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# Olfactory learning modifies the expression of odour-induced oscillatory responses in the gamma (60–90 Hz) and beta (15–40 Hz) bands in the rat olfactory bulb

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## Abstract

This study addressed the question of the possible functional relevance of two different oscillatory activities, beta and gamma (15–40 and 60–90 Hz, respectively) for perception and memory processes in olfactory areas of mammals. Local field potentials were recorded near relay olfactory bulb neurons while rats performed an olfactory discrimination task. Signals reflected the mass activity from this region and characteristics of oscillatory activities were used as an index of local synchrony. Beta and gamma oscillatory activities were quantified by time-frequency methods before during and after odour sampling. In rats early in their training, olfactory sampling was associated with a significant decrease in power in the gamma band in parallel with a weak but significant increase in the beta band (centred on 27 Hz). Several days later, in well-trained rats, the gamma oscillatory depression was significantly enhanced both in duration and amplitude. It appeared within the 500 ms time period preceding odour onset and was further reduced during the odour period. Concurrently the beta oscillatory response (now centred on 24 Hz) during odour sampling was amplified by a twofold factor. The beta band response was modulated according to the chemical nature of the stimuli and rat's behavioural response. This study showed for the first time that odour sampling in behaving animals is associated with a clear shift in the olfactory bulb neuronal activity from a gamma to a beta oscillatory regime. Moreover, the data stress the importance of studying the odour-induced beta activity and its relation to perception and memory.

## Introduction

Perceptual and motor functions are based on distributed processes among multiple cortical and subcortical structures. This raises the question of how these distributed activities are functionally linked to generate the representation of objects in brain. It has been proposed that synchronization of neuronal discharges on an oscillatory mode could be the sign of assemblies of neurons representing the same stimulus (reviewed by Gray, 1999). Oscillatory activity in different frequency bands occurs in the EEG during various behavioural states (Singer, 1993; Basar *et al.*, 2000). Among these, gamma band activity has been proposed as a possible mechanism underlying sensory integration and object representation (Tallon-Baudry *et al.*, 1998; Tallon-Baudry & Bertrand, 1999). More recently, beta band activity has also been associated to the rehearsal of an object in visual short-term memory (Tallon-Baudry *et al.*, 2001).

In the mammalian olfactory system, prominent oscillations in field potential activities have been described initially by Adrian (1950). In awake animals, in the absence of any olfactory stimulation, the signal derived from the first relay of olfactory processing, the olfactory bulb (OB), exhibits a well structured oscillatory gamma activity (30–90 Hz). This activity consists of regular spindle bursts of oscillations occurring during each inspiration phase of the animal (see Fig. 2). Possible functional significance of the gamma activity in the olfactory bulb has been extensively investigated by the use of multiple intracerebral (Bressler, 1988) and pial recordings (Freeman, 1978; Freeman & Schneider, 1982; Viana Di Prisco & Freeman, 1985; Gray & Skinner, 1988). Interestingly, in each animal, the distribution of the amplitude of the gamma burst activity forms a stable map at the surface of the OB even in the absence of any olfactory stimulus. An odour presentation modifies this distribution but only if the odour has acquired a given significance following conditioning. Thus, changes in gamma bursts amplitude reflect more the behavioural significance of the stimulus rather than its chemical quality.

More recently, in mammals, odour sampling was also found to be associated with beta-frequency (15–35 Hz) activity in OB, piriform cortex and lateral entorhinal cortex (Boeijinga & Lopes da Silva, 1989; Zibrowski & Vanderwolf, 1997; Chapman et al., 1998). More or less prominent increase in the amplitude of this oscillatory activity was observed for odours behaviourally relevant (Zibrowski & Vanderwolf, 1997; Chabaud *et al.*, 2000), experimentally associated with a reward (Boeijinga & Lopes da Silva, 1989) or following repeated presentations of the same odour (Gray & Skinner, 1988; Chabaud *et al.*, 2000; Vanderwolf & Zibrowski, 2001). Thus, similar to gamma-band responses to odours, beta band responses seem related both to perception and some aspects of memory processes. However, studies devoted to understand the role of beta and gamma activity in the olfactory

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system have so far been carried out separately in different species and different experimental contexts. Consequently, no data is available on the way they could evolve in parallel in the context of perception and memory. We addressed this question in the OB using recordings of local field potential (LFP) activities in rats engaged in an olfactory discrimination learning task.

## Materials and methods

### *Subjects, surgery and histology*

Experiments were carried out in accordance with the European guidelines regarding care and use of animals for experimental procedures. Ten male Wistar rats (280–300 g) purchased from IFFA-CREDO (L'Arbresle, France) were implanted under anaesthesia with equithesin (a mixture of chloral hydrate and sodium pentobarbital, 3 mL/kg, i.p.) with one monopolar (80 µm, 100–500 kΩ) recording electrode in the output cell body layer of the OB (mitral cells layer) (antero-posterior −7 mm relative to the nasal suture; medio-lateral relative to the medial suture +1.5 mm). The depth of the electrode was adjusted at the level of the ventral mitral cell layer using electrophysiological monitoring of the characteristic large multiunit mitral cell activity (Pager, 1974a,b). The reference electrode was positioned in the contralateral skull bone. The electrodes were connected to a miniature socket fixed onto the rat's head by dental cement. Two weeks of recovery separated surgery from recordings. At the end of the experiment the rats were injected with a lethal dose of pentobarbital and electrocoagulation was performed (1 mA, 10 s) at each electrode. Then the animals were perfused intracardially with a 0.9% saline solution followed by a 10% formalin solution. The brains were dissected and stored in formalin for 1 week after which they were cut into 40-µm slices and stained with cresyl violet. For each rat, the position of each recording electrode was verified. All implanted electrodes were in the intended position, in or at vicinity of the mitral cell body layer

### *Behavioural apparatus*

Training, testing and recording were all performed in a rectangular arena (40 × 63 cm). One wall of the cage, in its width axis, was equipped with an odour port and the opposite wall with a food tray. Photodetectors mounted inside of the odour port monitored nose pokes (odour detector) and those mounted on the sides of the arena on its longitudinal axis, 40-cm away from the odour port, detected animal's passage at this point (behavioural response detector).

Detection of animal's nose into the odour port triggered a signal for odour delivery. Deodorised air constantly flowed through the odour port. Following nose poke detection, odorised air was added to the main flow for a 3-s period. This was controlled through the opening and closing of solenoid valves on a two-channel air-diluted flow-olfactometer. A vacuum system ensured the odour remained confined to the port. However, within this 3-s period, the animal itself determined the duration of odour sampling.

The odours used were eugenol and geraniol (Sigma–Aldrich, France). They were obtained from evaporation of pure compounds from saturated granules. At the beginning of the experiment, the percentage of saturated vapor pressure introduced in the airflow was adjusted for each compound to be judged moderate and balanced by the experimenters. The values were 53% for eugenol and 44% for geraniol. Spontaneous sampling duration of each compound was also studied on a group of rats to confirm that the concentration of each odour determined in that way was associated with a similar spontaneous sampling duration. The food tray delivered standard food-pellets (40 mg) (Medical Associates Inc., Georgia, Vermont).

### *Behavioural procedures*

Food-deprived animals were engaged in a two-odour discrimination task. The animals were divided in two groups of five rats according to the value assigned to each odour. For group A, the animals learnt to go immediately (Go response) to the food tray following sampling of geraniol odour ($S^+$) and to stay nearby (No go response) when the odour sampled was eugenol ($S^-$). Correct Go responses were reinforced with one food pellet. For group B, odours contingency was inverted: eugenol became the $S^+$ and geraniol the $S^-$. Each daily session included from 20 to 30 trials (50% $S^+$/50% $S^-$ randomly) with a 1-min intertrial interval. Each trial was initiated by the detection of a nose poke into the odour port. There was no sound or light signal indicating to the animal the onset of the trial. For each trial, the duration of odour sampling was determined by the animal own choice providing that it lasted for more than 100 ms. For each trial, the behavioural response latency was quantified as the time elapsed between interruption of the photobeam at the odour port entry and the one 40 cm away. Due to different rat behavioural strategies in solving the task, individual criteria were defined for determination of Go and No go response. For each rat and for each training session, the average ± SD values for the 10 $S^+$/Go responses was computed. For the other 10 trials, the behavioural response was classified as a No go response when the response latency was greater than the average Go value ±two SDs. Otherwise the response was classified as an incorrect Go response. Go response to $S^+$ and No go response to $S^-$ were considered as correct choices. The learning criteria were fixed at 80% of correct choices on two consecutive sessions including at least 70% of correct No go responses.

### *Electrophysiological recordings and data analysis*

The whole experimental arena was placed in a Faraday cage. Neural activity was acquired through unitary gain field effect transistors in the headstage of the recording cable to reduce movement artefacts. The cable was connected to a swivelling electrical connector that allowed free movements. The LFP signals together with events markers (nose poke in and out, behavioural response) were amplified, filtered (0.1–300 Hz), digitised (sampling frequency 2000 Hz) (Wavebook 512, Iotech, Inc., Cleveland, OH, USA) and stored on a computer. For each rat, recordings were obtained on the first 20–30 trials of training (beginners) and several days later following learning to the criterion (experts). In each rat, sessions selected for the expert condition corresponded to the first two consecutive sessions for which the learning criterion was achieved.

Signal analysis was carried out off-line by the use of two time-frequency methods. Morlet wavelet analysis was applied for signal analysis between 4 and 110 Hz in order to define precisely and without any *a priori* limits of the frequency band of interest (Tallon-Baudry & Bertrand, 1999). For each single trial, the signal was analysed in the time-frequency domain by convolution with complex gaussian Morlet's wavelets with a ratio *f*/*sf* of 7, the frequency ranging from 8 to 80 Hz in 1-Hz steps (Tallon-Baudry & Bertrand, 1999). At 8 Hz, this leads to a wavelet duration (2 st) of 278 ms and to a spectral bandwidth (2 sf) of 2.3 Hz; at 20 Hz, to a wavelet duration of 111.4 ms and to a spectral bandwidth of 5.8 Hz, and at 100 Hz, to a duration of 22.2 ms and a bandwidth of 28.6 Hz. The time resolution of this method thus increases with frequency.

In order to capture transient changes of power over time, a time-varying fast Fourier transform was applied using the Welch spectral estimation method (Welch, 1967). Power spectra were computed on 150 ms time window sliding by step of 50 ms. For each trial, significant changes in power signal were computed for three periods: (1) 500 ms

before odour onset (pre-period, pre); (2) the variable duration period detected by photodetectors activation which corresponds to the presence of the rat's nose inside the odourised port (odour-period, odour); and (3) one period of 500 ms following the end of the detection period (post-period, post). Some trials were excluded from the analysis according to the following criteria: odour sampling lasting for less than 100 ms or longer than 2 s, presence of any artefact on neural signals. Power baseline was calculated on 1 s of activity starting 2 s before odour onset. For each time window within the three periods, threshold for significant changes was the mean of the logarithmic value of the power during baseline ±two SDs. For each period, the occurrence rate of response was defined as the ratio of number of 150 ms time windows where significant changes occurred divided by the total number of time windows during this period. Thus the occurrence rate varied from 0 to 1 and was proportional to the whole duration of power variation within the period whatever the duration of odour sampling.

Finally, odour-induced changes were quantified in terms of amplitude, frequency and latency. Changes in amplitude during the odour period were expressed relative to the one in the pre-period. This was done using data provided by the time-varying FFT. The main frequency and precise latency of the maximum of the oscillatory burst were extracted by the use of the Wavelet analysis during the odour period of each trial.

### Statistical analysis

Comparisons of occurrence rate were performed using a four-factor ANOVA: three independent factors, the odour (eugenol vs geraniol), its behavioural valence ($S^+$ or $S^-$) and the level of training (beginners vs experts) and one paired factor, the period factor (pre, odour, post). Significant factor or interaction was then analysed by corresponding tests. Wilcoxon test was used for comparison of variable obtained from different periods of time. It allowed for example to determine whether an increase or a decrease in occurrence rate observed during the odour-period was significant compared to the pre-period. For other comparisons *t*-tests or $\chi^2$ tests were used.

## Results

### Behavioural performance

Ten rats were engaged in the olfactory discrimination task. They were separated in two groups (group A and group B, five rats each), according to the specific association between the odour and the expected behavioural response. Group A: geraniol $S^+$/eugenol $S^-$ vs group B: eugenol $S^+$/geraniol $S^-$. Only three rats in each group were kept for the analysis according to the level of behavioural performance reached and their stability together with the quality of the electrophysiological signals. In both the 'beginner' and the 'well-trained' conditions, animals approached the port in active sniffing which started well before the beginning of the 500-ms pre-odor period. According to animal's behaviour, three stages were observed: during the 'beginner' phase (first two sessions), the animal did not make a relation between odour sampling and the presence or absence of food reinforcement. Consequently, response latencies were high and similar for both odours. The second phase (corresponding to sessions 3–16 of Fig. 1) was characterised by low latencies of Go responses for both odours. The animal has made the link between odour sampling and the possibility to receive a food pellet. However, behavioural responses were the same following sampling $S^+$ and $S^-$ stimuli. In the last phase (session 17 to the end), the two latency curves began to diverge. The animal exhibited a differential behavioural response to the two stimuli: it went on running very fast to the food tray (Go response) in response to the $S^+$ and slowed down its motor behaviour or even inhibit it (No go response) after sampling $S.^-$ As it can be seen in Fig. 1, the average number of trials to reach the criterion was significantly higher for the version of the task used in the group B ($n = 628 \pm 136$) than for the one used in the group A ($n = 312 \pm 67$) ($F_{1,4} = 13.074$, $p < 0.05$). However, this difference was related neither to significant different sampling duration nor to changes in the motor response latencies. First, regarding sampling duration, absence of significant differences between eugenol and geraniol in group A and in group B allowed us to pool data from the two odourants within each group. Second, further analysis revealed no significant intergroup difference. However, within each group the average sampling duration decreased significantly from the beginner condition (730 ms, Fig. 1, left inset) to the expert condition (550 ms, Fig. 1, right inset) ($F_{1,399} = 14.47$, $p < 0.001$).

### Electrophysiological data

#### *Qualitative aspects and determination of frequency bands of interest*

In beginners, 108 trials were included for signal analysis. In experts, only trials with a correct Go or No go behavioural response ($n = 297$ trials) were taken into account. A small number of incorrect responses precluded analysis of corresponding neural signals. During spontaneous activity, OB signal presented a marked fast bursting activity appearing within each respiratory cycle (Fig. 2.1). Odour sampling was always characterised by the reduction in power signal in the fast activity, in parallel with the emergence of a much slower oscillation. Such changes were visible on single trials (Fig. 2.2). Wavelet analysis performed in a large number of trials in beginners and experts revealed that the fast activity ranged from 60 to 90 Hz (gamma band) while the slower one ranged from 15 to 40 Hz (beta band).

#### *Odor-induced changes in gamma and beta frequency bands*

This part concerns analysis of significant changes in power estimated by the calculation of the occurrence rates during the three previously defined periods: pre, odour and post. Data are first presented in the gamma band and then in the beta band.

In the gamma band (Fig. 3) statistical analysis revealed no significant difference between the two experimental groups of rats (group A vs group B) Indeed, there was no difference between them whether considering the period factor (pre, odor, and post) or the level of expertise (beginners vs experts). Thus, within each recording condition, data from the two groups were pooled. In beginners (Fig. 3a), sampling $S^+$ or $S^-$ was associated with a significant depression in gamma activity during the odour-period and the post-period. In addition, there was a significant interaction between the factor period and the behavioural valence associated with the odour ($F_{2,208} = 4.269$, $p < 0.05$). This effect consisted in a more important rate of significant reduction in signal power in response to $S^+$ than to $S^-$ both during the odour-period (*t*-test, $p < 0.05$) and during the post-period (*t*-test, $p < 0.005$). In experts (Fig. 3b), a more pronounced interaction between the period factor and the behavioural valence of the odour was obtained ($F_{2,590} = 21.789$; $p < 0.001$). The depression effect was present during both odour and post-periods (pre/odour, pre/post, $p < 0.001$). Interestingly, during the odour-period the rate of occurrence of significant reduction in power was no more different between $S^+$ and $S^-$. However, in the post-period, a difference similar to the one observed in beginners was maintained, with values higher for $S^+$ than $S^-$ ($p < 0.001$). More importantly, in experts, depression in gamma activity was more pronounced than the one observed in beginners for all periods (Significant interaction between time-period and level

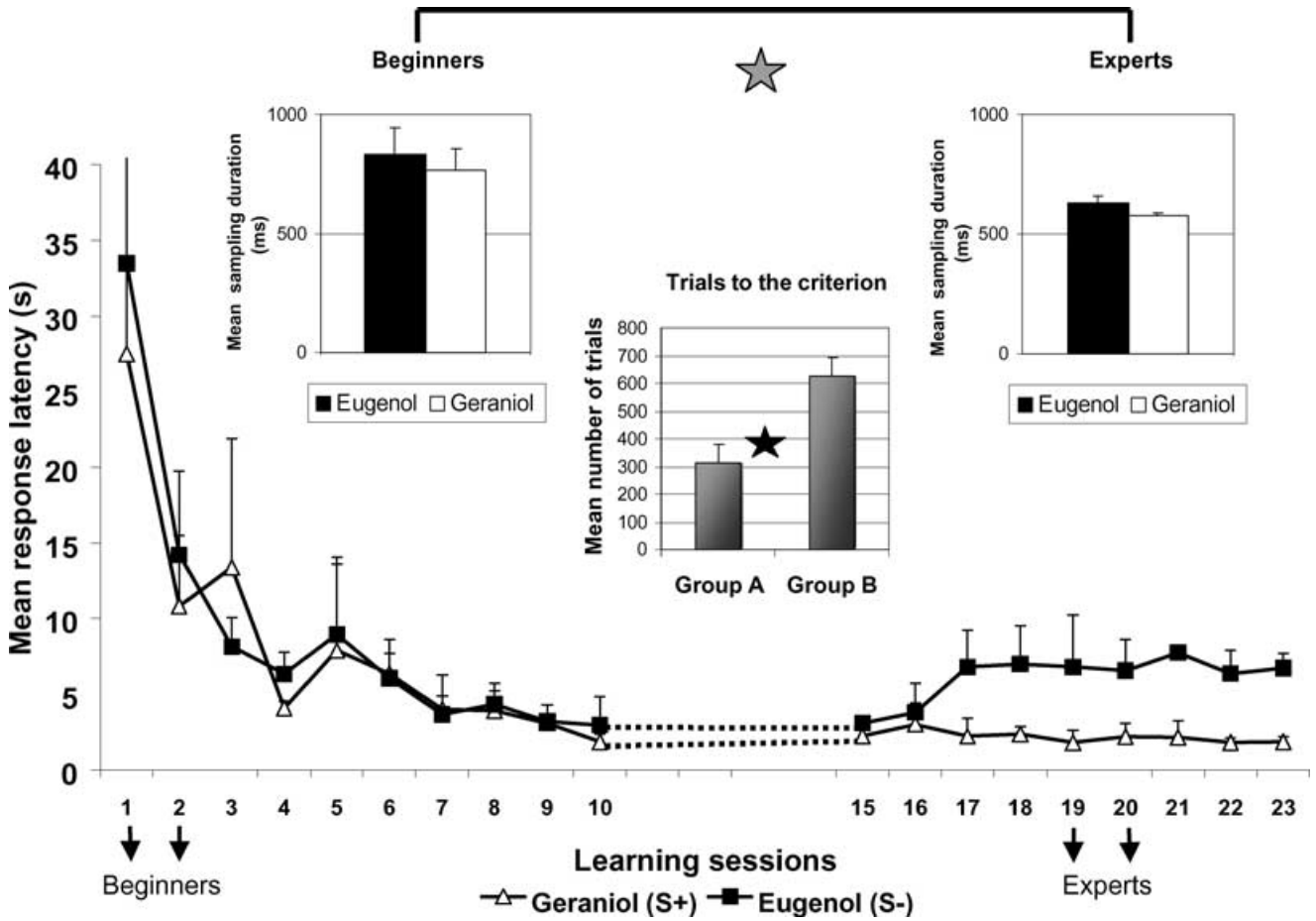



FIG. 1. Behavioural data. Mean motor response latencies (in s) in the group A following olfactory sampling of geraniol ($S^+$, white triangles) and eugenol ($S^-$, black squares) along daily training sessions. Group B (eugenol $S^+$ vs geraniol $S^-$) displayed a similar evolution in performances except that the learning criterion was reached on day 29 only. This difference was due to an increase in number of sessions (figured out by the dotted lines) for which the animal produced a Go response irrespective to what odour has been presented. The lower central inset presents the mean ± SEM number of trials to the criterion in the groups A and B ($^*p < 0.05$). Arrows under day 1, 2 and 19, 20 point to the sessions from which recordings were performed in beginners and in experts, respectively.The upper inset figures show values of mean ± SEM sampling duration (in ms) of each odourant in the beginner and in the expert conditions. The grey star indicates a significant ($p < 0.001$) reduction in sampling duration from beginners to experts.

$F_{2,802} = 20.858$, $p < 0.001$). This effect was not odour-induced as it was already observed on the pre-period (beginners/experts pre-period, $p < 0.001$).

Considering that the average duration of odour sampling lasted for 730 ms in beginner and for 550 ms in experts, the average duration of significant depression in gamma activity during the odour-period corresponds to 306 ms (occurrence rate $0.42 \times 730$ ms) and to 264 ms (occurrence rate $0.48 \times 550$ ms), respectively.

In the beta band (Fig. 4) and in beginners (Fig. 4a), statistics allowed to pool data of the occurrence rates from groups A and B. In contrast to what was observed in the gamma band, changes in power in the beta band were always expressed as increase in signal power. The ANOVA showed that values in occurrence rate were significantly increased, during the odour-period, whatever its chemical nature or its association to a defined reward ($F_{2,208} = 9.789$, $p < 0.001$; pre/odour, $p < 0.001$). In the expert condition, an obvious important increase in beta oscillation was observed in almost all-individual raw traces (Fig. 2.2). However, it was not possible to pool data from the two groups because the ANOVA revealed a significant interaction between the group factor and the period factor ($F_{2,586} = 3.534$, $p < 0.05$). This indicated that the occurrence rate of response within the odour-period or/and within the post-period differed among the two groups. One reason could be that the response to an odour differed according to its behavioural valence. Thus, we analysed the data for each odour separately to evaluate the impact of their behavioural valence. In the case of eugenol, sampling caused an increase in beta oscillation power restricted to the odour-period ($F_{2,266} = 75.051$, $p < 0.001$, pre-period vs odour-period, $p < 0.001$). This was true whatever eugenol was assigned as $S^+$ (group A) or as $S^-$ (group B) (Fig. 4b,c). In the case of geraniol, the analysis revealed a significant interaction between the period factor and the behavioural valence ($S^+$ or $S^-$) ($F_{2,320} = 9.626$, $p < 0.001$). When geraniol was $S^+$, the significant increase in occurrence rate found during the odour-period was still present during the post-period. (pre/odour, pre/post1 and odour/post1, $p < 0.001$) (Fig. 4b). This result was confirmed by the separate analysis of the response according to the valence. When considering Go response to $S^+$, there was a significant interaction between the period factor and the odour factor ($F_{2,320} = 4.314$, $p < 0.05$). This was due to a significant higher rate of response to geraniol when compared with eugenol (*t*-test, $p < 0.05$). In contrast, no odour difference was detected in the case of $S^-$.

Due to the group effect in expert animals, the impact of training on occurrence rate for each three periods of analysis was tested within

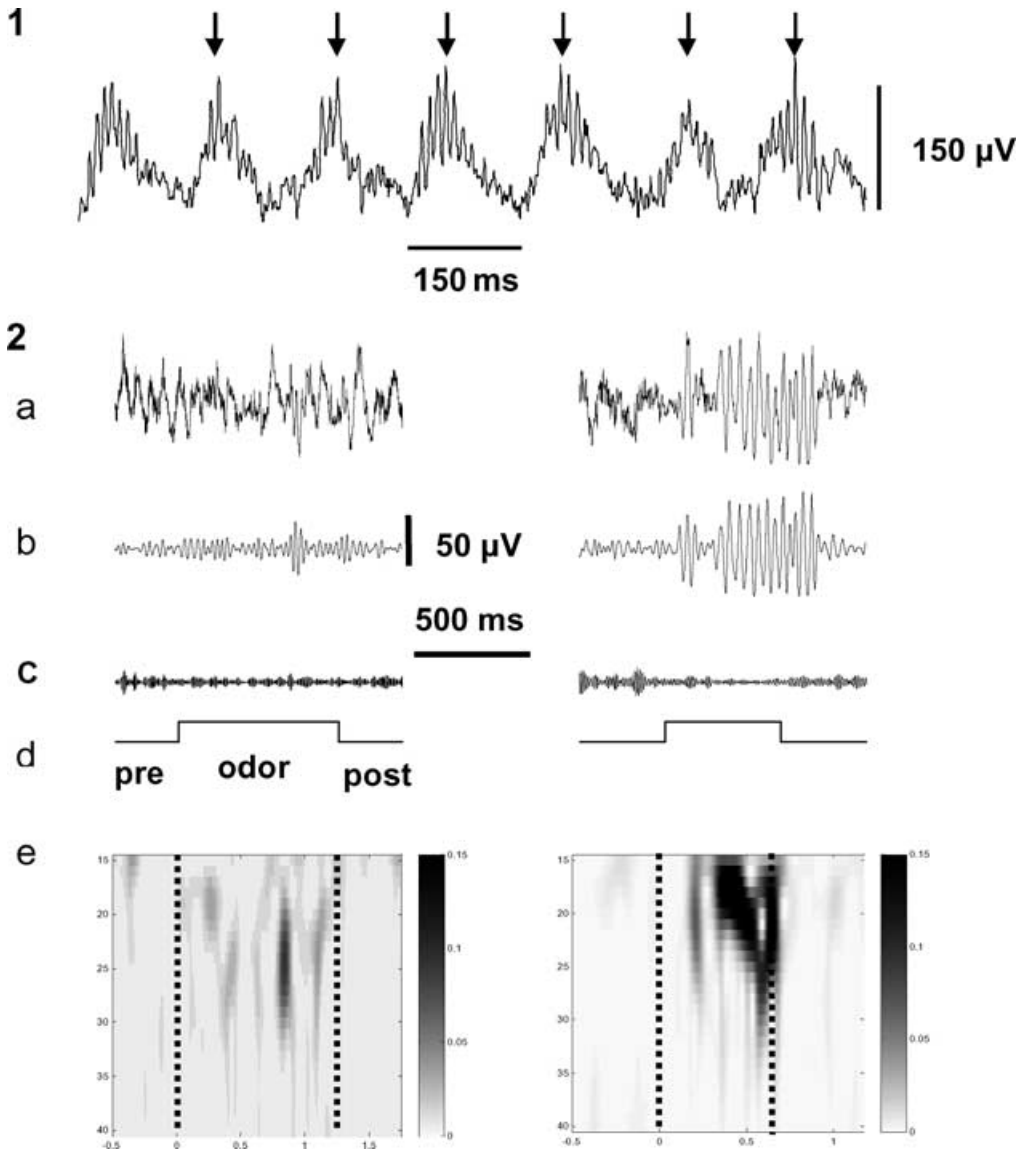



FIG. 2. Example of local field potentials recorded from the same animal in the two different conditions. (1) During spontaneous activity. Raw trace (1–100 Hz). Arrows point at bursts of gamma activity present at each respiratory cycle. The horizontal bar indicates the duration of one cycle. (2) During odour sampling in the beginner (left column) and in the expert (right column) conditions. (a) raw signals (1–100 Hz); (b) filtered signals in the beta band (15 and 40 Hz); (c) filtered signals in the gamma band (60–90 Hz) (d) succession of the three periods: 500 ms preodor, odour and 500 ms postodor-period; (e) corresponding wavelet transform in the beta band. *x*-axis: time in seconds, *y*-axis: frequency from 15 to 40 Hz from top to bottom. Grey colour code expresses signal power (µV2) within values indicated in the grey bar. Same time scale from a to e. Same amplitude scale from a to c.

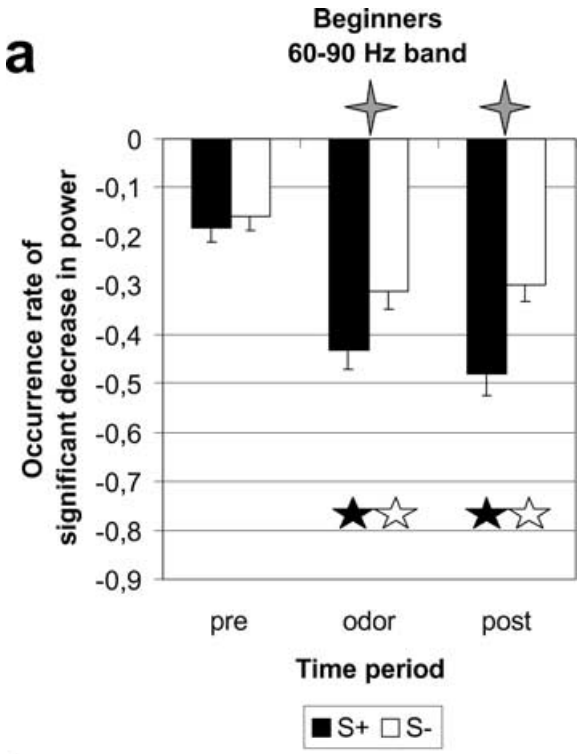
each experimental group. There was no significant effect during the pre-period. In contrast, learning modified the response in the odour and in the post-period. In the group A (Geraniol $S^+$/Eugenol $S^-$), there was a significant interaction between the level of expertise and the period factor (Fig. 4b) ($F_{2,514} = 13.044$, $p < 0.001$). This indicated that, according to these two factors, learning did not enhance response rate in a homogeneous manner. Passage from the beginners condition to the experts condition increased occurrence rate restricted to the odour-period for eugenol (*t*-test, $p < 0.005$) whereas for geraniol, it was significant for both periods (*t*-test, odour, $p < 0.001$; post1, $p < 0.01$) (Fig. 4a vs 4b). In the group B (Eugenol $S^+$/Geraniol $S^-$) the effect of expertise factor was restricted to eugenol ($F_{2,134} = 3.563$, $p < 0.05$) and concerned exclusively the odour-period (*t*-test, $p < 0.05$). For geraniol, there was no effect of learning (Fig. 4a vs 4c).

Considering average values in odour sampling and corresponding occurrence rates, duration of beta band response in the odour-period was 182 ms in beginners. In experts, this value varied from 176 ms for geraniol in group B to 319 ms for geraniol in group A.

A final set of analysis focused on characterization of the beta response in terms of frequency, latency and amplitude.

### *Learning-related changes in the beta band response characteristics*

During the odour period, beta band responses were also quantified in term of amplitude and frequency. For each trial, this was done both in beginners and experts conditions. Changes in amplitude were expressed as the ratio of average power for 150 ms time windows showing significant increase over the average power in the pre-period.



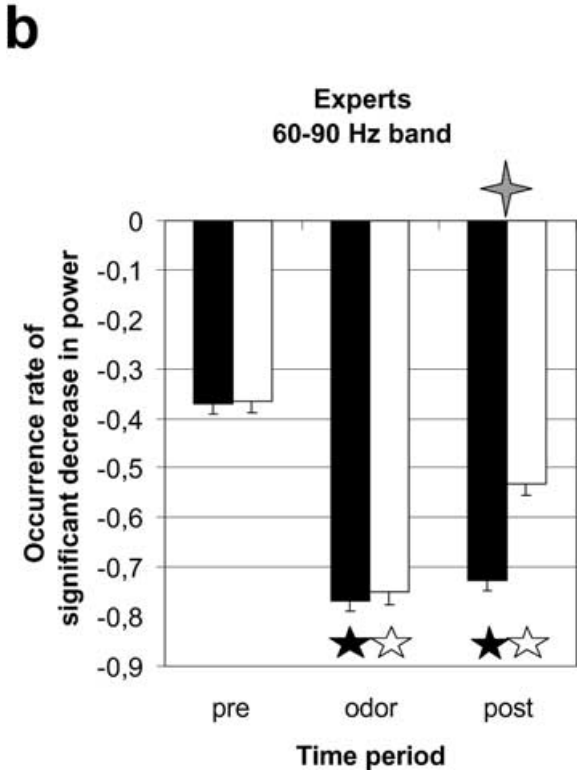




FIG. 3. Mean occurrence rate of significant decrease in power in the 60–90 Hz band. In each case, mean (± SEM) of occurrence rate in depression in signal power is symbolised by down-orientated bar during the three periods of signal analysis. Top panel: in beginners; bottom panel: in experts. Black and white stars indicate significant differences compared to the pre-period (at least $\imath_{\bullet} < 0.05$) in response to $S^+$ and to $S^-$, respectively. Grey stars indicate a significant difference between the two odours within the same period ($\imath_{\bullet} < 0.05$). Data have been collected from six rats.

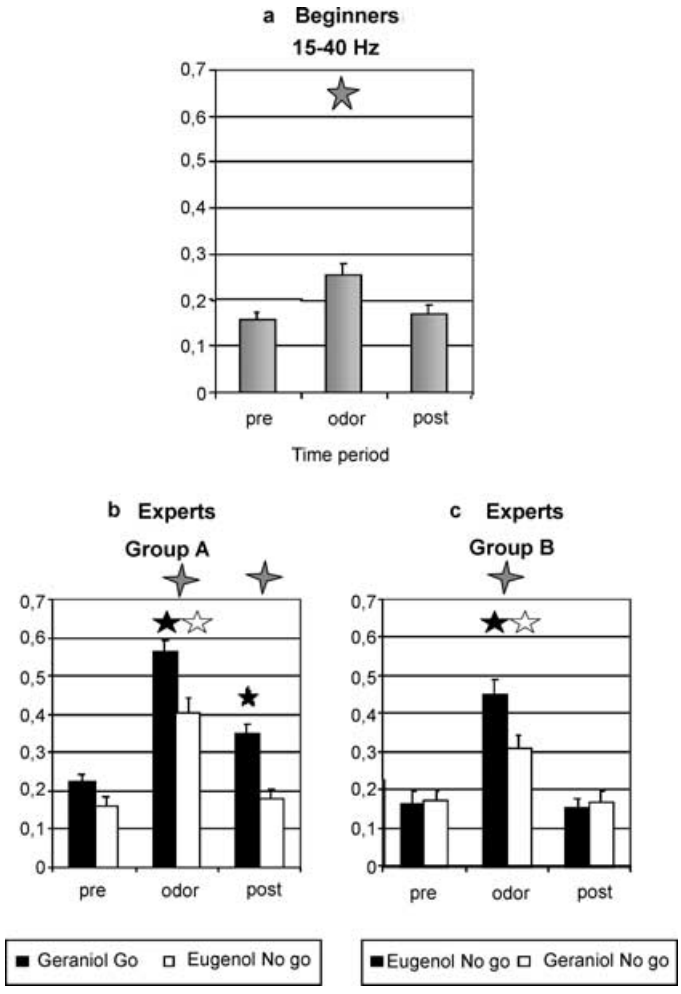



FIG. 4. Mean occurrence rate of significant increase in power in the 15–40 Hz band. In each case, mean (± SEM) of occurrence rate in increase in signal power is symbolised by up-orientated bar during the three periods of signal analysis. (a) In beginners for which data from the two odours, groups A and B, $S^+$ and $S^-$ trials are pooled ($n = 108$ trials). The grey star indicates a significant increase in occurrence rate during the odour-period vs the pre-period ($\imath_{\bullet} < 0.05$). (b) In group A, expert animals (Geraniol, $S^+$ black bars $n = 115$, Eugenol, $S^-$, white bars, $n = 88$). (c) In Group B expert animals (Eugenol, $S^+$ black bars $n = 47$, Geraniol, $S^-$, white bars, $n = 47$). In both (b) and (c), black and white stars indicate significant difference relative to the pre-period for the $S^+$and the $S^-$, respectively (at least $\imath_{\bullet} < 0.05$). Grey stars on the top of the figure indicate a significant difference in the rate of response between the two odours ($\imath_{\bullet} < 0.05$). Data have been collected from six rats.

Figure 5 summarised the results. While odour sampling induced significant increase in power, no difference was observed neither in beginners, nor in experts, related to the chemical nature or the valence of the stimuli. The only significant factor was the level of expertise ($F_{1,349} = 28.699$, $\imath_{\bullet} < 0.001$). Indeed, in beginners, amplitude of beta oscillation increased by a 1.6 factor, while this value reached 3.6 in experts ($F_{1,349} = 28.699$, $\imath_{\bullet} < 0.001$).

For each burst of activity with significant increase in the 15–40 Hz band, the frequency at the maximum power was extracted from the wavelet analysis. As it was the case for the amplitude, statistical analysis revealed no significant effect of the following factors: group (A vs B) odour quality (eugenol vs geraniol) odour valence ($S^+$ or $S^-$). The only significant factor of variation was the level of expertise ($F_{1,675} = 22.027$, $\imath_{\bullet} < 0.001$). Accordingly, data were pooled within

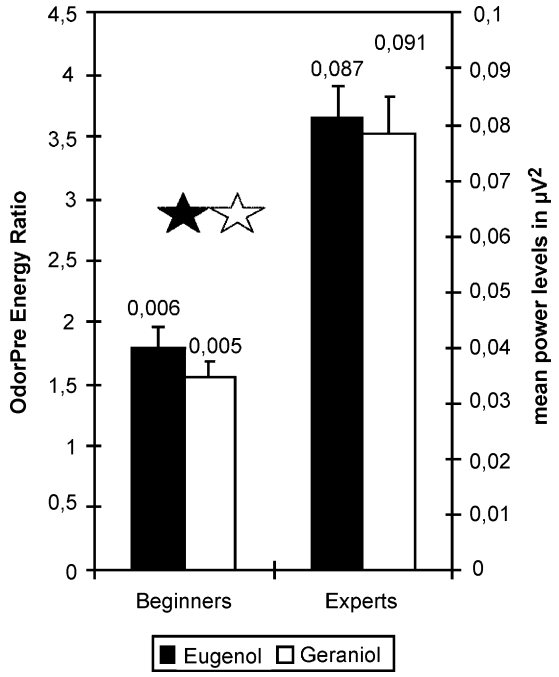



FIG. 5. Training-related changes in amplitude in the beta band response. Each bar expresses the average (± SEM) ratio in power signal during the odour-period over the one during the pre-period in response to eugenol and geraniol (black and white bars, respectively) (left scale). All trials have been pooled for each odour. In the beginner condition power increased by less than a twofold factor which did not differed between the two odours (geraniol, $n = 56$ trials; eugenol, $n = 52$ trials). In the expert condition, values reached a 3.6-fold factor for both odours (geraniol, $n = 162$ trials; eugenol, $n = 135$ trials) with in each case a significant difference compared to the beginner condition bars (Black star and white star, eugenol and geraniol, respectively, $p < 0.05$). The value on top of each bar indicates the mean power level of signals (µV2) during the odour period (right scale).

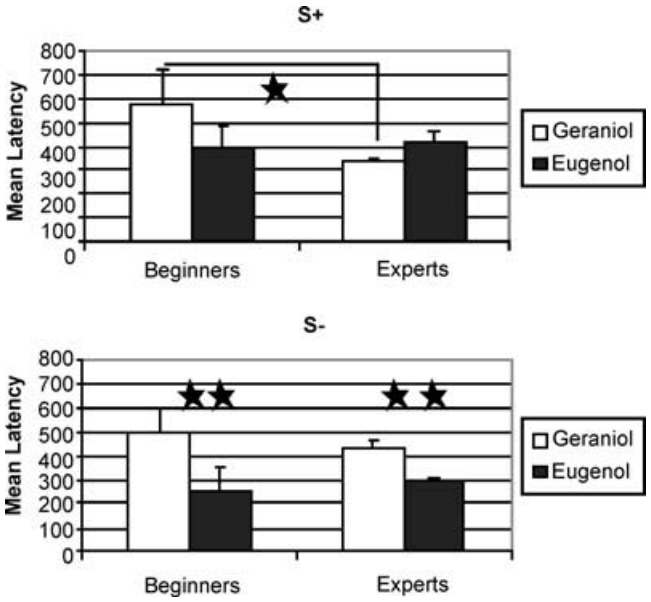



FIG. 6. Training-related changes in the latency of the beta band response. Upper graph: results obtained in the case of a reinforced odour ($S^+$). Lower graph: results obtained in the case of a nonreinforced odour ($S^-$). In each case latency values are expressed as the time of occurrence (in ms) of the maximum power of the signal in the beginners and in the experts conditions during sampling of geraniol (white bars) and eugenol (black bars). ($^*p < 0.02$; $^{**}p < 0.005$).

each level of expertise. In beginners, the frequency of the beta oscillation was around 27 Hz. In experts, this value significantly decreased to 24 Hz. This slight but very significant change was confirmed by the study of the distribution of values within the 15–40 Hz band by bin of 4 Hz each. The distribution and corresponding median value were then statistically compared by $\chi^2$ and Fisher tests. These analyses revealed a significant difference between beginners and experts ($\chi^2 = 39.31$, d.f. $= 4$, $p < 0.05$). The median value shifted from 26 to 24Hz. This difference in distribution was mainly due to an increase in experts in the burst number with maximum power between 15 and 24 Hz and a decrease of this value between 25 and 34 Hz.

The analysis of the latency of the maximum power of the beta burst (Fig. 6) revealed a significant effect of the chemical nature of the odour ($F_{1,272} = 8.314$, $p < 0.005$) and an interaction between the level of expertise and the nature of the odour ($F_{1,272} = 5.160$, $p < 0.05$). In beginners, the odour effect was observed but did not reach significance ($F_{1,52} = 2.965$, $p < 0.1$). In experts, there was a strong interaction between the nature of the odour and the associated valence ($F_{1,220} = 14.903$, $p < 0.0001$). Indeed, values in responses to $S^+$ vs $S^-$ led to statistically different results. In the case of response to $S^-$, latencies were not modified for any of the two odours in the course of learning. The only significant difference in that situation was a shorter latency of the response when eugenol was defined as the $S^-$ whatever was the level of expertise (odour effect $F_{1,110} = 9.561$, $p < 0.005$). In the $S^+$ situation, the analysis revealed a strong interaction between the level of expertise and the nature of the odour ($F_{1,162} = 5.717$, $p < 0.02$). This effect was due to a significant decrease in latency for geraniol selectively ($p < 0.02$) whereas no change was observed in response to eugenol.

To summarise, following training, the odour-induced beta oscillatory activity was significantly amplified by a twofold factor in power and its frequency decreased slightly but significantly. These effects were observed irrespective of the chemical nature of the odour and its valence. In the same time, however, the time course of this beta oscillatory response changed selectively in response to geraniol and only if this odour was used as $S^+$

## Discussion

This study describes, for the first time in parallel, two ranges of oscillatory activities observed at the OB level in response to odour sampling in freely behaving rats. Dominant gamma activity in resting activity shifted during odour sampling towards slower beta activity. This was observed when animals were 'naive' towards stimuli although learning the olfactory discrimination task amplified this pattern of oscillatory dynamics. Interestingly, at the level of piriform and entorhinal cortices a shift within the gamma band (35–85 Hz) from high to low frequency values was also observed following conditioning or repeated electrical stimulation of the lateral olfactory tract (Freeman, 1962; Bressler, 1988). Interestingly, the depression in gamma oscillations, which was initiated by olfactory sampling in beginners now developed within the 500-m period preceding odour onset. In addition, learning was associated with emergence of a very clear oscillation in the beta band around 24 Hz, which appeared during olfactory sampling and in some cases extended to the post-period. Following learning, odour-induced beta oscillatory activity increased in amplitude and its time-course changed specifically when geraniol was assigned as $S^+$.

### Gamma band response

In the present study, odour sampling induced a decrease in power in the gamma band. This type of depression in the high gamma band has also been observed at the level of the OB using different sets of odours in rabbit (Freeman & Schneider, 1982; Viana Di Prisco & Freeman, 1985; Gray & Skinner, 1988) and in rat (Kay & Freeman, 1998). Our results support the hypothesis previously suggested that changes in bulbar gamma activity is highly modulated by the behavioural context of the experiment (Freeman, 1978; Freeman & Schneider, 1982; Viana Di Prisco & Freeman, 1985). Indeed, in both beginners and experts, patterns of gamma response did not depend on the chemical nature of the stimuli. In experts, the occurrence rate of depression response was amplified by nearly 60% for both $S^+$ and $S^-$ odours relative to what was observed in beginners. Behavioural performances were much more reliable in experts than in beginners despite a 25% reduction in odour sampling duration. So, one can hypothesis that a marked and even short ($\approx$ 260 ms) depression in high gamma oscillations is of functional importance for information processing. Whether the depression is functionally related to attention process, olfactory discrimination or to sensory-motor coupling is still unknown. The fact that in experts, significant depression in gamma activity can be observed before odour onset while the animal was approaching the odour port is in accordance with the attention hypothesis. Such a phenomenon could be related to the expression of top-down influences through temporal organization of ongoing activity (Engel *et al.*, 2001). Modulations of OB gamma activity are mediated by action exerted by neuromodulatory systems (Gray *et al.*, 1986; Gervais *et al.*, 1990) and feedback from the piriform and the entorhinal cortices (Haberly & Price, 1978). Recent findings showing early neural correlates of acquisition of a Go/No go olfactory task at the level of the baso–lateral amygdala (Schoenbaum *et al.*, 1999), suggested that this structure could also play a role in the expression of the gamma depression in beginners. Indeed, we showed that in beginners the occurrence rate of gamma depression was significantly higher in $S^+$ trials than in $S^-$ trials even though animal's performances were far from reaching the learning criteria.

### Beta band response

In beginners, odour sampling was associated with a slight but significant increase in power in the beta band. This response is amplified by a twofold factor following learning. The beta band response is unlikely due to sniffing itself. Indeed, in contrast to the gamma response, beta oscillation was never observed before the animal sampled the odour and emerged systematically a few hundred ms following port entry. In addition, port approach was always associated with active sniffing while power in the beta band was unchanged.

The beta oscillations can be induced by different odourants such as pure chemical compounds like eugenol and geraniol (Chapman *et al.*, 1998; this study) as well as more complex and natural compounds (Boeijinga & Lopes da Silva, 1989; Zibrowski & Vanderwolf, 1997). The present study revealed the modulation of the response according to the chemical nature of the stimuli or/and its behavioural significance similarly to what was obtained by the use of single cell mitral cell recordings in behaving rats (Pager, 1983; Kay & Laurent, 1999).

The origin of beta oscillations at the OB level is still uncertain. However, some sets of data bring evidence for the involvement of modulatory actions exerted from the olfactory epithelium and from central structures together with intrabulbar generators. Odor-induced oscillations similar to those recorded in this study have been described in several species, both in the olfactory epithelium and in the OB (Dorries & Kauer, 2000; for a review, Nikonov *et al.*, 2002). The results of Kauer's group established a clear relationship between oscillations observed in the two structures. Odor-induced oscillations in the olfactory epithelium were still elicited following its isolation from the bulb by the section of the olfactory nerve. This indicates that, at least in Salamander, some oscillatory activity can be generated at the periphery and thus might modulate or even drive OB oscillations. However, in mammals, evidence for an epithelial generator are very weak (Nikonov *et al.*, 2002) and is hardly compatible to learning associated modulation we have observed. Indeed, learning modulation of OB response required top-down influences exerted through action of centrifugal fibers (Pager, 1974a, 1974b, 1978; Gervais & Pager, 1983). In freely behaving animals, modulation of OB beta oscillations could originate from piriform and entorhinal cortices (Boeijinga & Lopes da Silva, 1989; Kay *et al.*, 1996; Kay & Freeman, 1998; Chabaud *et al.*, 2000). Although OB activities are under centrifugal influences, the OB circuitry has the potency for generation of the whole range of oscillatory regimes (Freeman, 1975; Li & Hopfield, 1989; Eeckman & Freeman, 1990). More recently, intracellular recordings on OB slices showed that the beta oscillations could originate at least partly from mitral cells. Indeed, mitral cells display subthreshold oscillations of their membrane potential in the beta range (between 20 and 40 Hz) (Chen & Shepherd, 1997). Moreover, the frequency of these oscillations depended on the membrane potential, and reached 20 Hz when the membrane potential approaches the threshold for spikes triggering (Desmaisons *et al.*, 1999). In this perspective, the odour-induced phenomenon near 24–27 Hz we have observed could reflect synchronous oscillations in membrane potential of spiking and nonspiking mitral cells.

### Training-related changes of the beta band responses and possible functional significance

Training was associated with a global twofold factor increase in both amplitude and duration in the odour-induced oscillation in the beta band. This is unlikely due to the sole repeated exposure to odours. Indeed in awake rabbit 30 presentations of the same unreinforced odour over several days led to no change in amplitude and frequency in bulbar response in the 15–25 Hz frequency band (Gray & Skinner, 1988). In the present experiment, changes in the time-course of responses depended on the nature of the odours and their behavioural valence. This suggests that response modulation following training was due in part to associative learning itself.

Considering the hypothesis that oscillatory LFP activity reflects synchronous activation of the underlying neuronal population, amplification could be due either to a larger number of recruited neurons or to better synchronization. Recent data from insect olfactory system favour the latter hypothesis (Stopfer & Laurent, 1999).

The clear-cut odour-induced beta oscillation observed at the OB level could be functionally similar to stimulus-induced oscillatory activities already described in other sensory systems in mammals (Gray, 1994; Gray, 1999; Tallon-Baudry & Bertrand, 1999; Tallon-Baudry *et al.*, 2001) and in the insect olfactory system (Friedrich & Stopfer, 2001; Laurent *et al.*, 2001). As suggested by these authors, such coherent oscillations could facilitate transient formation of assemblies of synchronised output cell characterizing the stimulus. This is likely the case at the mammalian OB level as most odours evoked widely distributed patterns of activity (Jourdan, 1982; Royet *et al.*, 1987; Kauer & White, 2001). In addition, different compounds often activate nearby or overlapping regions (Yang *et al.*, 1998; Xu *et al.*, 2000; Friedrich & Stopfer, 2001; Kauer & White, 2001; Rubin & Katz, 2001). While optimised synchronization near 20 Hz has been shown to allow discrimination among chemically similar odours in the insect antennal lobe (Stopfer *et al.*, 1997), we here suggest that high

amplitude odour-evoked beta oscillation could facilitate olfactory recognition in freely behaving rat. In the mammalian olfactory system, most studies have focused on the functional signification of oscillations in the gamma range. The present work stressed the importance of studying the odour-induced beta activity. As a whole, the results suggest that oscillations in the gamma range (60–90 Hz) and in the beta range (15–40Hz) are expressed in a complementary mode during odour sampling: a marked depression of fast gamma activity being a prerequisite for the emergence of an oscillatory activity in the beta range correlated with odour recognition.

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