of the coherence (Z-coherence = tanh⁻¹(coherence)) to distribute the values from zero to infinity instead of zero to one, as we and others have previously (Boeijinga 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 µm depths per rat with and without odor stimulation. We used the power spectrum to determine if the frequency structure changed with location, electrode size or depth. We used coherence to estimate the relative magnitudes 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 vs. 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 Figure 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 ± 0.12 Hz, mean ± standard deviation).

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Power

We used the power spectrum from 10s 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 µm) across all leads. At deeper locations, a peak at approximately 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 pre-odor segments showed only small differences in gamma band power, with slightly higher power during odor stimulation than without odor (Supplementary Fig. S1A).

Coherence

Coherence relies on consistent phase relationships between two signals. Therefore, the size of the data window analyzed may significantly affect the results. For phenomena that show temporally local phase relationships that change quickly, such as gamma oscillations, the relative phase pattern may look random when averaging across large time windows. We therefore
calculated coherence from both long and short time windows (10s windows or 20 non-
overlapping 0.5s windows within and before each odor stimulation period at each depth). We
obtained different results from the two methods, particularly in the gamma band (Figs. 2B, 3A).

Coherence analysis over all 10s windows at a given depth across the 10 rats showed a prominent
peak in the beta frequency band both within and across locations, with almost indiscernible
differences between odor and non-odor periods (Fig. 2B, Supplementary Fig. S1B). In the most
superficial locations, coherence within sites was the same as that across sites for the outer
electrode comparisons (Fig. 2B, depths 200-600 µm). At deeper penetrations of the two
electrodes, coherence within locations across outer-inner electrode pairs remained high, but
across locations, coherence decreased significantly.

Previous studies have suggested that within gamma bursts there is a measurable phase gradient
across spatial locations at the pial surface, but that across bursts the spatial source of this phase is
random (Freeman and Baird 1987; Freeman and Skarda 1985). Such a phenomenon would lead
to an underestimation of coherence when using long time segments, because phase relationships
between two locations across a 10s period would appear random. We therefore repeated the
analysis dividing the 10s periods into 20 half-second windows for coherence estimation. A peak
in the gamma band emerged (40-50 Hz; Fig. 3A), and this gamma peak was as strong as the beta
peak in the deeper locations. The low gamma or high beta peak (30-35 Hz) that was shown in
the power spectra from individual leads was also present in these coherence spectra, particularly
in the deeper locations. The coherence in both of beta and gamma frequency bands was much
stronger within a location across outer and inner electrodes than across locations for either outer
or inner electrodes. The coherence spectra for 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. 2008; 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
µm radius but also contains the influence from neurons up to 250 µm away from a given
recording site, on the scale of a cortical column. The LFP around a small high impedance
electrode tip (~1 µm; 1-4 MΩ) thus may sum only over the column in which it resides, while
larger electrodes with larger recording surfaces and lower impedance (~100 KΩ) likely sum over
much larger areas. With a 200 µm diameter, the electrode may include the activity of several
cortical columns, up to 700 µm or more in diameter. This corresponds to an area ~2-7 times
larger than that covered by the smaller electrodes, assuming as a first approximation hexagonal
packing of cortical columns. We therefore examined whether previous results regarding the
similarity of olfactory bulb oscillations across the cortical surface estimated from large low-
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 µm) and inner
(3 µm) 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 10s 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 (10s) we
found coherence peaks primarily in the beta and respiratory bands. Using short time windows
(0.5s) 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

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 (0.5s), but that beta band events are globally
coherent in time windows as long as 10s. 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

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
relationship in the gamma band even within local regions; coherence across outer and inner leads
showed no peak when using 10s segments but did show a peak for 0.5s 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, then
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 then suggest that while 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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**Figure Captions**

**Figure 1. Electrodes, recording sites and sample data. A) Left:** Concentric electrode- an outer insulated stainless steel cannula with the end ring exposed (200 µm diameter, ~100 KΩ impedance) surrounds an inner tungsten microelectrode with a 1-3 µm tip (~1-4 MΩ impedance).  
**Right:** Electrode positions relative to the 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 two 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 two concentric electrodes (ant- anterior, med- medial, O- outer, I- inner; 5 seconds of data are shown). Top 4 traces- raw LFPs (0.3-300 Hz). Note that the two anterior and the two 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).**

**Figure 2. Power and coherence spectra by depth. A) Power spectra, 10s windows. Power was computed from 6 ten-second windows across the 10 rats at each depth for each recording site (ant- anterior, med- medial, O- outer, I- inner). Semilog plots for each depth are displayed with the depth in micrometers above each plot. Error bands around each power estimate represent the 95% confidence range from jackknife power estimates. Note the primary gamma peak at ~50 Hz and the lower gamma or high beta peak at ~35 Hz, particularly in the deep layers.**

**B) Z-Coherence, 10s windows. Z-coherence was computed from the same 10s time windows**
as in Figure 2A, and the error bands are the 95% confidence estimates from jackknife z-
coherence estimates. Note that the only prominent peaks from 10s windows are in the lower
frequency ranges, beta and theta. A weak 35 Hz peak also emerges in the deep layers. Z-
coherence in the first 3 layers is as high for the outer cross-site pair of leads as it is for the outer-
inner within-site pairs. By 1000-1200 μm in depth, the outer and inner cross-site pair coherence
values are the same. The gray traces in the bottom of the upper left panel are the coherence
values from shuffled data.

**Figure 3. Short time window coherence spectra by depth. A) 0.5s windows, odor periods.**
Z-Coherence was computed from 20 non-overlapping 0.5s windows during odor stimulation
comprising the same 10s windows as in Fig. 2, and gamma peaks are now evident at all depths
but not on every pair of leads. The largest coherence values are from within-site (outer-inner)
estimates. Prominent peaks are also present in the beta band and at ~35Hz as a shoulder to the
beta peak. Error bands are the 95% confidence estimates from jackknife z-coherence estimates.
The gray traces in the bottom of the upper left panel are the coherence values from shuffled data.

**B) 0.5s windows, prestimulus periods.** Z-coherence was computed from 20 non-overlapping
0.5s windows comprising the 10s prestimulus periods just before each odor period. Note that
gamma band peaks are less prominent when odors are absent.