Neuronal Activity of Mitral-Tufted Cells in Awake Rats During Passive and Active Odorant Stimulation

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Fuentes RA, Aguilar MI, Aylwin ML, Maldonado PE. Neuronal activity of mitral-tufted cells in awake rats during passive and active odorant stimulation. *J Neurophysiol* 100: 422–430, 2008. First published May 21, 2008; doi:10.1152/jn.00095.2008. Odorants induce specific modulation of mitral/tufted (MT) cells' firing rate in the mammalian olfactory bulb (OB), inducing temporal patterns of neuronal discharge embedded in an oscillatory local field potential (LFP). While most studies have examined anesthetized animals, little is known about the firing rate and temporal patterns of OB single units and population activity in awake behaving mammals. We examined the firing rate and oscillatory activity of MT cells and LFP signals in behaving rats during two olfactory tasks: passive exposure (PE) and two-alternative (TA) choice discrimination. MT inhibitory responses are predominant in the TA task (76.5%), whereas MT excitatory responses predominate in the PE task (59.2%). Rhythmic discharge in the 12- to 100-Hz range was found in 79.0 and 68.9% of MT cells during PE and TA tasks, respectively. Most odorants presented in PE task increase rhythmic discharges at frequencies >50 Hz, whereas in TA, one of four odorants produced a modest increment <40 Hz. LFP oscillations were clearly modulated by odorants during the TA task, increasing their oscillatory power at frequencies centered at 20 Hz and decreasing power at frequencies >50 Hz. Our results indicate that firing rate responses of MT cells in awake animals are behaviorally modulated with inhibition being a prominent feature of this modulation. The occurrence of oscillatory patterns in single- and multiunitary discharge is also related to stimulation and behavioral context, while the oscillatory patterns of the neuronal population showed a strong dependence on odorant stimulation.

INTRODUCTION

Mitral and tufted cells (MT cells), the main olfactory bulb (OB) projection neurons, are embedded in a complex network of local interneurons that modify and integrate OB input. In anesthetized mammals, different odorant's molecular features or concentrations increase or decrease MT cells' firing rate (Buonviso and Chaput 2000; Imamura et al. 1992; Katoh et al. 1993; Motokizawa 1996; Wellis et al. 1989). In addition, MT cells activity also exhibit temporal patterns expressed as oscillatory synchronization of groups of neurons (Kashiwadani et al. 1999; Schoppa 2006). In insects, the spatiotemporal patterns evoked by odorants correspond to the precise firing with respect to an oscillatory local field potential (LFP) (Laurent 2002; Laurent et al. 1996; Wehr and Laurent 1996). In mammals, LFP oscillations have been extensively studied in anesthetized (Adrian 1950; Freeman and Barrie 2000; Neville and Haberly 2003) and awake animals (Chabaud et al. 1999; Gray and Skinner 1988; Martin et al. 2004).

Notwithstanding the wealth of data obtained from anesthetized animals, little is known about the response properties of MT cells in awake behaving animals. Odorant-elicited changes in MT firing rates are of greater amplitude and more frequently observed in anesthetized than in the awake mice (Rinberg et al. 2006). In awake rats, MT cells recorded over long periods exhibit a high degree of variability to the same stimuli (Bhalla and Bower 1997). The contextual meaning of the stimulus can also affect the MT cells response properties (Kay and Laurent 1999). Altogether, these results stress the importance of studies in awake animals not only because of the effects of anesthesia on neuronal activity, known since Adrian's studies (Adrian 1950), but also because the OB is modulated by centrifugal fibers (Gray and Skinner 1988; Martin et al. 2004), which may have a pivotal role in the awake state. In this study, we examined unitary activity and LFP in the OB of awake behaving rats in two behavioral paradigms: passive odorant exposure (PE) and active odorant exposure during a two-alternative (TA) choice olfactory discrimination task. We found that global firing rate and oscillatory properties of MT cells activity are behaviorally modulated.

METHODS

Electrophysiology

Unitary activity recording and LFP signals were obtained using custom-made nichrome tetrodes constructed with 12 μm wire, with an impedance of 1.5–2 MΩ at 1 kHz (Gray et al. 1995). Six tetrodes were mounted in a custom-made micromanipulator, which allowed independent movement of each tetrode. Low-weight custom-made headstage preamplifiers (noninverting amplifier circuit, 11×) were connected directly to each tetrode to reduce electrical artifacts produced by the rats' movements. To obtain LFP and unitary activity, the signals from the tetrode were amplified (1,000), filtered in two frequency bands (DC: 500 Hz for LFP and 100–5,000 Hz for unitary activity), and digitized at 3 and 27 kHz, respectively. Acquisition, spike detection, and spike extraction was accomplished using PC custom software written in Lab Windows/CVI (National Instruments).

Surgery

All experiments followed institutional (CBA No. 0154) and National Institutes of Health guidelines in accordance with protocols published in "Preparation and Maintenance of Higher Mammals During Neuroscience Experiments," National Institutes of Health publication 94-3207. Six trained adult male Sprague-Dawley rats (280 g) were implanted in a sterile surgery with a tetrode manipulator. Rats where anesthetized with ketamine (12 mg/kg ip), acepromazine (1

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mg/kg ip), and atropine (0.5 mg/kg). A six-tetrode bundle mounted on the micromanipulator was faced to the exposed dorsal surface of the left OB. The micromanipulator was anchored to the animal's head with stainless steel screws and dental acrylic. Two additional screws were fastened in the contralateral nasal and occipital bones for ground connections. Immediately after surgery, all tetrodes were lowered 200 μm. During the next 4 days, the rat received enroflaxine (5 mg/kg im) and ketoprofene (1 mg/kg im). Electrophysiological recordings were initiated one week after the surgery. Tetrodes were lowered until characteristic large-amplitude unitary activity was found (Kay and Laurent 1999; Pager 1974). Location of the tetrodes in or close to the mitral layer of the OB was confirmed by electrolytic lesion followed by histology. Due to the limitations imposed by the size of the preamplifiers, we recorded from up to three tetrodes simultaneously.

Olfactory tasks and odorant stimulation

Training and electrophysiological recordings of behaving rats was conducted in an operant conditioning chamber (25 $\times$ 30 $\times$ 31 cm). The chamber had a light bulb, an odorant delivery port and a food tray. For the PE task, rats were trained to poke their nose for 2 s in the odorant delivery port after a signal light was turned on. Successful trials were rewarded with a 45-mg food pellet delivered trough a dispenser (ENV-203, Med Associates). In every trial, an odorant was chosen pseudorandomly from a set of five odorants and delivered for 1.0 s. The onset of odorant delivery was set to 1.0 s after the rats initiated the trial with a nose poke. Electrophysiological recordings were triggered by the nose poke using an infrared detector installed in the odorant delivery port (Head Entry Detector Package, ENV-254, Med Associates). If the rats withdrew their nose before 2 s, the trial was aborted and no reward was delivered. Typically, rats performed $\sim$100 trials in a single session. On the TA task, rats were initially trained to associate one of two levers (left or right) with one of the two odorants presented following the same protocol as in the PE task. After the training, we recorded a series of trials were the rats had to nose poke in the delivery port. After a delay of 500 ms, one of the two odorants was delivered for 1.0 s, and the animals had to press the appropriate lever. Withdrawal from the delivery port before 1.5 s caused the trial to be aborted. A typical TA session consists of 100 trials with two odorants delivered in a pseudorandom sequence. Two rats performed the PE task, while four different rats performed the TA task.

Odorant stimulation was achieved through a custom made olfactometer, equipped with six 50-ml containers, five of them containing 1 ml of a 1:100 dilution of an odorant in mineral oil, while the sixth was left empty for control trials. The odorants for the PE task included isoamyl acetate (IAA), hexanol, cineole, limonene, and peppermint (all from Sigma Chemical). For the TA task, we used isoamyl acetate versus hexanol or R-carvone versus S-carvone. A flow of humidified air (5 l/min, 21 $\pm$ 2% O_2+N_2) was continuously delivered through the empty container to the delivery port. The airflow was directed to the desired odor container and to the delivery port by switching between a set of electromechanical valves (Parker). A personal computer, programmed with custom-made software, controlled the operant chamber devices, the pellet dispenser and the olfactometer through an I/O digital card (PCI-6503, National Instruments).

Data analysis

Unitary and LFP signals were analyzed off-line. For each data set, spike classification was achieved by an interactive custom software, where selected spike parameters for any two of the tetrode's recording channels are displayed in two dimensional scatter plots, revealing clustering of values. Once an isolated cluster was defined as a single cell, the spike train of each cell was computed by recovering the time stamp of each spike data in the cluster and stored with a 0.1-ms resolution. In the event that two clusters were largely overlapped, they were combined, and the resulting spike train was classified as multiunit activity (MU). To assess significant increment (excitatory response) or decrement (inhibitory response) in firing rate induced by odorants, the average firing rate of a 200-ms window slided every 1 ms in the STIM epoch was compared with the average firing rate of the first 500 ms of the PRE epoch (1-side *t*-test, $P < 0.001$ adjusted by sequential Bonferroni correction for multiple comparisons). Response onset was defined as the time at which the first significant different bin is detected, thus the onset values, expressed in ms, are relative to 200-ms bin (Fig. 3*B,* ordinate axis). Response duration was defined as the number of bins of the stimulation period which were significantly different to baseline.

The existence of a refractory period in the firing rate histogram was determined and used as criteria to confirm single-unit classification. We constructed autocorrelograms with 1-ms resolution and $\pm$128-ms time lag. The refractory period, defined as the time between the center of the peak and the time to reach half-amplitude of the secondary peak (Aylwin et al. 2005) was obtained from the autocorrelograms. Rhythmic discharge of MT cells was examined by computing the power spectrum of average autocorrelograms ($\pm$160-ms time lag) during PRE and STIM epochs. We extracted the frequency and amplitude of the peak values in the frequency range 10–100 Hz. The statistical significance of spectral peaks was obtained by comparing the power in every frequency band to the 99% highest value calculated from running 1,000 simulations of pseudo-random spike trains, using a random sample from an uniform distribution and the refractory period from the actual data (Egana et al. 2005).

To compare the significant frequencies of rhythmic discharge between PRE and STIM periods, we used Kolmogorov-Smirnov (K-S), a robust nonparametric test that makes no assumptions about the distribution of the samples. The two populations to be compared were bootstrapped, and the K-S test was performed (n = 1,000). The median of the resulting P value population was used as an estimation for significance ($P < 0.01$). Distributions of P values and the medians are shown in Supplementary Fig. S2.[1]

All LFP spectral analyses were carried out using a multitaper method with nine tapers (Percival and Walden 1993). Spectral power in 500-ms window before and after stimulation was compared in the range 1–100 Hz. For every TA session with performance >75%, correctly executed trials were grouped by stimulus, and the average spectrum (*B*) was computed with the mtspectrumc function with Jackknife error bars at $P < 0.01$ (Chronux script package, http://chronux.org). Figure 5*C* was constructed by subtracting the average PRE spectrum from the STIM spectrum and dividing the resulting amount by the error of the STIM spectrum for every recording site. Thus every row represents the trials (typically 20–50) executed with the same odorant during a single discrimination session (TA task).

RESULTS

We recorded the OB activity from six rats while engaged in one of the two tasks: PE versus TA. Figure 1 *A* and *B* shows an example of data obtained with one tetrode. Unitary activity as well as LFP was obtained by differentially filtering the signal (see METHODS). Single units (SU) were sorted using the amplitude difference in the tetrode channels as seen in Fig. 1, *C* and *D*. If clusters were not separable, all spikes were merged into a single group and classified as multiunit (MU). Up to three SUs could be extracted from a single tetrode. Analysis of this ongoing activity revealed that in the absence of odorants, MT cells fired at heterogeneous rates with a mean rate of 12.5 $\pm$

[1] The online version of this article contains supplemental data.

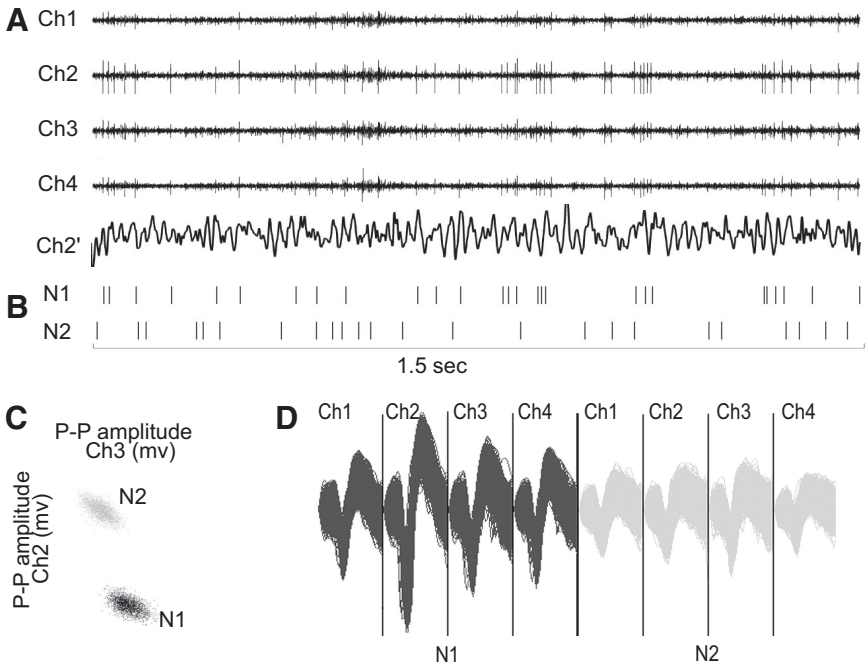

FIG. 1. Example of tetrode recording and sorting of single-unit activity of mitral/tufted (MT) cells of a behaving rat. *A*: the *1st 4 traces* correspond to the filtered signal (100–5,000 Hz) from each tetrode channel. The *5th trace* shows the local field potential (LFP) signal from channel 2 (band-pass: 10–100 Hz). *B*: sorted spike trains obtained from the traces shown in *A*. *C*: scatter plot of waveform peak-to-peak amplitudes recorded in channel 2 vs. those recorded in channel 3. Two clusters clearly emerge, each one corresponding to single-unit activity shown in *B*. *D*: waveforms of the spikes of the 2 sorted neurons in each channel.

5.3 Hz (range: 3.0–25.4, $n = 91$). However, no significant differences were found between the basal mean firing rates of the cells recorded from rats that participated in the PE task versus those recorded from rats in the TA task ($P = 0.11$, Student's *t*-test for independent samples).

Firing rate responses of MT cells during a passive odorant exposure task

We obtained 62 recordings (15 SU and 47 MU) from two rats performing the PE task. During this task, the rat poked its nose in the odorant port to trigger stimulus delivery. If the rat withdrew the nose before the end of the odorant exposure, the trial was discarded, and no reward was granted. We found excitatory and inhibitory responses of MT cells during the PE task. A multiunit excitatory response to butyric acid and hexanol is shown in Fig. 2*A*. The response to hexanol (top panel) was particularly robust. A single unit—shown in Fig. 2*B*—has a strong inhibitory response to butyric acid (*top*) and a weaker inhibitory response to peppermint (*bottom*).

Overall, we found that 51.6% (32/62) of the recordings showed significantly modulation in response to at least one odorant. Of the total number of responses, 59.2% (29/49) were excitatory, whereas 40.8% were inhibitory (20/49, Fig. 2*E*). When we examined the prevalence of response type by stimulus, we found that all odorants evoked a similar overall ratio (Fig. 2*F, top*), but there were larger variances if group by subject (Fig. 2*G, top*).

Response properties of MT cells during a two-choice discrimination task

During the PE paradigm, there is no behavioral correlate of the animal's perception neither is there a requirement for the animal to actually attend the olfactory stimulus. A discrimination task requires from the rat an active engagement and also provides a behavioral correlate of animal's perception. Thus we conjectured that the activity of MT cells during an active discrimination task should exhibit different properties when compared with the passive exposure to odorants. The activity of MT cells was examined during the TA task, where rats associated a left or right lever with each of two odorants. Only correct trials from sessions with accuracy >75% of correct behavioral responses were included in the analysis. We obtained a total of 61 recordings: 32 recorded during the discrimination of IAA versus hexanol (2 rats; 22 SU and 10 MU), and 29 recordings from discrimination of R- versus S-carvone (2 rats; 7 SU and 22 MU). Overall, 39.3% (24/61) recordings showed significantly modulation in response to at least one odorant in the TA task. As in the PE task, we also found excitatory (Fig. 2*C*) and inhibitory responses (Fig. 2*D*). Additionally, we found two biphasic responses. In contrast to PE task, where excitatory responses predominate, in the TA task, the proportion of inhibitory responses (76.5%) greatly exceeds that of excitatory responses (17.6%) as observed in Fig. 2*E*. When the percentage of excitatory/inhibitory responses per odorant and per animal was analyzed, the heterogeneity of the responses to each odorant or between animals was much larger for TA task than for the PE task (Fig. 2, *F* and *G*).

The temporal course of firing rate responses was also compared between tasks. Based on the procedure for detecting significant changes in firing rate, the onset and duration of responses were calculated (see METHODS). Fig. 3 summarizes the average firing rate for all units exhibiting responses to odorants in both tasks. The response onset, defined as the time of occurrence of the first 200-ms bin that significantly differed from baseline, is represented for every unit (Fig. 3*A*). TA excitatory responses occurred significantly earlier than excitatory and inhibitory PE responses (Fig. 3*B;* Kruskal-Wallis test followed by a nonparametric post hoc comparison, $P < 0.01$). Response duration (defined as the number of 200-ms bins significantly different from baseline) was compared between the tasks with the same approach. The results indicate that TA

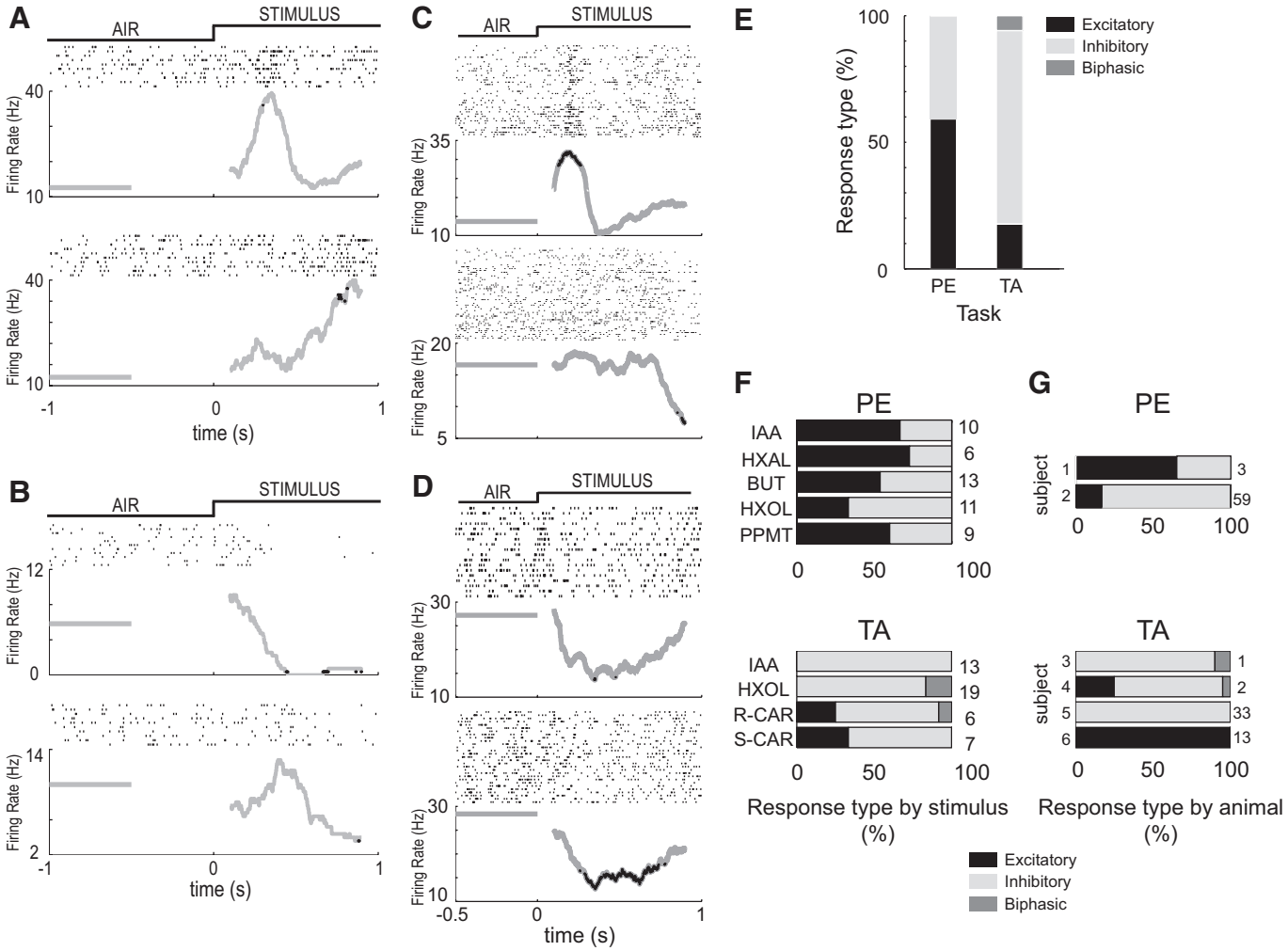

FIG. 2. Examples of modulation of MT cells firing rate during behavioral tasks. *A–D*: shown from top to bottom in each plot: stimulation protocol, raster plots for single trials, PRE-epoch average firing rate computed with a 500-ms window (*left,* straight gray trace) and STIM average firing rate, computed with a sliding 200-ms window (*right,* gray trace). Significant differences are shown in black ($P < 0.001$). *A*: multiunit excitatory responses during passive exposure (PE) task to hexanol (*top*) and butyric acid (*bottom*). *B*: single-unit inhibitory responses during PE task to butyric acid (*top*) and peppermint (*bottom*). *C*: single-unit response during discrimination (TA) of isoamyl acetate (*top*) and hexanol (*bottom*). *D*: single-unit inhibitory responses during discrimination of R- and S-carvone (*top* and *bottom,* respectively). *E*: percentage of excitatory, inhibitory and biphasic responses in both tasks. *F* and *G*: percentage of excitatory, inhibitory, and biphasic responses computed per stimulus and per animal.

inhibitory responses were significantly longer than PE excitatory and PE inhibitory responses.

Oscillatory activity and synchronization

Temporal properties of MT cells have been proposed as an additional coding feature of olfactory stimulus in the mammalian OB. We first examined a basic temporal property of spontaneous activity, namely the olfactory refractory period (RP). MT cells in anesthetized rats display a long silent period after each spike (Aylwin et al. 2005; Kay and Laurent 1999), setting a likely base for rhythmic population discharge. For single units only, we computed the RP duration as the time from zero to half the maximum activity of the first off-center peak in the 128-ms-lag autocorrelograms of the PRE stimulus period activity (Supplementary Fig. S1). The RPs in our sample data have an average duration of 9.0 ± 6.4 ms (range: 2–29, $n = 91$), which is comparable with the RPs observed in anesthetized animals (Aylwin et al. 2005).

In addition, we examined the magnitude and incidence of rhythmic discharge of SU and MU spike trains as well as oscillations in the LFP recordings. Studies in anesthetized preparations and in slices have shown that rhythmic discharge of MT cells is induced by odorant stimulation (Kashiwadani et al. 1999; Schoppa 2006). Thus we assessed rhythmic discharge of MT cells in the PRE and STIM epochs by examining the statistical significance of the peaks in the power spectrum of autocorrelograms of the recordings in the range 12–100 Hz (see METHODS). We found three types of responses in both tasks: recordings exhibiting rhythmic discharge only during STIM epoch (Fig. 4*A1*), only during PRE epoch (Fig. 4*A2*), and in both periods (Fig. 4*A3*). In the PE task, 79.0% (49 of 62) of the recordings showed rhythmic discharge. Of these, 24.5% (12/49) did so during PRE epoch, whereas other 24.5% (12/49) did it during STIM epoch. Surprisingly, >51.0% (25/49) of the neurons displaying rhythmic discharges did so during both PRE and STIM epochs. In the TA task, 68.9% (42 of 61) recordings

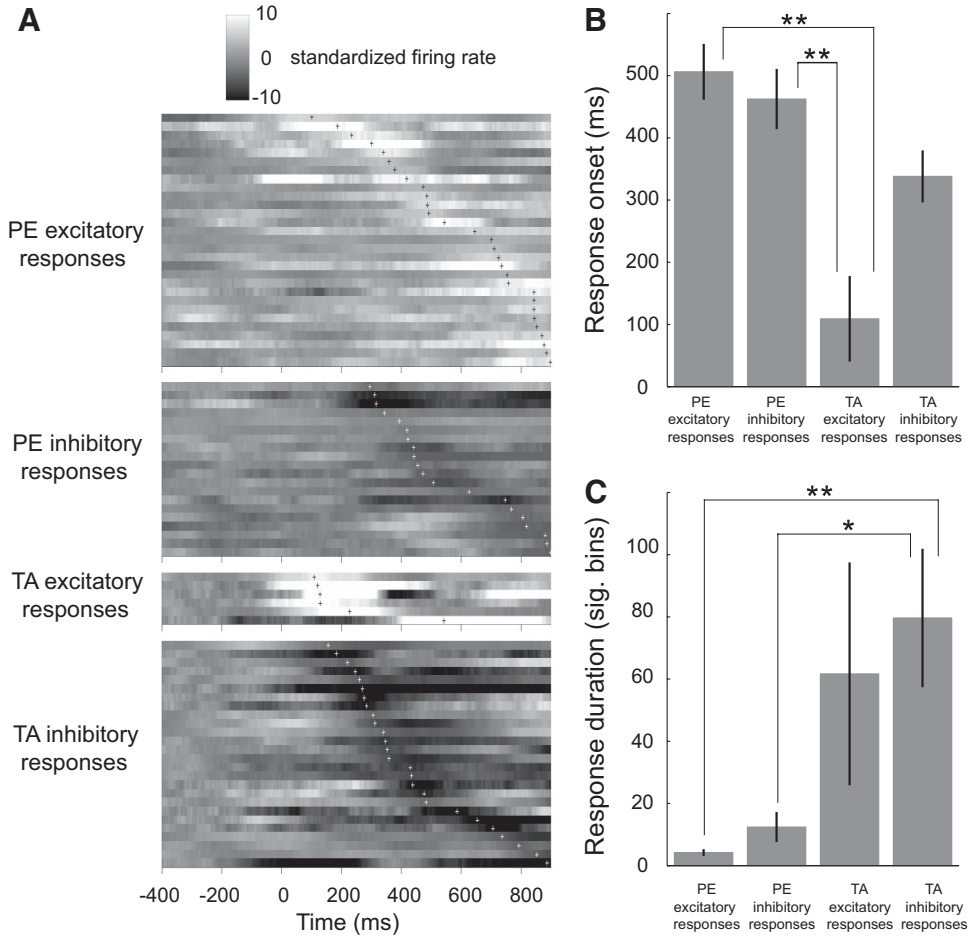

FIG. 3. *A*: summary of all MT cell responses showing significant changes in firing rate. Each row represents the average firing rate of a unit, standardized by the baseline (−400 to 0 s), in response to a particular odorant Responses are sorted according to onset, which are represented by a black (excitatory responses) or a white cross (inhibitory responses). *B*: bar graph depicting differences in response onset. *C*: bar graph depicting differences in response duration (double asterisk, $P < 0.01$; single asterisk, $P < 0.05$).

showed significant rhythmic discharge. Of those, 21.4% (9/42) correspond exclusively to PRE epoch, 19.0% (8/41) to STIM epoch and 59.5% (25/42) were found to discharge rhythmically both during PRE and STIM epochs.

To identify odorants that elicit changes in preferred frequency of MT cells rhythms, the distribution of frequency values was compared pair wise (see METHODS) between epochs in both tasks. In the PE task, we detected significant differences for IAA, hexanal, hexanol, and peppermint ($P < 0.01$; Fig. 4*B*). Visual inspection of frequency distributions suggests that an increment in the range >50 Hz underlies the observed differences. This is apparent in the distribution of frequency values pulled together for all odorants (Fig. 4*D*). In the TA task, we found significant differences in rhythmic discharge only for odorant IAA ($P < 0.01$), which produces an increment in frequencies <40 Hz (Fig. 4*C, top*).

We examined next whether LFP signals, reflecting the coordinated activity of a large number of neurons, exhibited odorant-dependent oscillations. We analyzed LFP signals from the TA task only because LFP signals from PE task were often fragmented with noise. For analysis, we selected 22 recordings from a pool of 13 discrimination sessions (TA task) executed by four animals. Only correct trials from sessions with performance >75% were included in the analysis. Fig. 5 shows an example of LFP traces corresponding to trials from a TA session where the animal was discriminating between R- and S-carvone. Short episodes of high-frequency oscillations are apparent previously and during odorant presentation, whereas episodes of low-frequency oscillations are clearly observed during the stimulus epoch. To identify stimulus-induced significant changes in LFP oscillations, we compared the average spectrum before and during the stimulation (see METHODS). Figure 5*B, left,* shows an example of TA session discrimination between IAA and hexanol. A significant increment in power in the frequency range 10–30 Hz are observed in both odorants, whereas hexanol stimulation exhibits a small but significant decrease in 70- to 100-Hz range. An example of TA session of discrimination between isomers, R- and S-carvone, is shown in Fig. 5*B, right.* Stimulation with both odorants exhibits a dramatic power increase in the range 10–40 Hz. Furthermore, S-carvone stimulation exhibits a clear power decrease at 45–75 Hz. The results of all the analyzed sessions are summarized in Fig. 5*C,* where every colored matrix represents the standardized difference between the PRE and STIM spectra for a single odorant across sessions. The count of significant changes of spectral power is shown at the bottom of every color matrix, revealing a common clear trend for all studied stimuli: odorant stimulation during discrimination task increases spectral power of LFP in the range <50 Hz (median: 20.5 Hz), while decreases in the range 50–100 Hz (median: 80.6 Hz).

DISCUSSION

The electrical activity of olfactory bulb neurons has been extensively described in anesthetized animals. However, few studies have described the OB activity in behaving animals

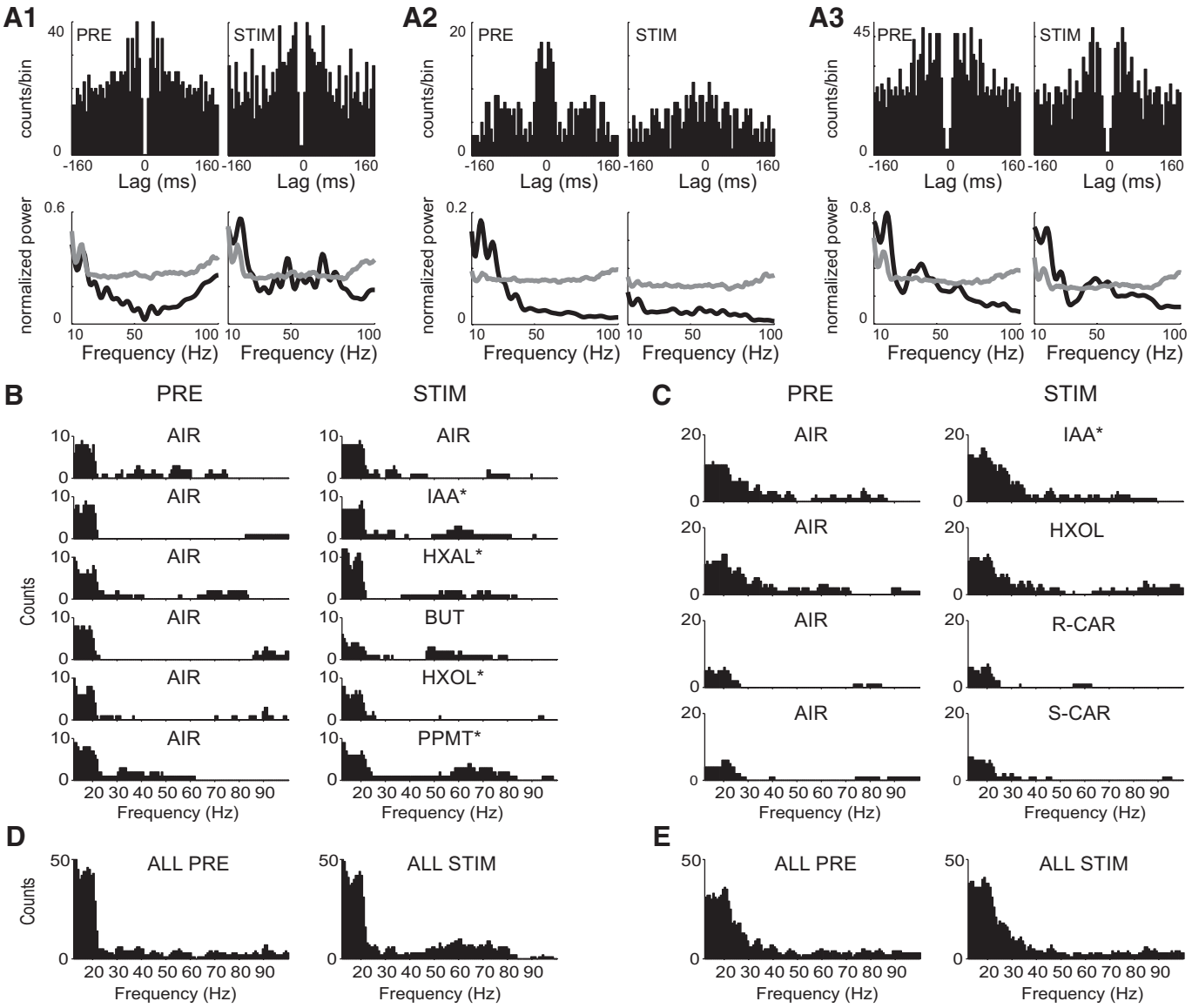

FIG. 4. Rhythmic discharge of MT cells is a robust event in the olfactory bulb (OB) of behaving rats. *A1*: single-unit displaying rhythmic discharge during stimulus epoch. *Top*: autocorrelation histograms of PRE and STIM epochs; *bottom*: actual power spectra of the autocorrelogram (black trace) and significance threshold (gray trace, $P < 0.01$). *A2*: single-unit displaying rhythmic activity before odorant delivery. *A3*: single-unit displaying rhythmic activity in both PRE and STIM epochs. Distribution of significant frequencies of rhythmic discharge during PRE and STIM periods for TE task (*B* and *D*) and for TA task (*C* and *E*). Pairs of distributions showing significant differences are labeled with asterisk ($P < 0.01$).

(Bhalla and Bower 1997; Kay and Laurent 1999; Lowry and Kay 2007; Rinberg et al. 2006). The aim of this study was to examine the firing rate and temporal properties of MT cells as well as LFP oscillations in the awake behaving rat during two different behavioral conditions, passive exposure to odorants and active olfactory discrimination.

We found that in the awake animal, the mean firing rates of MT cells in the olfactory bulb was similar than previously reported in anesthetized animals (Aylwin et al. 2005; Egana et al. 2005; Wilson 2000) and in awake behaving rats (Bhalla and Bower 1997; Kay and Laurent 1999). Similarly, we found no difference in the olfactory refractory periods between awake and anesthetized animals (Aylwin et al. 2005). These results demonstrate that anesthesia (urethan) does not alter significantly these MT neuronal properties and that centrifugal modulation of OB does not appear to modulate these parameters of neural activity.

Task dependency of neuronal responses

During passive exposure and odorant discrimination tasks 51.6 and 39.3% of the recordings showed rate modulation in response to odorants, respectively. While this incidence may depend largely in the collection of odorants used and the region of the OB recorded, we did find a striking difference between PE and TA regarding the directionality of the responses. While the majority (59.2%) of the responses in the passive exposure task was excitatory, in the discrimination task, 76.5% of the responses were inhibitory. Furthermore, inhibitory responses were not only more frequent in the TA task, but they also had significantly longer duration than any response type in the PE task. These results demonstrate that inhibition, as much as excitation, participates in odorant representation. Moreover, these results suggest that the balance of excitatory and inhib-

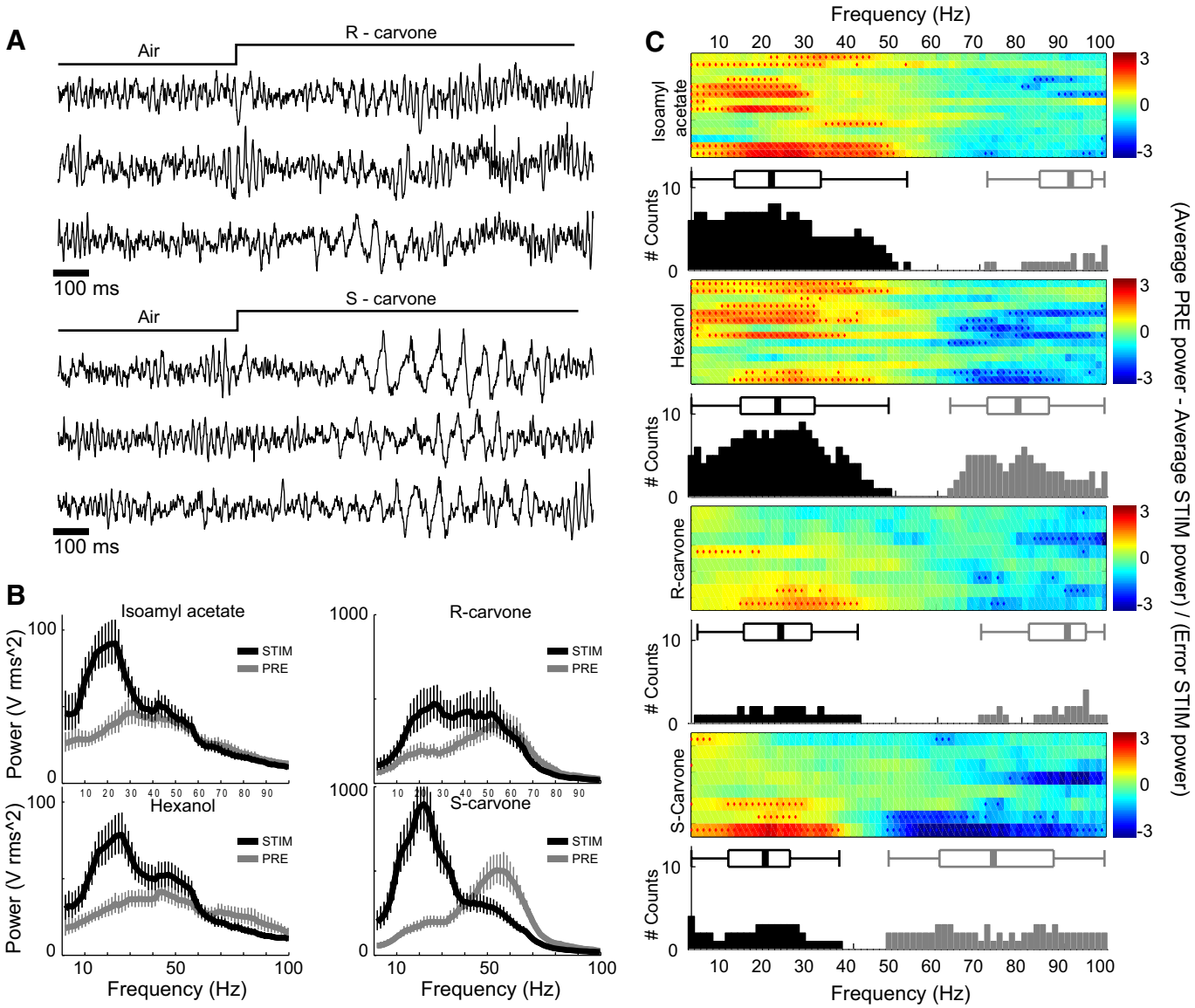

FIG. 5. LFP oscillations are modulated by stimulus application during olfactory discrimination. *A*: examples of LFP traces (low-pass filtered at 150 Hz) from 6 trials of a discrimination session between R- and S-carvone. *B*: spectral power before and during stimulation during 2 different TA sessions (*right* and *left*). Solid traces are average power spectrum from PRE (gray) or STIM (black) epoch. Vertical bars represent Jackknife error at $P < 0.01$. *C*: summary of spectral power modulation for 22 TA sessions. Color-code panels represent the power difference between STIM and PRE divided by the STIM error. Warm colors indicate a positive difference, that is, an increment of power, whereas cool colors indicate a decrease in spectral power after stimulation. Significant changes, established by nonoverlapping error bars (see *B*) are indicated with red dots (increments) and blue dots (decrements). Histograms below each color code indicate the incidence of frequency values where significant increments (black bars) and decrements (gray) where detected. Median, interquartile range, and maximum and minimum values are represented with a box and whiskers plot.

itory responses is dependent on the behavioral goal. Thus processes involved in the discrimination task are shaping the response of MT cells to odorants in a different way as they would during passive exposure to odorants. It is known that other behavioral paradigms, such as olfactory conditioning in early postnatal life, also generate a decrease in the proportion of excitatory responses, whereas increases inhibitory responses (Wilson et al. 1987). Therefore increased inhibition in the TA task may be reflecting the learning process associated to the TA task (Wilson 2000). The inhibition of MT cells can be the result of inhibitory interneurons activation at the glomerular or granular layer. The inhibition at glomerular layers, which involve mono and polysynaptic connectivity between MT cells and different periglomerular cells, is triggered by the input to the OB from olfactory receptor neurons, thus it is likely dependent on odorant stimulation. On the other hand, at the granular layer the inhibition of MT cells by granule cells could arise from feedback at dendro-dendritic synapses (Rall et al. 1966) or the modulation by centrifugal excitatory input (Nakashima et al. 1978). Centrifugal input from piriform cortex neurons can actually gate the dendro-dendritic inhibition by relieving the tonic Mg^{+2} block of NMDA receptors at MT-granule cell synapses (Balu et al. 2007). Thus the observed increase in MT cells inhibitory responses during the discrimination task may arise from the activation of the piriform cortex-OB circuit. It cannot be rule out, however, that because both PE and TA tasks required previous extensive learning, increased inhibition would not reflect learning but other cognitive process, such as attention, which would reflect as a top down

modulation of lower level structures (Delano et al. 2007; Reynolds and Desimone 1999).

Neuronal oscillations

Although the firing rate properties of MT cells in anesthetized animals do not differ from the properties observed in awake animals, one distinctive feature of MT cell activity in the awake rats is the ubiquitous rhythmic feature of LFP signals (Adrian 1950; Gray et al. 1986). However, in previous studies in anesthetized animals, we reported little or no rhythmic discharge of single unit spike trains in the absence or presence of odorant stimulation even though the olfactory refractory period hinted to its existence (Aylwin et al. 2005; Egana et al. 2005). Nonetheless, odorant stimulation induces rhythmic discharge of MT cells in anesthetized rabbits (Kashiwadani et al. 1999), and they have been induced by olfactory nerve stimulation in slices (Schoppa 2006). Here we found a high proportion of units with rhythmic discharge in both tasks (PE: 79.0%; TA: 68.9%). Surprisingly, the majority of these units (PE: 51.0%; TA: 68.9%) displayed rhythmic discharge both before and during stimulus delivery, suggesting that unitary rhythmic discharge is an intrinsic property of OB in awake animals and is not directly related to odorant stimulation. The finding that some units oscillated only during the epoch previous to odorant stimulation (PE: 24.5%; TA: 21.4%) supports this suggestion. Comparing the distribution of discharge frequencies before and during the stimulation, we found that four of five odorants used in the TE task produced an increment in discharge frequencies >50 Hz. In the TA task, we found only IAA produced an increment of rhythmic discharge around the 20 Hz. This is further evidence for behavioral modulation of OB unitary responses.

Synchronization of neuronal populations, measured as LFP oscillations, are thought to be an essential component of olfactory coding (Gray and Skinner 1988; Laurent 2002; Spors and Grinvald 2002; but see Christensen et al. 2000; Fletcher and Wilson 2005). Two frequency ranges of LFP oscillatory activity in the mammalian OB have been related to odorant stimulation: beta band, roughly between 15 and 35 Hz, and gamma band, between 35 and 100 Hz. Beta band oscillations power increase on odorant stimulation has been shown in anesthetized (Neville and Haberly 2003) and in awake animals exposed to odorants (Chapman et al. 1998; Gray and Skinner 1988; Zibrowski and Vanderwolf 1997) as well as in animals performing a go/no-go discrimination task between eugenol and geraniol (Martin et al. 2004; Ravel et al. 2003). Nevertheless an increment of beta power has not being observed during a TA discrimination task between pairs of compounds differing in a single methyl group (Beshel et al. 2007). It has been argued that this difference could originate in different behavioral strategies used in each type of discrimination task (go/no-go vs. TA). In this study, we consistently found increase power ~20.5 Hz during a TA discrimination task between IAA-hexanol and the isomers of carvone. The fact that the used odorant pairs are not the same across the mentioned studies may contribute to the distinct oscillatory patterns described in beta power modulation.

Gamma band oscillations has been found to decrease in the OB of awake rodents when stimulated with nonreinforced odors (Gray and Skinner 1988), conditioned odors (Bressler 1988; Kay and Freeman 1998) or during a go/no-go discrimination task (Martin et al. 2004; Ravel et al. 2003) However, LFP gamma oscillatory power has been found to increase during TA discrimination of molecularly highly similar odorants but not during discrimination of less similar odorants (Beshel et al. 2007). Although the former result seems to be incompatible with ours, the difference on used odorants may explain and even conciliate both results. According to Beshel et al., the gamma power induced by odorants during discrimination is modulated by the degree of molecular similarity between the odorant pair, and they show that stimulation with ketones or alcohols differing only in one methyl group increase gamma power, whereas ketones or alcohols differing more than one carbon in their chains do not. Following the same reasoning, more dissimilar pairs, like IAA and hexanol, used in this study, should not produce any increment in gamma power. Furthermore, we found that during IAA-hexanol discrimination, odorant stimulation decreases gamma power. Other studies using dissimilar odorants pairs for discrimination such as geraniol and eugenol also reported decrease of gamma power on stimulation (Martin et al. 2004; Ravel et al. 2003). The second pair we used for discrimination, R- and S- carvone, are isomers. In terms of similarity, they are not directly equivalent to a pair differing in a single methyl group. Stimulation during discrimination of the R and S-carvone showed less pronounced changes than the IAA-hexanol pair but still kept the trend of beta increments and gamma decrements. In recordings performed in rats while they remain in their cages while apparently resting, we observed strong LFP gamma oscillations associated to the respiration cycle (data not shown).

Coda

Our results indicate that the odorant induced changes in the firing rates of MT cells in awake active animals are behaviorally modulated with inhibition being a prominent feature of this modulation. In the same trend, the occurrence of oscillatory patterns in unitary activity of MT cells showed a weak but significant modulation by odorant stimulation and behavioral context, whereas the population oscillatory patterns from LFP signals, which may include other neuronal types, exhibit strong dependence on odorant stimulation.

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