Chemical factors determine olfactory system beta oscillations in waking rats

Catherine A. Lowry \textsuperscript{1} and Leslie M. Kay \textsuperscript{1,2}

\textsuperscript{1} Committee on Neurobiology
\textsuperscript{2} Department of Psychology
The University of Chicago
Chicago, IL 60637
USA

Submitted: February 3, 2007
Revised: March 11, 2007; 2\textsuperscript{nd} revision March 30, 2007

\textbf{Running title:} Olfactory beta oscillations and odorant volatility

41 pages total: 32 text pages (incl. 37 references, Figure Legends, 1 table), 9 figures

\textbf{Keywords:} beta oscillations; olfactory bulb; piriform cortex; predator odor; partial pressure; coherence; sensitization

\textbf{Corresponding Author:}
Leslie M. Kay
Institute for Mind \& Biology
940 E. 57\textsuperscript{th} St.
Chicago, IL 60637

Tel. 773-702-6174
Fax 773-702-6898
Email: LKay@uchicago.edu
Abstract

Recent studies have pointed to olfactory system beta oscillations of the local field potential (15-30 Hz) and their roles both in learning and as specific responses to predator odors. To describe odorant physical properties, resultant behavioral responses and changes in the central olfactory system that may induce these oscillations without associative learning, we tested rats with 26 monomolecular odorants spanning six log units of theoretical vapor pressure (estimate of relative vapor phase concentration) and 10 different odor mixtures. We found odorant vapor phase concentration to be inversely correlated with investigation time on the first presentation, after which investigation times were brief and not different across odorants. Analysis of local field potentials from the olfactory bulb and anterior piriform cortex shows that beta oscillations in waking rats occur specifically in response to the class of volatile organic compounds with VPs of 1-120 mmHg. Beta oscillations develop over the first 3-4 presentations and are weakly present for some odorants in anesthetized rats. Gamma oscillations show a smaller effect that is not restricted to the same range of odorants. Olfactory bulb theta oscillations were also examined as a measure of effective afferent input strength, and the power of these oscillations did not vary systematically with vapor pressure, suggesting that it is not olfactory bulb drive strength that determines the presence of beta oscillations. Theta band coherence analysis shows that coupling strength between the olfactory bulb and piriform cortex increases linearly with vapor phase concentration, which may facilitate beta oscillations above a threshold.
Introduction

Odor-evoked olfactory bulb (OB) oscillations of the local field potential (LFP) were originally described by Adrian (Adrian 1950), and since then they have been noted in many species of waking and anesthetized mammals. These fast oscillations are centered at about 70 Hz in the rodent olfactory system, but can be lower or higher, depending on the species and presence or absence of anesthesia (Bressler and Freeman 1980). Gamma oscillations have been the focus of modeling efforts and theories of sensory binding in olfactory and other systems. However, recent studies have shown that beta oscillations (15-30 Hz) are robustly generated by the olfactory system in an olfactory Go/No-Go task (Martin et al. 2004; Ravel et al. 2003). These oscillations depend in the OB on an intact bidirectional connection between the OB and piriform cortex (PC), appear to have different sources of generation in the OB than gamma oscillations (Martin et al. 2006; Neville and Haberly 2003), and have been proposed to be transmitted to the OB from the PC (Bressler 1984).

Beta oscillations have also been seen in response to specific odorants without explicit associative learning, and it has been claimed that these odorants may be privileged in their representation in the olfactory system by virtue of the types of oscillations they induce. Several studies have proposed that beta oscillations are specific to a predator odor response, as they are found to be enhanced in the OB, PC and hippocampus in response to two putative predator odorants, 2-propylthietane, a component of weasel anal gland secretions, and 2,4,5-trimethylthiazoline (TMT), a component of fox feces (Heale and Vanderwolf 1994b; Heale et al. 1994; Vanderwolf and Zibrowski 2001; Zibrowski et al. 1998; Zibrowski and Vanderwolf 1997). However, these studies also found that several organic solvents, such as toluene and xylene,
evoke beta oscillations. Computational studies have suggested that beta oscillations present in distributed neural systems may depend on the strength of input drive or feedback (Olufsen et al. 2003; Whittington et al. 2000).

To address innate beta oscillatory responses to odorants and the system properties associated with generation of these oscillations, we examine the physical properties of odorants which evoke enhanced beta responses in the olfactory system independent of associative learning. We show that the response is not specific to odorants generated by potential predator species; it is instead related to their airborne concentration. We also show that the beta oscillatory response is more robust in waking than in anesthetized rats and develops in waking rats over several presentations in a sensitization-like fashion.

Methods

Subjects were 13 adult male Sprague-Dawley rats (~450 g; purchased from Harlan HSD), maintained in the colony room on a 14-10 hr light/dark schedule (lights on at 8:00 CST). Rats were housed singly after electrode implantation, and all animals had access to unlimited food and water for the course of the experiments. All animal procedures were done with approval and oversight by the University of Chicago animal care and use committee with strict adherence to AAALAC standards.

Electrode implants

Seven of the rats were implanted with chronic electrodes, and the remaining six were used acutely for study of the effects under urethane anesthesia. The surgical procedure was the same for the two groups, except that no headstage was constructed for those studied acutely. Anesthesia protocols differed for the two groups. For chronic implants, each rat was given a
presurgical induction dose of Ketamine cocktail (35mg/kg Ketamine, 5 mg/kg Xylazine and 0.75 mg/kg Acepromazine) and was maintained with hourly intraperitoneal (IP) injections of 25 mg/kg pentobarbital (Nembutal). Acute procedures were done under urethane anesthesia (1.2-1.3g/kg dissolved in physiological saline). Bipolar stainless steel (100µm wire; ~1 mm vertical tip separation) electrodes, formvar insulated, were placed unilaterally (left) in the OB (8.5 mm anterior to bregma, 1.5 mm lateral) and aPC (0.5mm anterior to bregma, 3.0mm lateral, 15° angle), guided by concurrent stimulation of the lateral olfactory tract (LOT; 2.7mm anterior, 1.6mm lateral, 7mm deep, 14° angle). Electrodes were positioned across the mitral cell layer in the OB and across layer 2/3 pyramidal cell layer in the aPC by lowering the electrode perpendicular to the cell layer until the evoked potential from LOT stimulation was reversed across the two leads of the electrode. Reference and ground stainless steel screws were placed in the right anterior and posterior regions of the skull, and additional screws were used for securing the headstage to the skull. For chronic implants connector pins for each lead were inserted into a round plastic receptacle (Ginder Scientific, Ottawa, Canada), and the assembly was embedded in dental cement. Chronically implanted rats were allowed to recover for at least 2 weeks after surgery before beginning the experimental protocol.

**Verification of electrode placements**

After experiments were complete, rats were given an overdose of Nembutal and electrode placements were marked by passing current through the tip of the stainless steel wires. Electrode tips were marked using the Prussian Blue reaction. Prior to sectioning, brains were extracted from the skull and the electrode array was removed. Both OB and aPC placements could often be confirmed by visual examination of the external or cut brain for the blue stain marking the
electrode tip. Additional confirmation of placements was obtained by sectioning the brain coronally and staining with neutral red.

**Experimental design: waking rats**

Each rat was placed in a large clean polycarbonate cage with a headstage cable (Neuralynx HS-27) connected to the recording setup (Neuralynx Cheetah-32), and was allowed to move freely in the cage throughout the experiment. For each odor presentation an odor-saturated cotton swab was held under the rat’s nose for 10 seconds, or until the rat turned away from the swab, whichever was first. Turning away was defined as either turning the head away from the swab or backing more than 1 inch away from the swab and ceasing sniffing behavior after presentation under the snout. The amount of time spent sniffing the odorant, or the latency to turn away from the swab, on each trial was measured in seconds and recorded with a timer during the experiment. Each odorant was presented 12 consecutive times followed by a single dry swab presentation. Inter-trial time was at least 20 seconds.

Each test session consisted of 6 different odorants in a block design (12 trials for each odorant, as described above) with a block of 12 dry swab presentations before and after each set of 6 odorants. All animals were presented with a total of 6 sets of 6 odorant blocks (36 odors). Most animals were presented with 2 sets of 6 blocks (12 odorants) per day, and no rat was presented with more than 3 sets (18 odorants) in one day. At least two hours separated the end of the first odorant test set and beginning of the second odorant test set for an animal in a day. All tests took place during the light phase (8:00- 22:00 CST).

Odorant blocks were presented in balanced order across subjects, subject to the following restrictions for choosing the 6 odorants for each session (one session = six odorants). Only one
odorant of each functional group (carboxylic acids, ketones, aldehydes and alcohols) was presented within each session. Odorant mixtures and odorants determined by published studies to reliably elicit beta oscillations (toluene, xylene, TMT) were distributed across sessions so as to avoid giving more than one in each set of 6 odorant blocks. Within these parameters, odorants were assigned randomly to test sessions. Odorant vials were coded and the experimenter was blind to the identity of the odorants during testing.

Experimental design: anesthetized rats

The design was the same as that used for the waking rats, with a few exceptions. Each odorant block consisted of 12 consecutive presentations followed by a dry swab presentation, as in the awake condition, but the length of all odorant exposures was 10 seconds. There were at least 20 seconds between odorant trials, as in the awake condition. Odorants were presented in sets of 6 odorant blocks with a block of 12 dry swab presentations before and after each set. Most anesthetized animals were tested on 3 sets (18 odorant blocks). The number of tests varied slightly across subjects due to various technical problems and anesthetic depth.

Odorants

Test odorants spanned approximately 6 log units of vapor pressure (VP). Table 1 lists the 26 monomolecular odorants tested in these experiments with their theoretical VPs (at 25°C). We used the chemical structure of each monomolecular odorant to estimate various chemical properties, including mass, molecular weight, boiling and melting points, solubility, etc. (ChemDraw Ultra 2001). We also determined theoretical VPs at 25°C (Advanced Chemistry Development, Inc. (ACD/Labs 2003)). MSDS information was used when available to confirm
these data. Ten odor mixtures were tested: fox urine, male rat urine, Froot Loops®, Rat Chow, apple juice, formaldehyde, vanillin mixed in mineral oil, indole mixed in mineral oil, water, and mineral oil. Male rat urine was collected less than 3 days prior to testing and stored in airtight vials. This urine was collected in a nearby laboratory from rats to which the subjects had not had prior exposure. All monomolecular odorants were tested at 100% concentration, as were the urines and apple juice; vanillin, indole and the solid food odorants were mixed with mineral oil for presentation on a cotton swab. The subset of odorants used for anesthetized rats is noted in table 1.

Electrophysiology

All data were recorded using Neuralynx Cheetah-32 hardware and software, and for waking rats behavior was also recorded using a Logitech webcam. Local field potential (LFP) data were recorded via a multichannel headstage (Neuralynx HS-27), with a digital sampling rate of 2003 or 2016 Hz. Each lead was recorded with reference to a skull screw in the contralateral dorsal skull, posterior to the olfactory bulb. Analog filters were set at 1-475 Hz for waking rats and 0.1-475 Hz for anesthetized rats. The experimenter tapped a piezoelectric strip (output recorded on one data channel and strip visible in the frame of the webcam) immediately prior to and immediately after delivering the saturated swab under the rat’s nose to make a clear record within the data of time of odorant delivery. The piezo and video records were used to verify times when necessary.

Data analysis

Data with obvious movement artifacts were discarded (approximately 10% of the data recorded from waking rats). Odorants without complete 12-trial datasets for the same six waking
rats were discarded from the LFP analysis, resulting in a smaller set of odorants for the LFP analysis (six mixtures, mineral oil, Froot Loops®, male rat urine, indole, vanillin and water; 19 monomolecular odorants) than for the behavioral analysis (Figure 1; 26 monomolecular odorants). For each rat, the quality of signals across the two leads from each brain area were assessed. The lead with the best quality signals was chosen from each pair and used for analysis across the entire set of experiments. Quality was assessed for both leads by examining the record for significant movement artifact and line noise, as we have reported previously (Kay 2005). For the OB, the lead with most prominent theta and gamma rhythms was chosen. Sometimes this was the lead near the pial surface and sometimes it was the lead deep to the mitral cell layer, assessed by the polarity of the theta rhythm relative to the gamma burst during pre-experimental recordings (positive or negative going theta peaks). For the aPC, the presence of gamma bursts coherent with the OB bursts during exploratory behavior was used to assess the quality of signals, in addition to the criteria described above.

Power spectra were estimated from the FFTs applied to Hanning tapered half-overlapping data windows (IgorPro, Wavemetrics). For average power used for statistical assessment, the window size was 512 data samples (~0.25 sec), stepped by 256 samples, yielding 7 half-overlapping windows per second. This gave a frequency resolution of the FFT of 3.9 Hz. For a continuous representation of power (Figure 2) data windows of 4096 data points (~2 seconds) were used; the power spectra from seven half-overlapping 1024 point windows were then averaged to estimate the spectrum of the larger ~2 sec window, yielding a frequency resolution of ~2 Hz. The entire span was then stepped 1024 points to create a continuous measure of beta power through the course of the odorant block.
For each odorant trial we calculated the power spectral density of a frequency band during odor presentation (1-10 sec) using the area under the power spectrum curve (trapezoidal integration of 3-12 Hz for theta in waking rats; 1-6 Hz for theta in anesthetized rats; 15-32 Hz for beta). Because the LFP amplitudes differed across subjects and days - due primarily to differences in electrode placement across subjects and electrode drift within subjects - all power values were normalized as follows. Power in a given band for each odor trial was divided by the power from a period equal in length that ended two seconds before each test trial began, so that a value of one indicates no change from baseline. This normalized power was used as the amount of power increase over baseline in further statistical analyses. Coherence analysis was performed for the OB-aPC pair of leads as described previously (Kay 2005; Kay and Freeman 1998).

Briefly, the tapered FFTs from all the half-overlapping individual data windows (512 sample windows, ~0.25 sec) were used to produce averaged auto and cross spectra for the odor sampling periods. Coherence was estimated from the square of the averaged cross spectrum divided by the product of the averaged autospectra. All analyses were performed using StatView 5.0.1 (SAS Institute) and IgorPro 5.03 (WaveMetrics, Lake Oswego, OR).

Results

Both behavioral and neural data were analyzed for differences across odorants and behavioral state. We assessed behavioral differences by analyzing investigation times, and differences in beta and theta band responses by analyzing the power and coherence spectra of data from odor sniffing periods relative to pre-odor periods. We tested for increases or decreases in sniffing time, spectral power and coherence relative to neutral odor exposure (mineral oil) and related these to the theoretical vapor pressure for each odorant. We use the theoretical vapor
pressure at 25° C (in mmHg) as an approximate measure of the volatility of the pure odorant, representing the airborne concentration of odorants on a relative scale, as we have in other studies (Kay et al. 2005).

**Sniffing Duration**

Each waking rat was allowed to sniff the odorized swab for up to 10 seconds. A repeated-measures ANOVA on odorant sniffing durations for all odorants across the 12 trials and seven rats revealed a significant effect of trial on sniffing duration \( F(11,2343) = 40.53, p < 0.0001; \) Figure 1a) due to the first, second and twelfth trials. (The elevation of sampling time on the twelfth trial may indicate anticipation of new odors as the rats became accustomed to the experimental design.) There was also a significant effect of odorant \( F(26,179) = 3.086, p<0.0001 \), driven by differences in the first trial. Sampling durations across the seven rats in the first presentation showed a significant negative linear correlation with the \( \log_{10} \) VP of the monomolecular odorants \( r = -0.749, p<0.0001; \) Figure 1b). There was no correlation in any of the subsequent trials, and the behavior averaged over all trials showed no significant correlation with \( \log_{10} \) VP.

**Beta oscillations**

LFPs recorded from the OB and anterior piriform cortex (aPC) can show large changes in beta oscillatory power between odor sniffing and resting or waiting conditions (Kay 2003). Figure 2 shows an example of the power spectrum as it changes from the beginning to the end of a set of presentations for one odorant (trials 1 and 11). While qualitative examination of these plots makes it clear that during odor presentation there is an increase in beta band activity in later trials, statistical analysis bears this out across subjects and odorants. Figure 3 illustrates the
method for estimating average power by taking the area under the curve of the spectrum in a
frequency band (by integration) divided by the power for a period equal in length ending two
seconds before the trial. Thus, a value of one indicates no change from baseline, and values
above and below one indicate increases and decreases in power, respectively.

Beta power was significantly higher in response to some odorants, and there were
differences across trials (described below). A one-way repeated-measures ANOVA in a nested
design incorporating all 12 presentations of each of the odorants was used to analyze beta power
in the LFP responses to different odorants. Due to the exclusion of any odorant which did not
have useable data from the same 6 rats for all 12 trials, 25 of the original 36 odorants were
included in this analysis, spanning the entire range of VPs tested, including four complex
mixtures, plus water and mineral oil (Table 1). During odor sniffing, beta power varied with
odor in the OB (F(24,1704) = 11.490, p<0.0001) and aPC (F(24,1704) = 12.383, p<0.0001).
Post-hoc analysis (Fisher’s PLSD) indicates that there was greater power in the beta frequency
band in the OB and aPC during presentations of TMT, anisole, amyl acetate, ethyl methyl
butyrate (EMB), hexanal, hexanoic acid, toluene, and butanone as compared to mineral oil (Fig. 4).
In the OB the response to nonanone also showed elevated beta power. All of the odorants that
showed significantly higher beta power were in the 1-120 mmHg range of VPs, except for
nonanone.

A similar nested repeated-measures ANOVA on data from anesthetized rats showed a
small but significant beta power difference across odorants in the OB (F(12,432) = 2.461;
p=0.0041; Fig. 4c) and aPC (F(12,432) = 3.144; p=0.0003; Fig. 4d). Elevated power for fox
urine, nonanoic acid, hexanoic acid, TMT and toluene over water drove the effect in the OB;
only toluene was significantly elevated over water in the aPC, although there were marginal effects for nonanol and methyl salicylate.

**Beta sensitization and suppression across trials**

We found a significant difference in beta power across the twelve trials in both the OB (F(11,1639) = 3.785; p<0.0001) and aPC (F(11,1639) = 2.877; p = 0.0009) of the waking rats (Fig. 5a,b). As suggested by qualitative assessment in previous reports (Heale and Vanderwolf 1994a; Heale et al. 1994; Vanderwolf and Zibrowski 2001; Zibrowski et al. 1998; Zibrowski and Vanderwolf 1997), these differences were due to an increase of beta power in later over earlier trials. Beta power in the first trial was significantly lower than in trials 4-12 (OB) and 5-12 (aPC). These effects were driven by odorants in the 1-120 mmHg range (Fig. 5c,d). There was a small but significant negative correlation between sniffing duration and beta power (R = -0.157, p < 0.0001).

The LFPs from anesthetized rats also showed a significant difference in beta power across the twelve trials in both the OB (F(11,429)=4.32; p<0.0001) and aPC (F(11,429)=3.577; p<0.0001). However, these differences were due to greater beta band power in the first trial over later trials (Fig. 5a,b). On the first trial the average beta band power elevation during odorant presentation was the same in anesthetized and waking conditions. In the anesthetized rats, beta oscillations were then significantly decreased, while in the waking condition they were significantly increased in a sensitization-like fashion.

Coherence analysis is a measure of cooperativity between signals, normalized for the power of each signal. The level of beta band coherence between the signals in the OB and aPC was elevated when beta oscillatory power was elevated (Fig. 6a,b). The level of coherence for
monomolecular odorants was linearly correlated with log_{10}VP (Fig. 6c; r = 0.73, p = 0.0006), although the two odorants with the highest pressure (acetone, 348.5 mmHg; ammonia, 5990.5 mmHg) showed a general decrease in coherence. Beta band coherence also differed significantly across the 12 trials (F(11,1639) = 10.652; p < 0.0001), with trials 2-12 significantly higher than trial 1 and trials 5-12 higher than trials 2-4, (Fig. 6d). In anesthetized rats, from a 2-way ANOVA across trials and odors there was no significant difference in beta band coherence across trials (F(11,204)=1.275; p=0.3404), but there was a significant difference across odorants (F(17,204)=5.292; p<0.0001) driven by increases in coherence during exposure to odorants both within and outside of the 1-120 mmHg range. There was no interaction between trials and odorants.

**Odor-evoked gamma activity**

Recent studies have suggested a tradeoff between beta and gamma oscillations (Martin et al. 2004; Neville and Haberly 2003). We therefore analyzed gamma band activity to determine if such an effect was evident in our study. In waking rats, gamma band power (65-100 Hz, to avoid contamination by 60 Hz noise) varied significantly across odorants in the OB (F(24,1440)=23.824; p<0.0001; Fig. 7a) both within and outside of the 1-120 mmHg VP range, with no significant variation across trials (F(11,1440)=0.882; p=0.5619; Fig. 7c) and no interaction between odorants and trials (although pairwise comparisons across trials suggests a trend toward decreasing gamma power after the first two trials). Gamma power in the aPC also varied significantly across odorants (F(24,1440)=22.195; p<0.0001; Fig. 7b), with increases within and outside of the 1-120 mmHg VP range. Gamma power in the PC decreased significantly across trials (F(11,1440)=7.653; p=0.0130; Fig. 7c), with a significant interaction
between odorants and trials \( F(264,1440)=1.245; \ p=0.0083 \). Examination of individual trials on a finer timescale than the trials shows that strong gamma and beta oscillations alternate, as illustrated in Fig. 3.

Anesthetized rats also show enhanced gamma oscillations to some odorants. In the OB gamma band power varied significantly across odorants (2 way ANOVA, odorant x trial; \( F(17,204)=17.049, \ p<0.0001 \) for odorants; \( F(11,204)=0.329, \ p=0.9623 \) for trials, with no interaction between odorants and trials), and the aPC showed a similar effect (\( F(17,204)=20.955, \ p<0.0001 \) for odorants; \( F(11,204)=1.115, \ p=0.4252 \) for trials, with no interaction between odorants and trials). In the OB nonanal and hexanal responses were significantly larger than those to mineral oil \( p<0.0001 \). In the aPC, these same two odorants also show enhanced responses \( p<0.0001 \), and the response to toluene was slightly but significantly enhanced \( p=0.0084 \).

**Theta coupling of OB and aPC during odor sampling**

We considered the possibility that differences in sniffing behavior beyond sampling duration might account for the differences in beta oscillations. In the OB, the theta band rhythm (2-12 Hz) is strongly correlated with respiratory activity associated with breathing and sniffing (Kay 2005; Klingberg and Pickenhain 1965; Macrides and Chorover 1972). Thus, theta frequency represents the frequency of afferent drive to the system. Although the power can represent intensity of the afferent input, this is complicated by gain control mechanisms that exist in the glomerular layer and release of neuromodulators by the trigeminal nerve (Aroniadou-Anderson et al. 1997; Ennis et al. 2001; Schaefer et al. 2002). Therefore, the power of the theta rhythm is more accurately the intrabulbar representation of the afferent drive.
In order to examine the role that effective afferent drive might play in eliciting beta oscillations, we examined the power of the theta rhythm. We also examined the coherence between the OB and aPC in this band as a measure of functional coupling strength associated with sniffing. Theta band power in the OB and aPC showed significant variation across odorants (OB $F(24,1704) = 2.938, p < 0.0001$; aPC $F(24,1704) = 2.947, p < 0.0001$), but power did not vary systematically with VP (Figure 8a,b) or across trials ($F(11,1639) = 1.148, p = 0.3193$; $F(11,1639) = 1.488, p = 0.1292$). Coherence within the theta frequency band did vary significantly across odorants ($F(24,1704) = 2.826, p < 0.0001$; Figure 8c), and posthoc comparisons of all odorants to the mineral oil response showed a significant increase only for ammonia, the most volatile odorant. However, the level of theta band coherence correlated linearly with $\log_{10} VP$ ($r = 0.70; p = 0.0006$; Fig. 8c). Theta band coherence between OB and aPC also varied across trials ($F(11,1639) = 7.468, p < 0.0001$; combined analysis of all rats and odorants, Fig. 8d) with a significant increase for trials 2-12 over the first trial and trials 6-11 over the second trial.

Power in the theta band varied across odorants in the anesthetized rats (OB: $F(17,391)=24.819, p<0.0001$; aPC: $F(17,391)=1.939, p=0.014$; Fig. 8a,b), as did OB-aPC theta band coherence ($F(17,391)=3.943, p<0.0001$), driven by significant decreases in coherence for fox urine and hexanol and an increase for propionic acid (Fig. 8c). Across trials combined for all rats and odorants, there was a significant difference in theta power in the OB ($F(11,539)=2.013, p=0.0254$; from 2 way ANOVA, trial x odorant) along with an interaction between trial and odor ($F(17,187)=1.388, p=0.0062$), but no pairwise comparisons of trials were
significant (Fisher PLSD). Theta power did not vary over trials in the PC (F(11,726)=1.131, p=0.3332), neither did OB-aPC theta band coherence (F(11,726)=0.493, p=0.9084).

**Discussion**

While many recent studies have shed considerable light on how odor receptors and olfactory bulb architecture contribute to odor coding, we still know relatively little about how intrinsic responses to specific odorants and odorant properties may affect odor processing in waking mammals. We present several significant findings relating odorant vapor phase concentration and olfactory behavioral and neural responses (summarized in Fig. 9). First, when presented with a novel odorant (first presentation) the duration of a rat’s investigation is inversely correlated with that odorant’s partial pressure as estimated from the theoretical vapor pressure (Fig. 1b). Similarly, human subjects sniff longer for lower concentration odorants (Sobel et al. 2000). While humans increase the length of inhalation, rodents increase investigation time without changing sniffing frequency. The net result on this first exposure is likely to regulate the amount of odor stimulus available to bind to receptors during this first sniffing bout, because odorant diffusion in the epithelial mucous is slow enough that a series of brief sniffs (~125 msec each at ~8 Hz) should produce a sustained level of odorant in the mucous (Scott et al. 2006).

Our second finding is that pure monomolecular odorants with VPs of 1-120 mmHg (0 to ~2 log\(_{10}\)VP) induce significantly enhanced beta oscillation power in olfactory structures (Fig. 4a,b and Fig. 6a,b). This increase of power does not appear with the first presentation, but instead appears after 3-4 trials in a sensitization-like fashion (Figs. 5, 6). Our third finding shows that coupling of the OB and aPC in the theta frequency band associated with the sniff
cycle is enhanced after the first trial and its magnitude positively correlated with \(\log_{10} VP\), without any systematic increase in the amplitude of the theta rhythm (power) across odorants (Fig. 8) or trials. The link between odorant volatility and beta and theta response patterns is enhanced in waking rats, and is greatly diminished in urethane anesthetized rats.

**Theta band coupling of olfactory bulb and cortex varies with airborne concentration**

The interplay of sniff duration and theta band coupling are related, because the theta rhythm in the OB represents the frequency and effective bulbar afferent drive from the olfactory nerve (Bressler 1987; Macrides and Chorover 1972). After the first trial in our study, sniffing durations were brief and not statistically different across odorants (Fig. 1a). However, the effective coupling strength in both the theta and beta frequency bands increased after the first trial, and the magnitude of OB-aPC theta band coupling in these later trials correlated with vapor phase concentration. Theta oscillation power (amplitude) in the olfactory bulb did not vary across presentations and did not vary systematically across odorants. We may therefore infer that the increase in coupling strength between the two systems is not driven by a systematic increase in effective intrabulbar afferent intensity and may involve a separate mechanism. However, this does not rule out differences not detectable at the level of the theta band or the LFP signal in general.

Since all of our monomolecular odorants were delivered at 100% concentration, and most odorants at high enough concentration affect the trigeminal nerve in the nasal epithelium (Brand 2006), we suggest the trigeminal system as a possible parallel mechanism to effect changes in connection strength. Recent reports have noted in anesthetized rodents that respiratory linking of olfactory bulb and cortex cycles between “up” and “down” states similar to thalamocortical
coupling (Fontanini and Bower 2006; Fontanini and Bower 2005; Kay and Sherman 2007), and there are “sentinel” trigeminal receptors in the nasal cavity that may serve to track respiration and irritation (Finger et al. 2003). It is therefore possible that activation of the trigeminal system could facilitate a change in coupling strength in waking animals either by direct neural activation or by neuromodulators released into the olfactory areas by brainstem nuclei, the basal forebrain or the trigeminal nerve itself (Brand 2006; Breer et al. 2006).

**Beta oscillations in waking rats are restricted to highly volatile odorants**

The beta oscillatory response has been proposed to be a specific predator response (Heale et al. 1994). Our data argue against this interpretation, indicating that odor-specific beta oscillations are not specific to predator odorants but rather to highly volatile odorants (1-120 mmHg). Given that vapor phase concentration can predict beta oscillation power, this suggests that beta oscillations may be produced simply in response to strong or pungent odorants. However, our results also show that odorants with the highest VPs (acetone and ammonia) did not elicit a significant beta response. The very high VP odorants are also very strong trigeminal stimulants, and sensory and cortical responses to presentations of odorants with extremely high vapor pressures can be modulated by the trigeminal system by several mechanisms, as described above. The most obvious role of trigeminal activation would be to decrease sniffing (intensity or duration) due to the irritant nature of trigeminal stimulation, but theta band power, which is an approximation of effective afferent intensity, was not lower for the highest intensity odorants.

Variations in sniffing behavior not detectable by theta rhythm analysis could vary the placement of odorants across the olfactory epithelium or change the temporal dynamics of the
incoming afferent signal, contributing to a change in the OB activation patterns. In particular, the negative correlation between sniff duration and beta power supports the conclusion of an earlier study, showing that lower concentration odorants induce beta oscillations in anesthetized rats (Neville and Haberly 2003). If for higher VP odorants, less odorant is delivered to the nasal mucosa, this would result in a lower concentration stimulus. However, the constancy of theta oscillation power across odorants suggests that the results of inhalation are not different at the intrabulbar level.

**Relationship to odor learning**

Beta oscillations can also be produced in response to odorants not in the range of highly volatile odorants and at much lower concentrations than those used in our study when rats reach criterion performance a Go/No-Go associative odor discrimination task, suggesting that beta oscillations may represent a form of population synchrony involved in learning over many trials and sessions (Martin et al. 2004). In our study, beta oscillations arose only after several presentations in waking rats and were specific to a given odorant, resetting when a new odorant was presented, so this could represent a form of short term memory process similar to that seen in the analogous insect system in a similar familiarization paradigm (Stopfer and Laurent 1999).

Previous studies showed that bidirectional connections between the OB and PC are essential for the production of beta oscillations (Martin et al. 2004; Martin et al. 2006; Neville and Haberly 2003). We show here that beta oscillations are predicted when coherence between the olfactory bulb and piriform cortex is higher in the theta/respiratory frequency band (Figs. 5, 7). This suggests that coupling strength may be a determining factor or threshold for beta
oscillations, even when the effective input strength (OB theta oscillation power) is constant. That the same type of beta oscillations can occur with learning, suggests that the effective connection strength may also be increased during the learning process. This result is consistent with computational studies which show that beta, rather than gamma, oscillations occur in hippocampal networks dependent on Hebbian changes in pyramidal cell connections and input and feedback drive strength (Olufsen et al. 2003).

We found that beta oscillatory responses, measured by power in the beta frequency band, varied across odorants in the anesthetized condition as well. However, these responses were much weaker, with power significantly decreased after the first trial. Interestingly, while the odorants in the aPC that evoked beta oscillations followed the general pattern of the waking condition, being mostly restricted to the 1-120 mmHg range (Fig. 4D), those that evoked such oscillations in the OB were distributed among several odorants both within and outside of this range (Fig. 4C). Furthermore, unlike the responses in waking rats, OB-aPC theta band coherence and power did not vary systematically with vapor pressure.

In previous studies it was reported that gamma oscillations, as measured by average gamma band power over many trials, often disappear when beta oscillations are enhanced and vice versa (Martin et al. 2004). Our examination of the gamma oscillatory band shows that in waking rats, when results from all odorants are considered, odor-evoked gamma oscillations decrease in the aPC after the first trial and show a modest decrement in the OB as well (Fig. 7c). However, during odorant exposure we find enhanced gamma power in both the OB and aPC for several of the same odorants that induce enhanced beta oscillations (Fig. 7a,b). Further inspection of the data reveals that many trials show within a single investigation period
alternating gamma and beta events (e.g., Figs. 2b, 3a). In anesthetized rats, enhanced gamma oscillations are seen only to a limited number of specific odorants, two in the OB and three in the aPC (Fig. 7a,b). The two strongest responses in both structures were chemically related odorants (hexanal and nonanal), suggesting that the gamma response in anesthetized rats may be related to odorant input structure.

The relationship between beta oscillations and unit activity has not been extensively studied, but several published reports shed some light on which cells may be involved in supporting these oscillations in the OB. Studies in frogs, rabbits and rats have shown that as concentration is increased, more mitral cells show significant responses to tested odorants, with the strongest effects being increases in the number of cells showing inhibitory responses to odorants during inhalation and in the number of cells showing excitatory responses to odorants during exhalation (Chaput and Lankheet 1987; Duchamp-Viret and Duchamp 1997). Another study showed that the beta oscillation response in urethane-anesthetized rats is restricted to the exhalation phase of respiration, dominated by firing of neurons in the internal plexiform and granule cell layers (Buonviso et al. 2003). This suggests that OB beta oscillations evoked in response to high concentration odorants may be supported by cells outside of the mitral cell layer. This is consistent with the necessity for an intact bidirectional pathway between OB and aPC in producing beta oscillations (Martin et al. 2006; Neville and Haberly 2003), since centrifugal input to the OB from the aPC is restricted to the granule cell layer.

**Comparisons between waking and anesthetized results**

The data show that neural responses to odorants in waking rats are very different from those seen under urethane anesthesia. These effects are consistent with previous studies in which
we have shown effects due to learning and behavioral state, which are not seen in the anesthetized preparation (Kay 2003; Kay and Freeman 1998; Kay and Laurent 1999). The most dramatic difference in these data are in the specificity of beta oscillatory increases to volatility in the waking condition, which are not repeated in the anesthetized condition (Figs. 4, 6). Furthermore, the increases seen in anesthetized rats are much smaller than those seen in waking rats. These results suggest that the increase in beta oscillations in waking animals is not directly evoked by the strength of the odorant input signal but rather by the rat’s internal response to the highly volatile odorants. This hypothesis is supported by the consistency of theta oscillatory power across odorants (Fig. 8). A recent study has suggested that glomerular circuitry serves to normalize the strength of inputs to the olfactory bulb across wide ranges of concentration, consistent with our observations of the theta rhythm power (Cleland et al. 2007). If the trigeminal system mediates part of the beta oscillatory response, we would again expect a similar effect in the anesthetized rats. We speculate that if the trigeminal system is involved it is at the level of the attentional or perceptual circuitry, rather than a simple reflex-like circuit (Brand 2006).

In conclusion, these data show that the relative airborne concentrations of pure odorants and associated linear increases in coupling strength between the OB and aPC represent some of the principal parameters that may effect beta oscillations in the olfactory system of waking rats independent of explicit associative learning. These oscillations have the same frequency, duration, amplitude and apparent system properties as those produced in response to some types of odor learning, suggesting that the effect in our study represents a type of short term perceptual...
Lowry & Kay, *Olfactory beta oscillations and odorant volatility*

learning that may serve information transfer between cortical areas. Whether the beta oscillations reported here are produced by the same neural circuits associated with Go/No-Go odor learning remains to be determined and should be the object of future studies.

**Acknowledgments**

We thank Claire Martin for helpful comments on the manuscript. CL was supported by the Brain Research Foundation, an Erma Smith Fellowship and T32 GM07839. LK was supported by NIDCD R01DC00795. Current address for CL: Neuroscience Associates, 10915 Lake Ridge Drive, Knoxville, TN 37934; cfranssen@nsalabs.com.
References


Figure Legends

Figure 1. A) Length of odor investigation times across trials. The sniffing duration was significantly longer in the first trial than the second trial and longer in both the first and second trials than all later trials (p<0.0001; posthoc Fisher PLSD). The sampling duration for the twelfth trial was significantly longer than the fifth (p=0.0178), sixth (p=0.0400), seventh (p=0.0270), tenth (p=0.0240) and eleventh (p=0.0178) trials. B) Vapor pressure and sniff duration. There was a significant correlation between log\textsubscript{10} VP of monomolecular odorants and behavior (length of time spent investigating the odorant) on the first trial (n = 26; not shown are mineral oil, water, and the six mixtures for which the VP is undetermined). Error bars represent S.E.M. across the seven rats; * p<0.05, ** p<0.01, longer duration than other trials.

Figure 2. Sample time-frequency power plots from one rat during presentations of a single odorant (hexanone). The amount of power from the olfactory bulb signal is shown in color; warm colors indicate high power. Vertical axis of top plots is frequency, horizontal axis is time; LFP data from the OB (top) and aPC (bottom) are shown below. A) First trial, with six-second odor sampling period marked with a solid line and raw data below. B) 11\textsuperscript{th} trial with one-second odor sampling period. Ten seconds of data are shown for each trial. Robust beta oscillations, as in B, are common for highly volatile odorants in later trials and are not seen in the first trial. The approximate ranges for theta, beta and gamma oscillations are shown on the left (gamma 1 and gamma 2 are high and low frequency gamma, respectively, as defined elsewhere (Kay 2003)).
Figure 3. Power spectral analysis: Data are from the 10th trial of the data set displayed in figure 2. A) Sample sniffing period lasting 1.2 sec (“odor” shaded region; ~293-294 sec) is matched with a prestimulus control period (“control” shaded region; ~290-291 sec). Raw data (top), beta band filtered data (filters set at 15-32 Hz to capture the 15-30 Hz beta band; bottom). A ~0.5 second burst of beta activity is visible during the odorant presentation. Minimal power is apparent in the beta band during the time before and after the odorant presentation. B) Power spectra for the 1.2 second of odorant presentation (solid line) and the control period (dashed line) from A. Units of power are in $\mu V^2/Hz$, but for analysis the area from the odor period spectrum is divided by that from the prestimulus control period for the frequency band of interest yielding a dimensionless ratio.

Figure 4. Beta power for all odorants. A) Normalized OB beta band power from waking rats for each of the odorants arrayed in order of VP. B) aPC beta band power from waking rats. Dashed vertical lines divide the classes of odorants into mixtures, low volatility odorants (< 1 mmHg VP), highly volatile odorants (1-120 mmHg) and very highly volatile odorants (> 120 mmHg). C) OB and D) aPC normalized beta band power from anesthetized rats. For waking rats, high power beta oscillations dominate in the highly volatile odorants over mineral oil. For urethane anesthetized rats, three of the seven odorants that induce a significant increase in OB beta power over mineral oil are in the 1-120 mmHg range. In the aPC induced beta oscillations are larger in power overall, but only four odorants (3 in the 1-120 mmHg range) induce beta activity significantly above the mineral oil control in anesthetized rats. Odorants that did not have 12 trials from the same six rats (A and B) or from three or four rats (C and D) are not
included in the analysis. Note that for anesthetized rats, four of the 18 odorants are different from those used for the waking rats (indicated with dashes around the odorant names). Asterisks indicate posthoc significance (Fisher PLSD): * p<0.05, ** p<0.01 (error bars throughout: SEM).

**Figure 5. Beta power builds across trials in waking rats and decreases in anesthetized rats.**

A,B) **Average beta band power by trial** during odor sampling in the OB and aPC, respectively, averaged over all odorants. Solid line is from waking rats, dashed line from anesthetized rats.

C,D) **Trial averages for waking rats divided by vapor pressure class** as in Fig. 4. The increase in beta band power across trials is driven by the highly volatile odorants (1-120 mmHg VP).

**Figure 6. Power and coherence in the beta band is directly related to log_{10}VP.**

A,B) **OB and aPC beta power** data (as in Figure 4), displayed as a scatterplot by log_{10}VP. Solid circles represent data from waking rats, and surrounding single and double circles indicate p < 0.05 and p < 0.01 significance limits, respectively, as compared to mineral oil (downward arrow and horizontal line). Squares represent data from anesthetized rats, with filled squares indicating significant differences from the response to mineral oil. Vapor pressure classes as described elsewhere. The leftmost segment is for complex mixtures or odorants dissolved in mineral oil, for which VP is not determined.  

C) **OB-aPC beta band coherence by log_{10}VP.** In waking rats, odorants within the range of 1-120 mmHg VP show enhanced coherence relative to mineral oil. The lowest VP odorant (nonanoic acid) and several of the mixtures showed decreased coherence levels. There is also a significant linear correlation between beta band coherence and log_{10}VP.
for waking rats. The line fit is to the odorants up to 120mmHg ($r=0.73$, $p=0.0006$), not including mixtures on the left; a fit to the entire range of monomolecular odorants is still significant ($r=0.62$, $p=0.003$), but there is a large deviation for the very highest VP odorants. Anesthetized rats show significant decreases in coherence associated with methyl salicylate, hexanoic acid, nonanal, hexanol, hexanal, toluene and propionaldehyde. 

**D) Beta band coherence across trials.** In waking rats (solid line; ** p<0.01 as compared to trial 1) there is a significant increase in coherence for trials 2-12 over trial 1 and in trials 5-12 over trials 2-4. Data from anesthetized rats (dashed line) shows no significant variation over trials. Responses to water are indicated by the upward arrow.

**Figure 7. Gamma band power is elevated for some odorants in the OB and aPC in waking rats.**

**A)** Exposure to two of the odorants, nonanal and hexanal, produced enhanced gamma power in the OB (*p < 0.05, **p<0.01). Data from anesthetized rats is indicated by the markers with S.E.M. overlaid on the plot (# p < 0.05, ## p < 0.01).  

**B)** Three odorants (nonanal, hexanal and toluene) induced enhanced gamma power in the aPC. Two of these are the same as those in the OB. 

**C)** In waking rats, gamma power in the OB was not significantly decreased across trials, but a trend toward decrease for trials 4-8 and trial 12 relative to trial 1 was shown by pairwise posthoc comparisons (Fisher PLSD; $p<0.05$). In the aPC, gamma power decreased significantly after the first trial ($p<0.0001$ for trials 2-12 relative to trial 1). There was no significant change in gamma power across trials for anesthetized rats.
Figure 8. Power and coherence in the theta band. A,B) Theta band power data displayed as in Fig. 6, for OB and aPC, respectively (symbols defined as in Fig. 6). There is little difference in theta power in both structures across odorants in waking rats. There are differences in theta power induced by some odorants in the OB of anesthetized rats (hexanol, EMB, hexanal and toluene). Only propanol shows enhanced theta power in the aPC of anesthetized rats. C) Theta band coherence by log_{10}VP. There is a significant linear correlation between theta band coherence and log_{10}VP in waking rats (r=0.70; p=0.0006), not including the mixtures. There is no systematic difference across odorants for anesthetized rats (fox urine and hexanol evoke significant decreases in coherence, relative to mineral oil). D) Theta band coherence across trials. There is a significant increase in theta coherence for trials 2-12 over trial 1 and in trials 6-11 over trial 2 in waking rats (solid line; ** p<0.01 as compared to trial 1). There is no difference across trials for anesthetized rats (dashed line). For waking rats, the frequency band used for analysis is 3-12 Hz; for anesthetized rats the frequency band is 1-6 Hz, due to the slower respiratory rate under anesthesia. Upward and downward arrows indicate responses to mineral oil and water, respectively.

Figure 9. Respiratory frequency coupling may facilitate beta oscillations. Each of the twelve LFP plots (left and right columns) contains 1 second of data from both the OB (top trace) and aPC (bottom trace) during odor sniffing, intended as examples of the statistical results in figures 4-7 (all data from the same rat, amplitudes normalized by prestimulus control periods). Odorants (top to bottom): nonanoic acid, nonanol, TMT, hexanone, butanone, and acetone. Left column: from first exposure of each of the six odorants; right column: from later trials. Relative
airborne concentrations are represented as solid squares in the center column (size proportional to $\log_{10} \text{VP}$ for the six odorants; see table 1). Sniff duration on the first trial decreases (Fig. 1b) and theta coherence on subsequent trials increases (Fig. 8c) linearly with increasing $\log_{10} \text{VP}$. Rightmost filled circles represent average beta power for each of the six odorants (size proportional to average OB power; Fig. 4a).
Table I. Vapor pressures of tested odorants

<table>
<thead>
<tr>
<th>Tested odorants</th>
<th>Theoretical VP at 25°C mm Hg</th>
<th>log&lt;sub&gt;10&lt;/sub&gt;VP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral oil *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fox urine*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Froot Loops ®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat Chow *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male rat urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanillin *</td>
<td>0.00195</td>
<td>-2.061</td>
</tr>
<tr>
<td>Indole *</td>
<td>0.696</td>
<td>-1.979</td>
</tr>
<tr>
<td>Water *</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Formaldehyde soln.</td>
<td>3463</td>
<td></td>
</tr>
<tr>
<td>Nonanoic acid *</td>
<td>0.00869</td>
<td>-2.061</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0.0105</td>
<td>-1.979</td>
</tr>
<tr>
<td>Geraniol</td>
<td>0.0133</td>
<td>-1.876</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>0.0266</td>
<td>-1.575</td>
</tr>
<tr>
<td>Nonanol *</td>
<td>0.0408</td>
<td>-1.389</td>
</tr>
<tr>
<td>Methyl salicylate *</td>
<td>0.0701</td>
<td>-1.154</td>
</tr>
<tr>
<td>Citral</td>
<td>0.0713</td>
<td>-1.147</td>
</tr>
<tr>
<td>Hexanoic acid *</td>
<td>0.159</td>
<td>-0.799</td>
</tr>
<tr>
<td>Nonyl aldehyde *</td>
<td>0.533</td>
<td>-0.273</td>
</tr>
<tr>
<td>Nonanone</td>
<td>0.646</td>
<td>-0.190</td>
</tr>
<tr>
<td>Hexanol *</td>
<td>0.948</td>
<td>-0.023</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.975</td>
<td>-0.011</td>
</tr>
<tr>
<td>TMT *</td>
<td>1.32</td>
<td>0.121</td>
</tr>
<tr>
<td>Propionic acid *</td>
<td>4.23</td>
<td>0.626</td>
</tr>
<tr>
<td>Anisole</td>
<td>4.25</td>
<td>0.628</td>
</tr>
<tr>
<td>Amyl acetate</td>
<td>5.68</td>
<td>0.754</td>
</tr>
<tr>
<td>Ethyl methyl butyrate *</td>
<td>7.86</td>
<td>0.895</td>
</tr>
<tr>
<td>Hexanal *</td>
<td>10.9</td>
<td>1.037</td>
</tr>
<tr>
<td>Hexanone</td>
<td>13.3</td>
<td>1.124</td>
</tr>
<tr>
<td>Xylene</td>
<td>14.5</td>
<td>1.161</td>
</tr>
<tr>
<td>Propanol *</td>
<td>26.3</td>
<td>1.420</td>
</tr>
<tr>
<td>Toluene *</td>
<td>27.7</td>
<td>1.442</td>
</tr>
<tr>
<td>Butanone</td>
<td>114.6</td>
<td>2.059</td>
</tr>
<tr>
<td>Propionaldehyde</td>
<td>299.4</td>
<td>2.476</td>
</tr>
<tr>
<td>Acetone *</td>
<td>348.5</td>
<td>2.542</td>
</tr>
<tr>
<td>Ammonia</td>
<td>5990.5</td>
<td>3.777</td>
</tr>
</tbody>
</table>

* odorant used for both waking and anesthetized rats
Figure 1. A) Length of odor investigation times across trials. The sniffing duration was significantly longer in the first trial than the second trial and longer in both the first and second trials than all later trials (p<0.0001; posthoc Fisher PLSD). The sampling duration for the twelfth trial was significantly longer than the fifth (p=0.0178), sixth (p=0.0400), seventh (p=0.0270), tenth (p=0.0240) and eleventh (p=0.0178) trials. B) Vapor pressure and sniff duration. There was a significant correlation between log10VP of monomolecular odorants and behavior (length of time spent investigating the odorant) on the first trial (n = 26; not shown are mineral oil, water, and the six mixtures for which the VP is undetermined). Error bars represent S.E.M. across the seven rats; * p<0.05, ** p<0.01, longer duration than other trials.
Figure 2. Sample time-frequency power plots from one rat during presentations of a single odorant (hexanone). The amount of power from the olfactory bulb signal is shown in color; warm colors indicate high power. Vertical axis of top plots is frequency, horizontal axis is time; LFP data from the OB (top) and aPC (bottom) are shown below. A) First trial, with six-second odor sampling period marked with a solid line and raw data below. B) 11th trial with one-second odor sampling period. Ten seconds of data are shown for each trial. Robust beta oscillations, as in B, are common for highly volatile odorants in later trials and are not seen in the first trial. The approximate ranges for theta, beta and gamma oscillations are shown on the left (gamma 1 and gamma 2 are high and low frequency gamma, respectively, as defined elsewhere (Kay 2003)).
Figure 3. Power spectral analysis: Data are from the 10th trial of the data set displayed in figure 2. A) Sample sniffing period lasting 1.2 sec (odor shaded region; ∼293-294 sec) is matched with a prestimulus control period (control shaded region; ∼290-291 sec). Raw data (top), beta band filtered data (filters set at 15-32 Hz to capture the 15-30 Hz beta band; bottom). A ∼0.5 second burst of beta activity is visible during the odorant presentation. Minimal power is apparent in the beta band during the time before and after the odorant presentation. B) Power spectra for the 1.2 second of odorant presentation (solid line) and the control period (dashed line) from A. Units of power are in μV²/Hz, but for analysis the area from the odor period spectrum is divided by that from the prestimulus control period for the frequency band of interest yielding a dimensionless ratio.
Figure 4. Beta power for all odorants. A) Normalized OB beta band power from waking rats for each of the odorants arrayed in order of VP. B) aPC beta band power from waking rats. Dashed vertical lines divide the classes of odorants into mixtures, low volatility odorants (< 1 mmHg VP), highly volatile odorants (1-120 mmHg) and very highly volatile odorants (> 120 mmHg). C) OB and D) aPC normalized beta band power from anesthetized rats. For waking rats, high power beta oscillations dominate in the highly volatile odorants over mineral oil. For urethane anesthetized rats, three of the seven odorants that induce a significant increase in OB beta power over mineral oil are in the 1-120 mmHg range. In the aPC induced beta oscillations are larger in power overall, but only four odorants (3 in the 1-120 mmHg range) induce beta activity significantly above the mineral oil control in anesthetized rats. Odorants that did not have 12 trials from the same six rats (A and B) or from three or four rats (C and D) are not included in the
analysis. Note that for anesthetized rats, four of the 18 odorants are different from those used for the waking rats (indicated with dashes around the odorant names). Asterisks indicate posthoc significance (Fisher PLSD): * p<0.05, ** p< 0.01 (error bars throughout: SEM).
Figure 5. Beta power builds across trials in waking rats and decreases in anesthetized rats. A,B) Average beta band power by trial during odor sampling in the OB and aPC, respectively, averaged over all odorants. Solid line is from waking rats, dashed line from anesthetized rats. C,D) Trial averages for waking rats divided by vapor pressure class as in Fig. 4. The increase in beta band power across trials is driven by the highly volatile odorants (1-120 mmHg VP).
Figure 6. Power and coherence in the beta band is directly related to log10VP. A, B) OB and aPC beta power data (as in Figure 4), displayed as a scatterplot by log10VP. Solid circles represent data from waking rats, and surrounding single and double circles indicate $p < 0.05$ and $p < 0.01$ significance limits, respectively, as compared to mineral oil (downward arrow and horizontal line). Squares represent data from anesthetized rats, with filled squares indicating significant differences from the response to mineral oil. Vapor pressure classes as described elsewhere. The leftmost segment is for complex mixtures or odorants dissolved in mineral oil, for which VP is not determined. C) OB-aPC beta band coherence by log10VP. In waking rats, odorants within the range of 1-120 mmHg VP show enhanced coherence relative to mineral oil. The lowest VP odorant (nonanoic acid) and several of the mixtures showed decreased coherence levels. There is also a significant linear correlation between beta band coherence and log10VP for waking rats. The line fit is to the odorants up to 120mmHg ($r=0.73$, $p=0.0006$), not including mixtures on the left; a fit to the entire range of monomolecular odorants is still significant ($r=0.62$, $p=0.003$), but there is a large deviation for the very highest VP odorants. Anesthetized rats show significant decreases in coherence associated with methyl salicylate, hexanoic acid, nonanal, hexanol, hexanal, toluene and propionaldehyde. D) Beta band coherence across trials. In waking rats (solid line; ** $p<0.01$ as compared to trial 1) there is a significant increase in coherence for trials 2-12 over trial 1 and in trials 5-12 over trials 2-4. Data from anesthetized rats (dashed line) shows no significant variation over trials. Responses to water are indicated by the upward arrow.
Figure 7. Gamma band power is elevated for some odorants in the OB and aPC in waking rats. A) Exposure to two of the odorants, nonanal and hexanal, produced enhanced gamma power in the OB (*p < 0.05, **p<0.01). Data from anesthetized rats is indicated by the markers with S.E.M. overlaid on the plot (# p < 0.05, ## p < 0.01). B) Three odorants (nonanal, hexanal and toluene) induced enhanced gamma power in the aPC. Two of these are the same as those in the OB. C) In waking rats, gamma power in the OB was not significantly decreased across trials, but a trend toward decrease for trials 4-8 and trial 12 relative to trial 1 was shown by pairwise posthoc comparisons (Fisher PLSD; p<0.05). In the aPC, gamma power decreased significantly after the first trial (p<0.0001 for trials 2-12 relative to trial 1). There was no significant change in gamma power across trials for anesthetized rats.
Figure 8. Power and coherence in the theta band. A, B) Theta band power data displayed as in Fig. 7, for OB and aPC, respectively (symbols defined as in Fig. 7). There is little difference in theta power in both structures across odorants in waking rats. There are differences in theta power induced by some odorants in the OB of anesthetized rats (hexanol, EMB, hexanal and toluene), although the magnitude of change in theta power relative to the prestimulus baseline is not as dramatic as in waking rats (a value of one indicates no change from baseline). Only propanol shows enhanced theta power in the aPC of anesthetized rats. C) Theta band coherence by log10VP. There is a significant linear correlation between theta band coherence and log10VP in waking rats (r=0.70; p=0.0006), not including the mixtures. There is no systematic difference across odorants for anesthetized rats (fox urine and hexanol evoke significant decreases in coherence, relative to mineral oil). D) Theta band coherence across trials. There is a significant increase in theta coherence for trials 2-12 over trial 1 and in trials 6-11 over trial 2 in waking rats (solid line; ** p<0.01 as compared to trial 1). There is no difference across trials for anesthetized rats (dashed line). For waking rats, the frequency band used for analysis is 3-12 Hz; for anesthetized rats the frequency band is 1-6 Hz, due to the slower respiratory rate under anesthesia. Upward and downward arrows indicate responses to mineral oil and water, respectively.
Figure 9. Respiratory frequency coupling may facilitate beta oscillations. Each of the twelve LFP plots (left and right columns) contains 1 second of data from both the OB (top trace) and aPC (bottom trace) during odor sniffing, intended as examples of the statistical results in figures 4-7 (all data from the same rat, amplitudes normalized by prestimulus control periods). Odorants (top to bottom): nonanoic acid, nonanol, TMT, hexanone, butanone, and acetone. Left column: from first exposure of each of the six odorants; right column: from later trials. Relative airborne concentrations are represented as solid squares in the center column (size proportional to log10VP for the six odorants; see table 1). Sniff duration on the first trial decreases (Fig. 1b) and theta coherence on subsequent trials increases (Fig. 8c) linearly with increasing log10VP. Rightmost filled circles represent average beta power for each of the six odorants (size proportional to average OB power; Fig. 4a).