Neuronal activity of mitral-tufted cells in awake rats
during passive and active odorant stimulation

Abbreviated Title: Neuronal activity in the OB of awake rats

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Abstract

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 local field potentials (LFP) signals in behaving rats during two olfactory tasks: passive exposure (PE), and two-alternative choice discrimination task (TA). MT inhibitory responses are predominant in the TA task (76.5%) while MT excitatory responses predominate in the PE task (59.2%). Rhythmic discharge in the 12-100 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 above 50 Hz, while in TA, 1 of 4 odorants produced a modest increment below 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 greater than 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 multi-unitary discharge is also related to stimulation and behavioral context, while the oscillatory patterns of the neuronal population showed a strong dependence on odorant stimulation.
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 (Wellis et al., 1989; Imamura et al., 1992; Katoh et al., 1993; Motokizawa, 1996; Buonviso and Chaput, 2000). 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 spatio-temporal patterns evoked by odorants correspond to the precise firing with respect to an oscillatory LFP (Laurent et al., 1996; Wehr and Laurent, 1996; Laurent, 2002). In mammals, LFP oscillations have been extensively studied in anesthetized (Adrian, 1950; Freeman and Barrie, 2000; Neville and Haberly, 2003) and awake animals (Gray and Skinner, 1988; Eeckman and Freeman, 1990; Chabaud et al., 1999; 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 choice (TA) olfactory discrimination task. We found that global firing rate and oscillatory properties of MT cells activity are behaviorally modulated.

**Materials and 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 (non-inverting amplifier circuit, 11X) were
connected directly to each tetrode in order to reduce electrical artifacts produced by the rats’ movements. In order to obtain LFP and unitary activity, the signals from the tetrode were amplified (1k), filtered in two frequency bands (DC-500 Hz for LFP and 100-5000 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#0154) and NIH guidelines in accordance with protocols published in “Preparation and maintenance of higher mammals during neuroscience experiments”, NIH publication Nº 94-3207. Six trained adult male Sprague-Dawley rats (280g) were implanted in a sterile surgery with a tetrode manipulator. Rats were anesthetized with Ketamine (12 mg/kg i.p.), acepromazine (1 mg/kg i.p.) and atropine (0.5 mg/kg). A 6-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 four days the rat received enroflaxine (5 mg/Kg, i.m.) and ketoprofene (1 mg/Kg, i.m.). Electrophysiological recordings were initiated one week after the surgery. Tetrodes were lowered until characteristic large amplitude unitary activity was found (Pager, 1974; Kay and Laurent, 1999). 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 3 tetrodes simultaneously.

**Olfactory tasks and odorant stimulation**

Training and electrophysiological recordings of behaving rats was conducted in an operant conditioning chamber (25x30x31 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 seconds 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, Inc). In every trial, an odorant was chosen pseudo-randomly 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, Inc.). If the rats withdrew their nose before 2 seconds, the trial was
aborted and no reward was delivered. Typically, rats performed about 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 2 odorants delivered in a pseudo-random 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 CO). For the TA task we used isoamyl acetate vs. hexanol or R-carvone vs. S-carvone. A flow of humidified air (5 L/min, 21±2% O₂+N₂) was continuously delivered through the empty container to the delivery port. The airflow is directed to the desired odor container and to the delivery port by switching between a set of electromechanical valves (Parker Inc). 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 to the average firing rate of the first 500 ms of the PRE epoch (one-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 ±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 Mde et al., 2005) was obtained from the autocorrelograms. Rhythmic discharge of MT cells was examined by computing the power spectrum of average autocorrelograms (±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 1000 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, a robust non-parametric test which makes no assumptions about the distribution of the samples. The 2 populations to be compared were bootstrapped and the K-S test was performed (n=1000). 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. 2.

All LFP spectral analyses were carried out using a multitaper method with 9 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 (Fig. 5B) was computed with the ‘mtspectrunc’ 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 6 rats while engaged in one of the 2 tasks: passive exposure to odorants vs. two-alternative choice odor discrimination. Fig. 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 1 D. If clusters were not separable, all spikes were merged into a single group and classified as multiunit (MU). Up to 3 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±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 vs. those recorded from rats in the TA task (P = 0.11, Student 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 2 rats performing the passive exposure task (PE). 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, while 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 passive exposure paradigm there is no behavioral correlate of the animal's perception; neither there is 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 to 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 vs. hexanol (2 rats; 22 SU
and 10 MU), and 29 recordings from discrimination of R- vs. 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, G).

The temporal course of firing rate responses was also compared between tasks. Based in the procedure for detecting significant changes in firing rate, the onset and duration of responses were calculated (see Methods). Fig. 3 A 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 non-parametric 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 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, (Kay and Laurent, 1999; Aylwin Mde et al., 2005) 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. 1). 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 single and multi unit 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 3 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 recordings with rhythmic discharge in both
periods (Fig. 4 A3). In the PE task, 79.0% (49 out of 62) of the recordings showed
rhythmic discharge. Of these, 24.5% (12/49) did so during PRE epoch, while other
24.5% (12/49) did it during STIM epoch. Surprisingly, over 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 out of 61) recordings 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.

In order 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 below 40 Hz (Fig. 4 C, top panel).

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 since LFP signals from PE task wee often fragmented with
noise. For analysis we selected 22 recordings from a pool of 13 discrimination sessions
(TA task) executed by 4 animals. Only correct trials from sessions with performance
>75% were included in the analysis. Figure 5 A 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, while episodes of low frequency
oscillations are clearly observed during the stimulus epoch. In order to identify
stimulus induced significant changes in LFP oscillations, we compared the average
spectrum before and during the stimulation (see Methods). Fig. 5 B, left panels, 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, while hexanol stimulation exhibits a small but significant decrease in 70-100 Hz range. An example of TA session of discrimination between isomers, R- and S-carvone, is shown in Fig 5 B, right panels. 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 (Bhalla and Bower, 1997; Kay and Laurent, 1999; Rinberg et al., 2006; Lowry and Kay, 2007). The aim of this study was to examine the firing rate and temporal properties of MT cells as well as local field potential 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 (Wilson, 2000; Aylwin Mde et al., 2005; Egana et al., 2005) 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 Mde et al., 2005). These results demonstrate that anesthesia (urethane) 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. Whilst 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, participate in odorant representation. Moreover, these results suggest that the balance of excitatory and inhibitory 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, while 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 can not be rule out however, that since 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 (Reynolds and Desimone, 1999; Delano et al., 2007).

**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 cells activity in the awake rats is the ubiquitous rhythmic feature of local field potential 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 Mde 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 4 out of 5 odorants used in the TE task produced an increment in discharge frequencies greater than 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 upon odorant stimulation has been shown in anesthetized (Neville, 2003) and in awake animals exposed to odorants (Gray and Skinner, 1988; Zibrowski and Vanderwolf, 1997; Chapman et al., 1998) as well as in animals performing a Go/No-go discrimination task between eugenol and geraniol (Ravel et al., 2003; Martin et al., 2004). 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 around 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 non-reinforced odors (Gray and Skinner, 1988), conditioned odors (Bressler, 1988; Kay and Freeman, 1998) or during a Go/No-go discrimination task (Ravel et al., 2003; Martin et al., 2004). 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, while 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 upon stimulation (Ravel et al., 2003; Martin et al., 2004). 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, while the population oscillatory patterns from LFP signals, which may include other neuronal types, exhibit strong dependence on odorant stimulation.
REFERENCES


LEGENDS

Figure 1. Example of tetrode recording and sorting of single unit activity of MT cells of a behaving rat. (A) The first four traces correspond to the filtered signal (100-5000 Hz) from each tetrode channel. The fifth trace shows the 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 of one corresponding to single unit activity shown in B. (D) Waveforms of the spikes of the 2 sorted neurons in each channel.

Figure 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 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.

Figure 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 (**, P<0.01; *P<0.05).

Figure 4. Rhythmic discharge of MT cells is a robust event in the OB of behaving rats. (A1) Single unit displaying rhythmic discharge during stimulus epoch. Top panels: autocorrelation histograms of PRE and STIM epochs; bottom panels: 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, D) and for TA task (C, E). Pairs of distributions showing significant differences are labeled with asterisk (*, P<0.01).

Figure 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, while cool colors indicate a decrease in spectral power after stimulation. Significant changes, established by non-overlapping error bars (see 5 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) were detected. Median, interquartile range and maximum and minimum values are represented with a box and whiskers plot.

Supplementary Figure 1. MT cells exhibit refractory periods whose duration is correlated with the basal firing rate. (A) Autocorrelograms of 2 single MT cells during the PRE stimulus period (1.0 s duration, 100 trials) which reveals a silent period after each discharge of 6 and 5 ms duration. (B) Auto-correlograms of 2 single MT cell during PRE stimulus period. A long refractory period of 29 and 28 ms duration is observed. (C) Distribution of the refractory period and mean firing rate of 91 MT cells assessed during PRE stimulus period. (D) Refractory period and mean firing rate are negatively correlated (r = -0.56, Pearson correlation coefficient, P < 0.001).

Supplementary Figure 2. Distribution of P values obtained from bootstrapping the PRE and STIM populations of significant frequencies and comparing them by means of Kolmogorov-Smirnov test. Bin size of all histograms is 0.01. Two populations were considered different when the median of bootstrapped P values was smaller than 0.01.