Chem. Senses 25: 561–573, 2000

Exposure to Behaviourally Relevant Odour Reveals Differential Characteristics in Rat Central Olfactory Pathways as Studied through Oscillatory Activities

P. Chabaud, N. Ravel, D.A. Wilson[1], A.M. Mouly, M. Vigouroux[2], V. Farget[2] and R. Gervais

Institut des Sciences Cognitives, CNRS-UPR 9075, 67 Boulevard Pinel, F-69675 Bron, France, [1]Department of Zoology, University of Oklahoma, Norman, OK 73019, USA and [2]Neurosciences et Systèmes Sensoriels, ERS 5020, CNRS-UCB Lyon I, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France

Correspondence to be sent to: Rémi Gervais, Institut des Sciences Cognitives, CNRS UPR 9075, 67 Boulevard Pinel, F-69675 Bron, France. e-mail: chabaud@isc.cnrs.fr

Abstract

This study investigated how changes in nutritional motivation modulate odour-related oscillatory activities at several levels of the olfactory pathway in non-trained rats. Local field potential recordings were obtained in freely moving animals in the olfactory bulb (OB), anterior and posterior parts of the piriform cortex (APC and PPC respectively) and lateral entorhinal cortex (EC). Dynamic signal analysis detected changes in power during odour presentation for several frequency bands The results showed that in most cases odour presentation was associated with changes in a wide 15–90 Hz frequency band of activity in each olfactory structure. However, nutritional state modulated initial responses to food odour (FO) in the OB and EC selectively in the 15–30 Hz frequency band. Changes in nutritional state also modulated responses to repeated FO stimuli. Habituation was expressed differentially across structures with a clear dissociation between the two parts of the piriform cortex. Finally, systemic injections of scopolamine (0.125 mg/kg) selectively blocked expression of the nutritional modulation in the OB found in the β band. These results suggest that internal state can differentially modulate odour processing among different olfactory areas and point to a cholinergic-sensitive β band oscillation during presentation of a behaviourally meaningful odorant.

Introduction

Adrian initiated investigations on how odours modulate activity in mammalian central olfactory areas (Adrian, 1950). He pointed out that the olfactory bulb (OB) displayed prominent mass oscillatory activity in both the absence and presence of odours. Those activities were expressed as oscillations of field potentials ranging from 1 to 100 Hz. These observations raised the question of the possible functional importance of mass oscillations in olfactory processing. Freeman and Schneider found that in the rabbit olfactory conditioning induced specific iso-amplitude maps of 40–80 Hz oscillatory activities recorded from the OB (Freeman and Schneider, 1982). Also, parallel local field potentials (LFP) were recorded in several olfactory areas in trained animals. In the cat (Boeijinga and Lopes da Silva, 1989) and rat (Kay and Freeman, 1998) odour sampling was associated with a widespread increase in power in the β frequency band (15–20 Hz) together with a significant reduction in a broad γ band (35–120 Hz).

The first aim of this study was to characterize in several olfactory areas the effect of behaviourally relevant odours presented to non-trained animals as initiated by Gray and Skinner (Gray and Skinner, 1988b). Odours were not presented as brief puffs in front of the animal's nose, but instead introduced into the environment. Simultaneous LFP recordings were obtained from the OB, piriform cortex (PC) and lateral entorhinal cortex (EC). In the PC, electrodes were positioned in the anterior and posterior parts. This was to test for a possible functional dissociation between these two parts as suggested by some anatomical [reviewed by (Haberly, 1985)] and optical and electrophysiological recordings (Boudreau and Freeman, 1963; Litaudon and Cattarelli, 1995; Litaudon *et al.*, 1997; Mouly *et al.*, 1998; Rosin *et al.*, 1999). The second aim was to test for involvement of cholinergic input to olfactory areas which originate from the basal cholinergic nuclei (Woolf *et al.*, 1984; Zaborsky *et al.*, 1986; Luiten *et al.*, 1987). Cholinergic modulation is indeed suspected to be important because several lines of evidence suggest its role in selective attention and in some forms of short-term memory [reviewed by Everitt and Robbins (Everitt and Robbins, 1997)]. This is likely the case for olfactory information processing. Indeed, cholinergic modulation was found to be important for normal expression of short-term olfactory memory as measured through behavioural habituation (Hunter and

Murray, 1989; Paolini and McKenzie, 1993) and in a delayed matching-to-sample task (Ravel *et al.*, 1992, 1994). In the latter case, cholinergic modulation exerted at the level of the OB seemed particularly important. Thus, there is a possible functional dissociation of the action of the cholinergic system on its different olfactory targets. Cholinergic modulation could be expressed in mass oscillatory activities as observed in other brain structures for high frequency γ oscillations (30–60 Hz) (Buzsaki and Gage, 1989; Jones, 1993; Rasmusson *et al.*, 1994; Cape and Jones, 1998) and θ rhythms (5–10 Hz) (Vertes and Kocsis, 1997; Vinogradova *et al.*, 1998). Finally, modulation of neural responses to odours of different behavioural relevance was evaluated through changes in nutritional state. Indeed, previous studies showed that at the OB level, responsiveness to food odours is modulated according to the animal's internal state (food-deprived or satiated state) through the action of centrifugal nerve fibres (Pager *et al.*, 1972; Pager, 1974, 1978). In contrast, the way this type of modulation is expressed in other olfactory areas has not been investigated so far and the identity of centrifugal fibres mediating this modulation remains unclear (Gervais and Pager, 1983; Gervais *et al.*, 1984).

Materials and methods

Animals and surgery

Five male Wistar rats (280–300 g) purchased from IFFA-CREDO (l'Arbresle, France) were used in the present experiment. For surgery, they were anaesthetized with equithesin (a mixture of chloral hydrate and sodium pentobarbital, 3 ml/kg i.p.). The level of anaesthesia was held constant with regular injections of equithesin throughout surgery (0.3 ml/h). The animals were fixed in a stereotaxic apparatus, with the head flat. Four holes were drilled in the skull for implantation of recording electrodes, respectively over the OB, the anterior part of the piriform cortex (APC), the posterior part of the piriform cortex (PPC) and the entorhinal cortex (EC). The coordinates, according to Paxinos and Watson (Paxinos and Watson, 1996), were respectively: OB, antero-posterior (AP) 6.7 mm relative to bregma, medio-lateral (ML) 1.5 mm; APC, AP 2.2 mm relative to bregma, ML 4 mm; PPC, AP –2.3 mm relative to bregma, ML 5–5.5 mm; EC, AP –6.3 mm relative to bregma, ML 6 mm. Thus, in the AP axis each electrode was at least 4 mm apart from the others. In each recorded structure the electrode tip was positioned near the output cell body layer. In the OB the depth of the electrode was adjusted at the level of the ventral mitral cell layer using electrophysiological monitoring of the characteristic large multi-unit mitral cell activity (Pager *et al.*, 1972; Chaput and Holley, 1976). After fixation of the bulbar electrode, the depth of each recording site was determined using an evoked field potential induced in response to electrical stimulation of the OB electrode (0.1 ms pulse). The reversal point of the electrically evoked potential in APC, PPC and EC corresponds to the pyramidal cell layer (layer II) (Mouly *et al.*, 1998). The ground electrode was positioned in the skull bone contralaterally with regard to the recording electrodes and at an equal distance from the electrodes in the APC, PPC and EC. Monopolar recording electrodes consisted of 80 μm stainless steel wires insulated except at the cross-section. Their impedance varied between 100 and 500 kΩ. Electrodes were connected to a miniature socket fixed to the rat's head with dental cement. Two weeks of recovery separated surgery from recordings.

Stimulation and recording condition paradigms

For recordings rats were placed in a cylindrical cage that was constantly ventilated (Vigouroux and Royet, 1981). Odours were delivered from the top of the cage by an olfactometer (Vigouroux and Chaput, 1988). Two odours characterized by a difference in their behavioural valence were used: food odour (FO) and isoamyl acetate (ISO) (Sigma, France). The odour of food pellets consisted of 100 g of rat chow powder, made from chow pellets taken from the rats' daily food supply. The concentration of each odourant was chosen to evoke clear-cut exploratory responses for FO and a rapidly dimishing behavioural response to ISO with no sign of an aversive effect. The protocol was designed to study both the effects of isolated presentation of an odour and the effect of repetitive presentation. Thus, in each recording session FO and ISO were first presented once with a 3 min interval. Then, each odour was presented three times with a 1 min inter-stimulus interval (see Table 1). Each stimulation lasted for 15 s and the odourant reach the bottom of the cage in about 0.5 s following electrovalve opening. This relatively long odour presentation was chosen to favour habituation over repetition. According to the order of presentation, the first isolated stimulus was designated rank 0 and the three successive repetitions, rank 1, rank 2 and rank 3, respectively. No behavioural response was required from the rats, but for each odour presentation the intensity of the behavioural response, such as sniffing, exploration and rearing, was noted on a three step scale. The recording chamber was placed in a Faraday cage and the rat connected to a cable associated with a swivelling electrical connector that allowed free movement. A miniature source following a multi-channel JFET headstage was mounted on the animal's head to reduce movement artefacts. In this experimental set-up the contribution of the 50 Hz signal was very weak. The LFP signals were amplified, filtered (0.1–300 Hz), digitized (sampling frequency 1000 Hz) and stored on a PC computer using a CED 1401 data recording interface developed by CED (Cambridge Electronic Design, UK). Acquisition started when the rat was quiet.

Table 1 presents the schedule of the four successive recording days. On day 1 signal acquisition was performed after a period of free access to food (designated the satiated

Table 1 Time schedule summarizing the order of recording sessions (upper part) and the odor stimulation sequence (lower part)

	Day 1		Day 2		Day 3		Day 4	
Internal state	satiated		food-deprived		food-deprived		food-deprived	
Pharmacological treatment	no treatment		no treatment		NaCl rats nos 1–3; scopolamine rats nos 4 and 5		NaCl rats nos 4 and 5; scopolamine rats nos 1–3	
Odour	FO	ISO	FO	FO	FO	ISO	ISO	ISO
Rank	0	0	1	2	3	1	2	3
ITI (min)	3	3	1	1	1	1	1	

Scopolamine and NaCl treatments were counterbalanced on days 4 and 5.

FO, food odour; ISO, isoamyl acetate; ITI, inter-trial interval to the next presentation.

condition). From day 2 to day 4 access to food was restricted to a 4 h period following the recording session. In this case signal acquisition was performed after a 16 h fast (designated the food-deprived condition). The effect of cholinergic modulation was tested in food-deprived animals only. This is because previous experiments showed that food deprivation led to a high level of olfactory responsiveness due to the action of centrifugal fibres. Such a centrifugal control was not observed in the satiated condition (Pager *et al.*, 1972; Pager, 1978). In order to test for a possible involvement of the centrifugal cholinergic system in the phenomenon previously observed in the food-deprived condition, responses were obtained following injection of either scopolamine (0.125 mg/kg i.p. 20 min before recording) (Sigma, France) or the vehicle (0.9% NaCl). Recordings started 20 min after injection. This relatively low dose of scopolamine has been found to have no effect on olfactory discrimination but impairs short-term olfactory memory (Ravel *et al.*, 1992).

Dynamic signal analysis

Signal analysis was carried out off-line using custom made software developed with Matlab v.4.2c. Spectral analysis was done on 0.1–300 Hz wide band filtered signals. Changes in power associated with stimulus presentation were quantified for a 3 s period following odour arrival at the bottom of the recording cage. This 3 s period was chosen because we observed that it corresponded to a period of quiet olfactory processing with no or weak contribution of sniffing and exploratory behaviour. Consequently, 'spontaneous' activity used as the baseline for response determination was set to the 3 s period preceding stimulus onset. Thus, for each stimulation a 6 s period of activity was analysed.

Preliminary observations of data showed that, under our experimental conditions, changes in power related to odour exposure mostly appeared transiently for <0.5 s. In order to capture these transient changes, a dynamic sliding window method was developed. Extensive pilot studies were carried out in order to define optimal time window analysis (200, 400, 500 or 800 ms). This optimization aimed at fulfilling two constraints: detection of transient phenomenon together with a good estimation of all signal frequency components. A first series was performed on complex artificial signals very similar to biological ones. They were made of white noise mixed with several different oscillatory frequencies ranging from 20 to 80 Hz and with bursts lasting from 100 to 500 ms. This allowed selection of a limited number of sliding window possibilities. A second series performed on biological signals with well-contrasted different regimes led us to retain the following setting: a 400 ms time window sliding by steps of 200 ms. This was efficient to detect transient oscillatory regimes lasting for at least 200 ms in the 10–90 Hz frequency range and with a frequency resolution of 1.95 Hz. Nevertheless, choosing slightly different settings (for example 500/200 ms or 500/250 ms) did not modify the outcome of the signal analysis.

With these parameters, the 6 s period of LFP activity generated 29 overlapping time epochs: 14 during the baseline and 15 following odour onset. In each 400 ms time epoch the power spectrum was computed using a classical spectral estimation method, the Welch estimation procedure (Welch, 1967; Lopes da Silva, 1987; Marple, 1987; Kay and Freeman, 1998). In this procedure a Hamming window was applied to the data before computation of the fast Fourier transform (FFT). For each 400 ms time epoch a power spectrum was averaged from six successive 145 point periods which overlapped by 60% and padded with zeroes to 512 points. Analysis was restricted to a 0–90 Hz frequency band, which contained 98% of the whole energy of signals between 0 and 100 Hz. Finally, in order to evaluate possible participation of specific rhythms, power was first computed independently in six frequency bands, each 15 Hz wide: 0–15, 15–30, 30–45, 45–60, 60–75 and 75–90 Hz. These bands were chosen because each corresponds to an already identified regime in olfactory and non-olfactory areas: 0–15 Hz, respiratory and internally driven θ; 15–30 Hz, the β activity observed in olfactory areas (Boeijinga and Lopes da Silva, 1989; Chapman *et al.*, 1998; Kay and Freeman, 1998); 30–45 Hz, the so-called '40 Hz' found in visual areas (Singer, 1993); 45–60 Hz, the low γ bursts found in OB and

PC (Kay and Freeman, 1998); 60–75 and 75–90 Hz, the high γ bursts from OB and PC (Boudreau and Freeman, 1963; Bressler and Freeman, 1980; Kay and Freeman, 1988). Importantly, it was found that different choices in frequency band borders did not modify the outcome of the signal analysis.

Calculation of distribution of power during spontaneous activity

Spontaneous activity refers to the 3 s portion of activity preceding odour onset. Each signal was analysed using the dynamic method. For each structure, the power spectra of all individual 14 time epochs were averaged to obtain a *grand* average power spectrum. The distribution of power was described using two parameters: the amount of energy in each frequency band as a percentage of total energy between 0 and 90 Hz and the median value calculated between 15 and 90 Hz. Indeed, as will be seen in the results, data analysis was most often restricted to values between 15 and 90 Hz.

Determination of significant changes during odour presentation

For each stimulation, the mean and STD values of power obtained in the 14 time epochs of baseline activity were calculated and used as a reference to evaluate significant changes in power induced by odours. For each structure and for each frequency band a threshold value corresponding to the mean ± 2 STD was calculated (Figure 1A). Thus, during odour presentation only time epochs during which power exceeded the upper or lower thresholds were considered as significant changes (Figure 1B). Visual inspection of response patterns using this method revealed that significant changes rarely occurred for the whole 3 s period of odour stimulation. Indeed, in each frequency band and in each structure changes typically occurred in two to seven consecutive time epochs, thus corresponding to 600–1600 ms. The occurrence of a response was defined as at least two consecutive time epochs (thus at least 600 ms) with significant changes. With this criterion the response was classified as excitatory (1) or null (0). Significant decreases in power were fairly rare and their occurrence was too low to allow further statistical analysis.

Statistical analysis

For each recording site each odour presentation provided six responses, one in each of the predefined frequency bands. These measures were averaged across animals (n = 5). Comparisons between relevant factors were carried out using multi-factor ANOVA. Variables taken into account were the frequency band, the type of odour (ISO versus FO), the nutritional state of the animal (satiated versus food-deprived), the drug treatment (scopolamine versus NaCl) and the order of presentation (ranks 0–4). However, all variables were not tested in the same ANOVA. The variables which were considered in each analysis are given in Results. ANOVA analyses were followed by pairwise comparisons (Tukey) for the significant factor or interaction. Moreover, in each group evolution of the response according to the order of stimulation was tested by Wilcoxon matched pairs signed ranks test. As seen in the results, some ANOVA analyses revealed a significant effect of odour but with no significant band interaction. This means that expression of the response appeared homogeneously in each of the tested frequency bands.

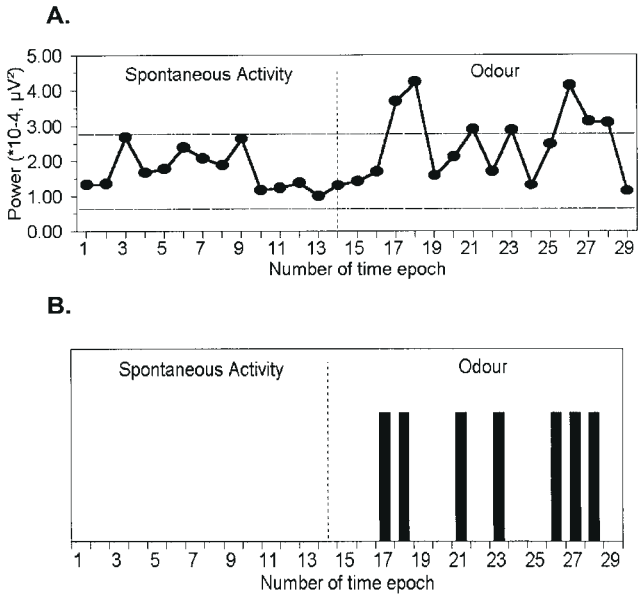

Figure 1 Method used to determine significant odour-related changes in power spectra values. **(A)** Example of power values in 29 time epochs (400 ms each overlapping by 200 ms) recorded from the OB in the 15–30 Hz frequency band. For each stimulation (in this example ISO presented in the food-deprived condition) there were 14 time epochs before and 15 after odour onset (vertical dashed line). The threshold value was determined using the 14 time epochs preceding odour onset. The threshold was equal to the mean ± 2 STD as indicated by two thick horizontal lines (upper and lower lines, respectively). **(B)** In this example, odour presentation was associated with a significant increase in power during seven time epochs (nos 17, 18, 21, 23 and 26–28). The occurrence of a response was defined as at least two consecutive time epochs (thus at least 600 ms) with significant changes. For clarity of representation successive time epochs are presented contiguously, although they overlap by 50%.

Histology

At the end of the experiment the rats were deeply anaesthetized 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 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.

Table 2 Distribution of energy during spontaneous activity in each frequency band and for each structure

Structure	Frequency bands (Hz)					
	0–15	15–30	30–45	45–60	60–75	75–90
OB (n = 546)	63.52 ± 19.33	8.68 ± 6.67	5.12 ± 3.66	8.35 ± 8.37	7.90 ± 6.48	6.42 ± 5.18
APC (n = 546)	60.54 ± 15.73	14.96 ± 6.61	7.52 ± 4.44	7.73 ± 5.81	5.35 ± 4.19	3.90 ± 3.53
PPC (n = 686)	66.31 ± 14.79	17.11 ± 8.15	6.49 ± 3.87	4.63 ± 3.67	3.16 ± 2.89	2.30 ± 2.45
EC (n = 406)	70.42 ± 13.22	18.23 ± 9.14	5.82 ± 3.66	2.81 ± 1.76	1.63 ± 1.30	1.09 ± 1.08

Table 3 Average median values for spontaneous activity between 15 and 90 Hz for each recorded structure under the three experimental situations

Structure	Experimental group		
	Satiated	Food-deprived	Food-deprived + scopolamine injection
OB	50.86 ± 12.11 Hz (n = 546)	51.58 ± 13.22 Hz (n = 1092)	51.58 ± 14.85 Hz (n = 546)
APC	38.01 ± 10.22 (n = 546)	40.38 ± 11.07 (n = 1092)	35.29 ± 10.24[a] (n = 546)
PPC	29.94 ± 7.89 (n = 686)	30.30 ± 7.75 (n = 1372)	28.13 ± 8.52[a] (n = 686)
EC	25.25 ± 4.56 (n = 406)	26.37 ± 5.07 (n = 812)	24.10 ± 5.61[a] (n = 406)

One can see a significant effect of scopolamine treatment on APC, PPC and EC. n refers to the total number of 400 ms time epochs used to evaluate average values STD for five animals. [a]$P < 0.005$ relative to food-deprived condition.

Results

Spontaneous activity

In each structure at least 60% of the energy resided in the 0–15 Hz frequency band (Table 2). Due to the difficulty of interpretation in behaving animals of the origin of these low frequencies (respiratory frequency or endogenous θ), further analyses were restricted to the 15–90 Hz frequency band. In the food-deprived condition, data were obtained from each animal after no treatment and following NaCl injection. There was no statistically significant difference between these two conditions. Thus, for the rest of the paper data from these two recording sessions are pooled together and are referred to as the food-deprived condition.

As seen in Figure 2 and Table 2, the distribution of power in each frequency band differed from one structure to the other. As a general feature, similarities were more pronounced between the OB and the APC on the one hand and between the PPC and the EC on the other. This feature can be observed qualitatively in Figure 2 from both unfiltered and filtered signals. We can observe that the OB and APC signals contain both low and high frequencies while the PPC and EC mainly express low frequencies. Indeed, the energy in high frequency bands (45–90 Hz), which dominated in the OB and APC, strongly decreased in the PPC and EC. For instance, the peak centred on 60 Hz displayed by the OB and APC was not observed in the PPC and EC. On the contrary, the power in low frequencies in the 15–30 Hz frequency band was relatively low in the OB and reached the highest value in the EC. The differential distribution of the power spectra can be expressed as the difference in the value of the median calculated from 15 to 90 Hz. The median value was preferred over the mean value due to the non-Gaussian distribution of energy between 15 and 90 Hz. In the satiated, food-deprived and scopolamine conditions the median frequency within the power spectrum decreased significantly from rostral to caudal structures [ANOVA: satiated, $F(3,2180) = 752.60$, $P < 0.0001$; food-deprived, $F(3,4364) = 1350.98$, $P < 0.0001$; scopolamine, $F(3,2180) = 699.38$, $P < 0.0001$] (see Figure 2 and Table 3).

Finally, the distribution of energy during spontaneous activity was sensitive to scopolamine treatment but not to change in nutritional state. Scopolamine treatment significantly reduced the median value of the power spectra in the APC, PPC and EC, with no change in standard deviation values when compared with the food-deprived group (see Table 3, column 3). This effect was principally due to an increase in power in the 15–30 Hz frequency band. This shift towards the left of the power spectra did not modify general characteristics of the distribution observed in the control condition.

Effect of odour presentation

First we describe the effect of the first presentation of odours. We examined to what extent nutritional state modified reactivity in each structure (Figure 3). The ANOVA revealed that nutritional state (food-deprived versus satiated) significantly affected responsiveness in two structures only: the OB [ANOVA: interaction Band × Odour × State,

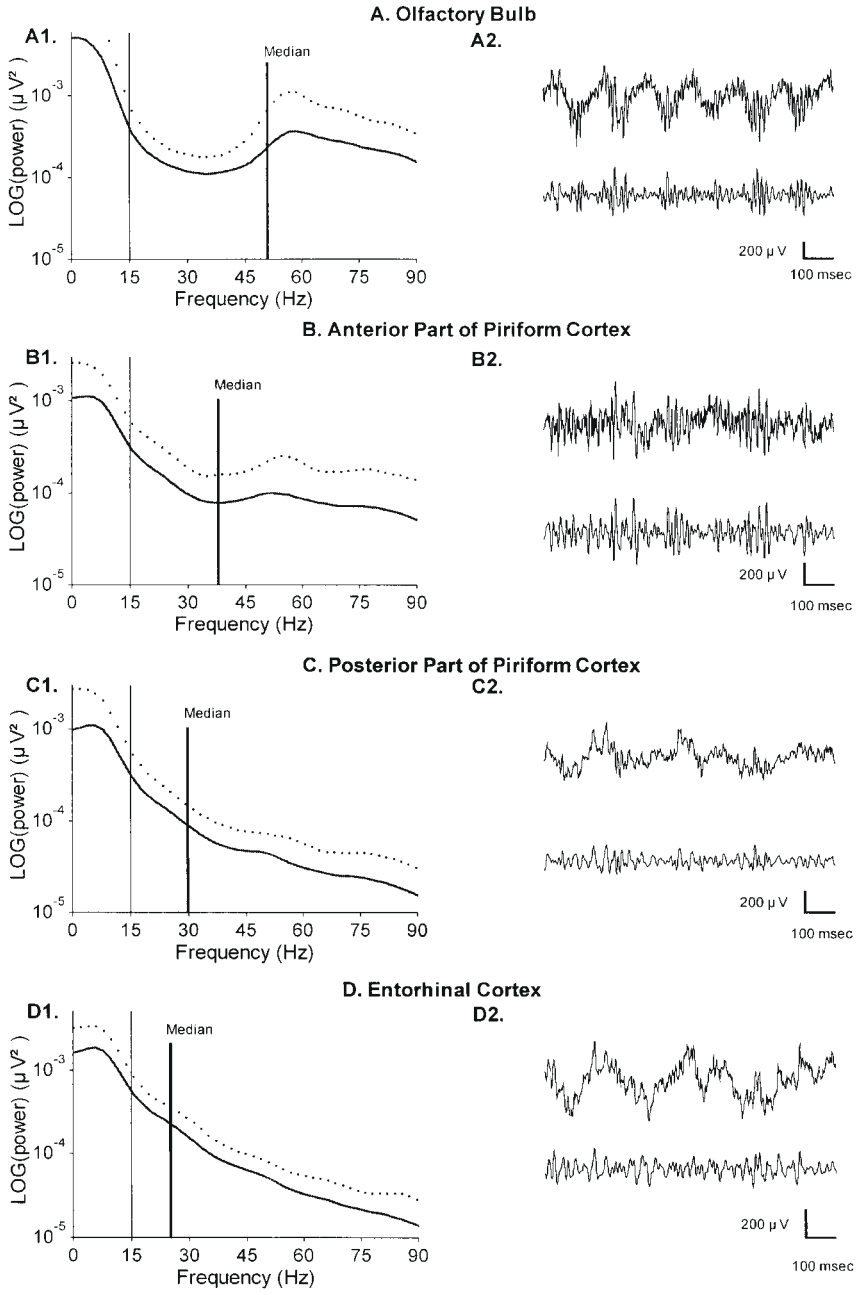

Figure 2 Average distribution of energy between 0 and 90 Hz at each recorded site during spontaneous activity. **(A)** Olfactory bulb; **(B)** anterior part of piriform cortex; **(C)** posterior part of piriform cortex; **(D)** entorhinal cortex. (Left) The grand average power spectrum (A1, $n = 546$ time epochs; B1, $n = 546$; C1, $n = 686$; D1, $n = 406$; in each case obtained in five rats). The thick and dotted curves represent the mean and the STD of power, respectively. The thick vertical line represents the median value between 15 and 90 Hz. (Right) Example of recorded signals for each site (A2, B2, C2 and D2). In each case, 1 s of activity is shown with two different filter settings: (upper) 0–300 Hz; (lower) 15–90 Hz. One can observe the dominance of fast oscillations in OB and APC and slow oscillations in PPC and EC.

$F(4,100) = 5.78$, $P < 0.0005$] and the EC [ANOVA: interaction Band × State, $F(4,70) = 2.70$, $P < 0.05$]. This means that the nature of the odour significantly affected reactivity in the OB but not in the EC (Figure 3A,D). Further ANOVA analysis allowed identification of which frequency bands were modulated by nutritional state in these two structures. It appeared that in both the OB and EC nutritional state selectively affected the 15–30 Hz frequency band [ANOVA: OB, interaction State × Odour, $F(1,20) = 16.13$, $P < 0.001$; EC, global effect of State, $F(1,16) = 4.60$, $P < 0.05$] (Figure 3A,B).

We then looked for potential different responsiveness between the first presentation of FO and ISO in each nutritional state taken independently. A differential rate of

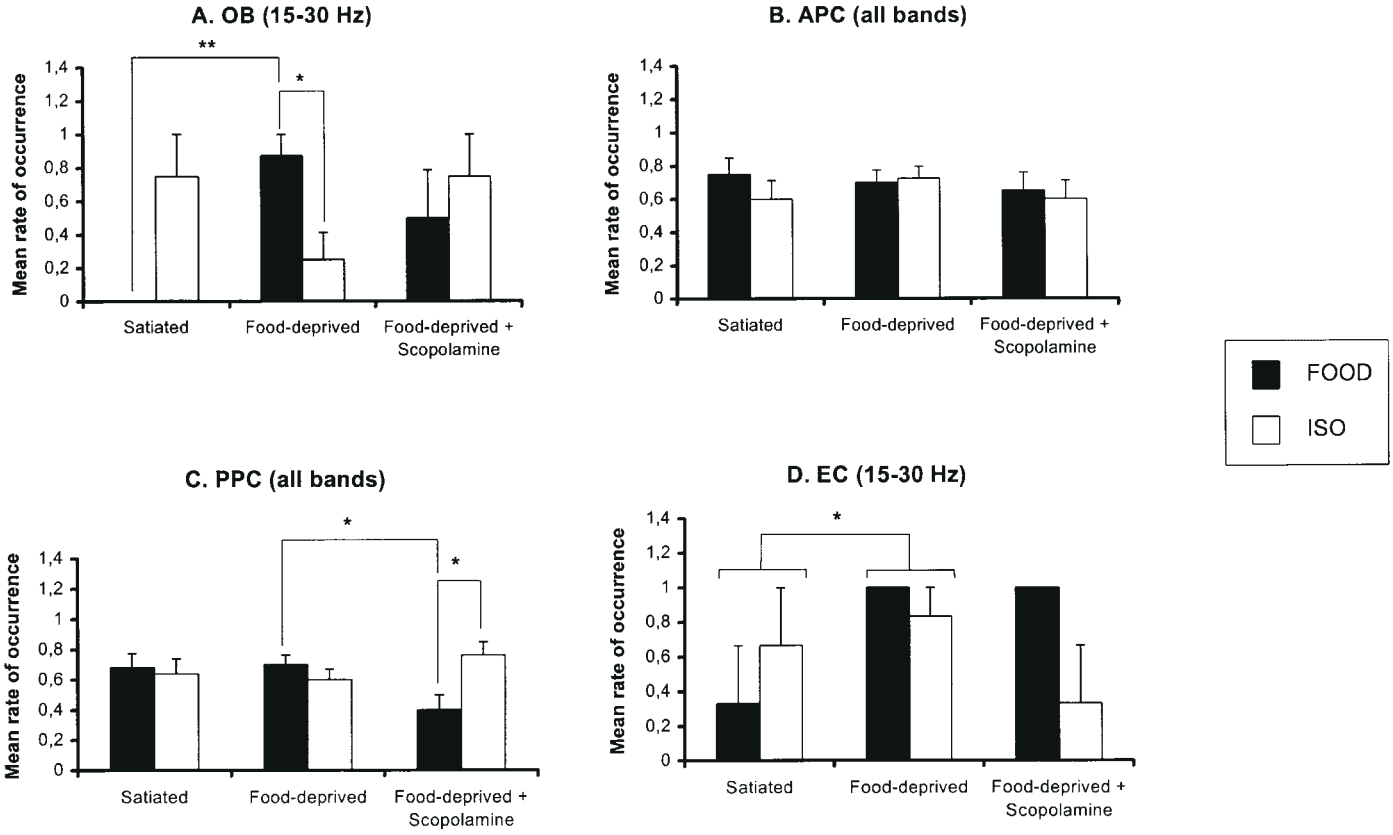

Figure 3 Responsiveness to the first presentation in each recorded structure. Each panel presents mean rate of occurrence of excitatory response to FO (in black, FOOD) and to isoamyl acetate (in white, ISO) in a given structure from five animals. For each structure data are presented as those obtained in the satiated condition (left), in the food-deprived condition (central) and in the food-deprived condition following systemic scopolamine injection (right). Depending on the structure, significant differential responsiveness was observed in different frequency bands: in a narrow 15–30 Hz band for OB **(A)** and entorhinal cortex **(D)** and in all bands between 15–90 Hz in APC **(B)** and PPC **(C)**. *$P < 0.05$; **$P < 0.01$.

response between FO and ISO was observed in the OB only (Figure 3A). Indeed, in satiated rats the rate of response to ISO was higher than to FO (for which there was no response) although the difference did not reach a significant threshold (pairwise comparison, $P = 0.06$). However, in the food-deprived condition there was a greater response to FO than ISO (pairwise comparison, $P < 0.05$).

In the 15–30 Hz frequency band we estimated the average value of frequency in which significant changes in power following odour onset were detected. In the OB and EC the average values were 20.2 ± 4.8 Hz ($n = 63$ time windows) and 18.4 ± 3.9 Hz ($n = 76$ time windows), respectively. Figure 4 presents a typical odour-related modulation in signal amplitude recorded at the OB level.

One can note that responsiveness to the first presentation in both APC and PPC did not vary either according to nutritional state or according to odour. Odour exposure increased power homogeneously in each frequency band, noted as 'all bands' in Figure 3B,C. This likely corresponds to a general increase in the power signal.

Response to the first presentation in the food-deprived condition was affected following scopolamine treatment in two structures only: the OB and PPC (Figure 3A,C). The most interesting effect appeared at the OB level, for which responsiveness significantly differed between the scopolamine-treated and control conditions [ANOVA: interaction Treatment × Odour, $F(1,20) = 4.95$, $P < 0.05$]. As seen in Figure 3A, scopolamine suppressed the differential rate of response between FO and ISO normally observed in controls in the 15–30 Hz frequency band. In the PPC (Figure 3C) the effect was observed in the broad 15–90 Hz frequency band [ANOVA: interaction Treatment × Odour, $F(1,130) = 7.88$, $P < 0.01$]. The effect of scopolamine in the PPC was a specific decrease in the rate of response to FO (pairwise comparison, $P < 0.05$). As a consequence, a differential rate of response between FO and ISO appeared in scopolamine-injected rats ($P < 0.05$).

Second, we examined how nutritional state modulated habituation of neural responses to repeated odour presentations (Figures 5 and 6). Specifically, the aim of this section was to investigate how the previously described (Pager *et al.*, 1972; Pager, 1978) nutritional state modulation of OB

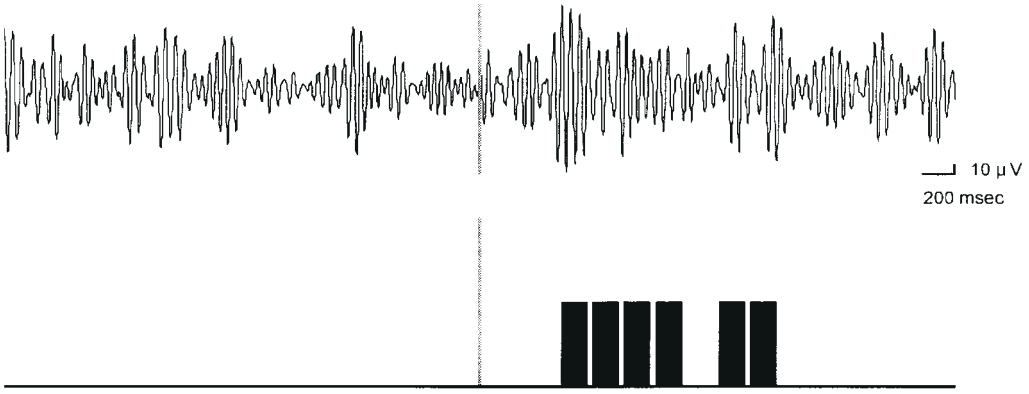

Figure 4 Example of β band oscillations in the olfactory bulb before and during odour exposure. (Top) LFP signal filtered in the 15–25 Hz frequency band for a 6 s period of activity: 3 s before and 3 s following odour onset (thick vertical bar). Odour presentation is associated with a transient increased amplitude in β band activity. (Bottom) During odour exposure each vertical black bar represents a 400 ms time window in which power signal differed significantly from the baseline (see Materials and methods). As in Figure 2, successive time epochs are presented contiguously, although they overlap by 50%.

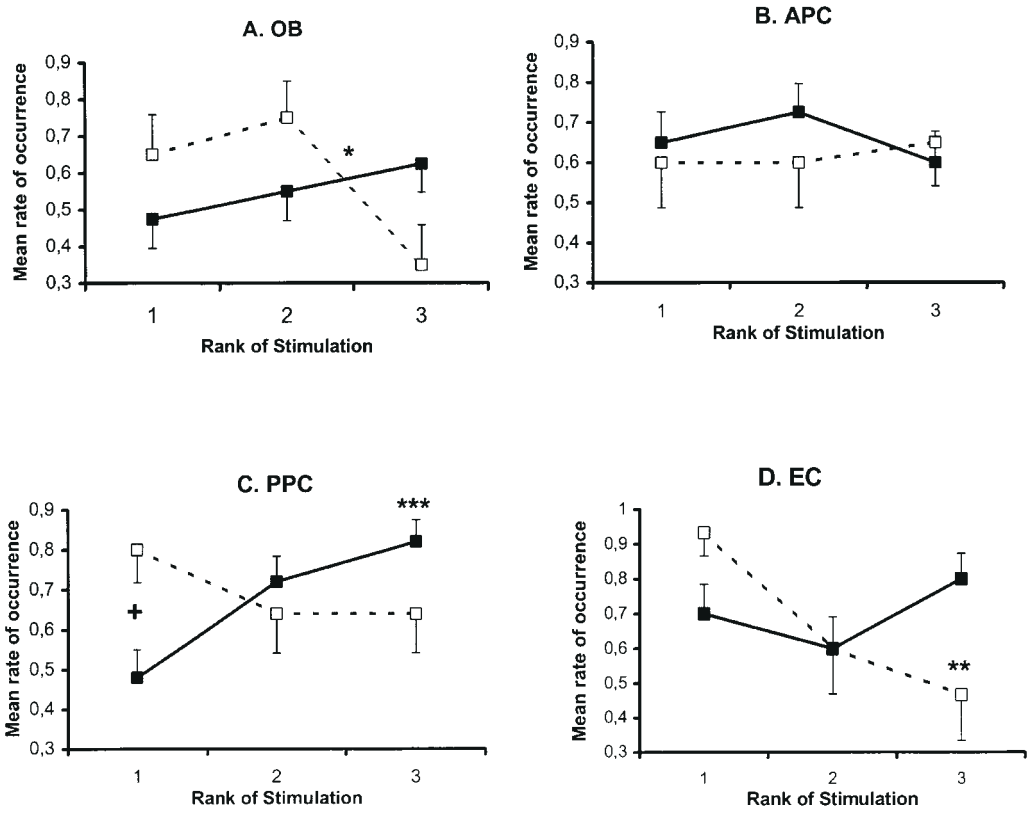

Figure 5 Time course of habituation to food odour in each structure in each nutritional state. Food odour was presented three times with a 1 min inter-stimulus interval in the satiated (dotted line) and in the food-deprived (full line) conditions. Each point represents rate of occurrence of excitatory response (mean ± SEM) for each site in all animals ($n = 5$). The course of habituation was not band specific. Asterisks point to significant differences under the same experimental condition (OB, $*P < 0.05$, between R2 and R3 in satiated condition; EC, $**P < 0.01$ between R1 and R3 in satiated condition; PPC, $***P < 0.005$ between R1 and R3 in food-deprived condition). The cross indicates a significant difference between the two experimental conditions for the same rank of stimulation ($^{+}P < 0.05$ in PPC for rank 1).

habituation to FO was expressed in other olfactory areas. We also looked for possible selective oscillatory regimes which could express the modulation and its sensitivity to scopolamine treatment. Since a difference in habituation according to nutritional state is expressed towards FO but not to ISO (Pager *et al.*, 1972; Pager, 1978), the present study

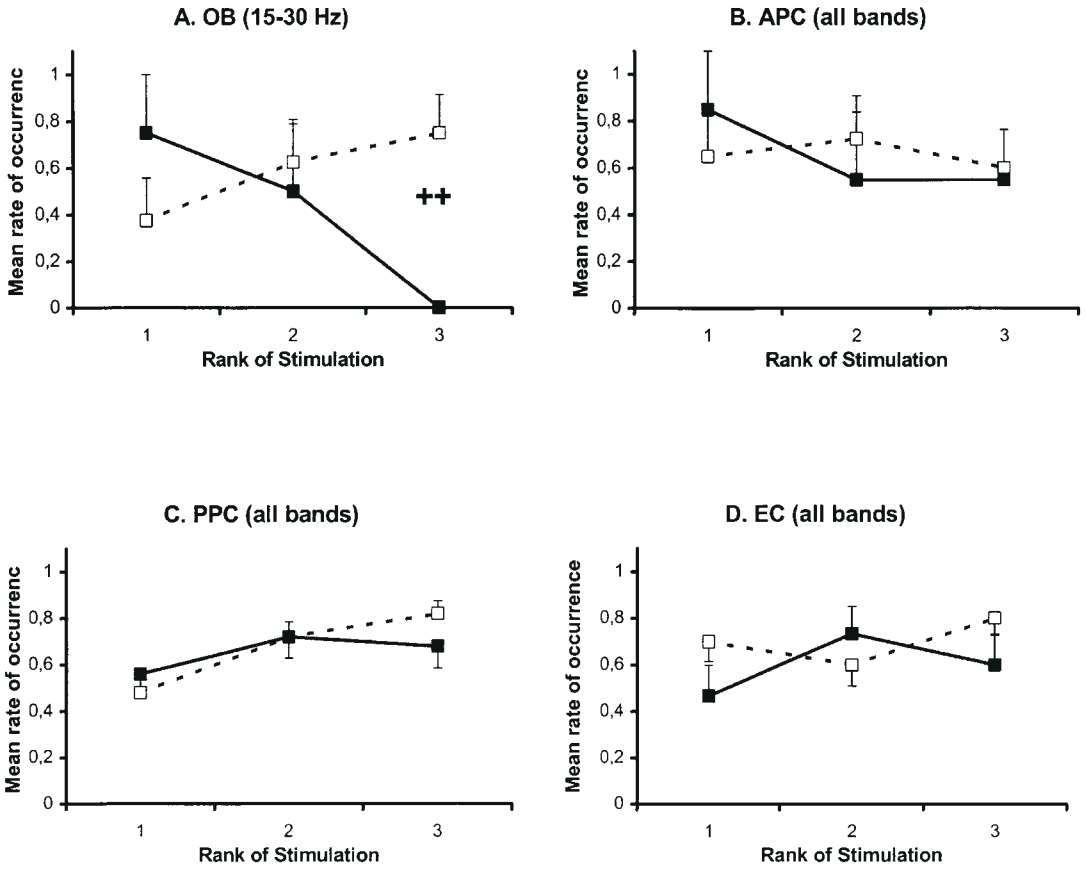

Figure 6 Effect of scopolamine treatment on time course of habituation to food odour in each recorded site in the food-deprived condition (mean ± SEM). Data were obtained under the control condition (dashed line) and after scopolamine injection (scopolamine, full line). Scopolamine induced significant changes in the OB only, restricted to the 15–30 Hz frequency band. As seen in **(A)**, the rate of occurrence of an excitatory response declined abruptly with repetition in the scopolamine condition while it had a tendency to increase in the control condition. ($^{++}P < 0.01$ between the two groups for the same rank of stimulation). In the other three structures there was no band selective effect.

tested for a differential habituation to FO only. This was done on responses obtained during three successive odour presentations at 1 min intervals.

Figure 5 summarizes the results obtained in the food-deprived and satiated conditions. In these two conditions repetition of the stimulus induced significant variations in response rate which were not frequency band selective. Consequently, habituation was described for the broad 15–90 Hz frequency band. A first global ANOVA analysis revealed that time courses of habituation differed according to the nutritional state in three structures: the OB [ANOVA: interaction State × Rank, $F(2,174) = 3.94$, $P < 0.05$], the PPC [ANOVA: interaction State × Rank, $F(2,195) = 5.33$, $P < 0.005$] and the EC [ANOVA: interaction State × Rank, $F(2,105) = 3.68$, P 0.05]. In the APC habituation occurred in a similar manner in both nutritional states.

Then, we examined habituation to FO in each nutritional state taken independently. In satiated rats a significant reduction in the rate of response over repetitions was observed in two structures: the OB [ANOVA: global effect of Rank, $F(2,57) = 3.85$, $P < 0.05$] and the EC [ANOVA: global effect of Rank, $F(2,30) = 4.88$, $P < 0.01$]. As seen in Figure 5A,D, habituation was less pronounced in the OB than in the EC. Indeed, in the OB a significant decrease in response was noted between ranks 2 and 3 only (pairwise comparison, $P < 0.05$). In the EC there was a clear tendency for a reduction in reactivity between ranks 1 and 2 (pairwise comparison: $P = 0.6$) and a very significant drop between ranks 1 and 3 (pairwise comparison, $P < 0.01$). In the food-satiated condition no habituation was observed in the APC (Figure 5B) or PPC (Figure 5C). Finally, in the food-deprived condition responsiveness was maintained across repetitions in the OB, APC and EC. Surprisingly, in the PPC responsiveness significantly increased with repetition (rank 1 versus rank 3, pairwise comparison, $P <$ 0.005) (Figure 5C). As a whole, it thus appeared that habituation to FO was expressed differentially between the OB, PPC and EC and each of these structures was sensitive to changes in nutritional state.

Finally, we tested the hypothesis that the action of centrifugal cholinergic projections to olfactory structures could participate in the maintenance of responsiveness

to FO observed in the food-deprived condition. Figure 6 summarizes the effects of scopolamine injection. Interestingly, the drug selectively affected the OB. In addition, ANOVA analysis revealed that the effect of scopolamine was selectively observed in the 15–30 Hz frequency band [ANOVA: interaction Treatment × Rank, $F(2,30) = 3.63$, $P < 0.05$]. As seen in Figure 6A, scopolamine treatment induced a decline in response rate over repeated trials in a manner similar to that noted in the satiated condition. For instance, at rank 3 the rate of response was significantly inferior to that noted in the control condition ($P < 0.01$). In other words, scopolamine injection into food-deprived rats abolished maintenance of the high level of OB reactivity to FO normally observed in controls.

Discussion

Differential distribution of energy in spontaneous activity along the rostro-caudal axis

Analysis of LFP recordings during spontaneous activity clearly showed a heterogeneous distribution of energy along the rostro-caudal axis. The OB–APC complex expressed a high level of fast 45–90 Hz oscillatory activity while the PPC–EC complex was dominated by lower 15–30 Hz frequencies. The peak centred on 60 Hz displayed by the OB–APC complex represents fast burst activity classically observed by others during inspiration in the cat (Boudreau, 1964; Becker and Freeman, 1968), rat (Bressler and Freeman, 1980) and rabbit (Bressler, 1984). The fast 45–90 Hz burst activities are largely determined by the OB intrinsic circuitry but are also under the control of centrifugal influences (Gray *et al.*, 1986; Gray and Skinner, 1988a). The fact that both the density of OB projections to the olfactory cortex (Schwob and Price, 1978) and the conductance velocities (Kerr and Dennis, 1972) decrease gradually along the rostro-caudal axis could explain the progressive decrease in the γ band power from the APC to the EC (Freeman, 1975). In contrast, the origin of the slower 15–30 Hz β activity is more controversial. Earlier studies suggested that it is prominent in the piriform cortex in the cat (Freeman, 1960; Becker and Freeman, 1968) and rabbit (Bressler, 1984) and transmits to the OB by feedback pathways. More recent data in awake rats showed that presentation of a noxious olfactory stimulus or electrical stimulation of the OB induced coherent 15–35 Hz activities in olfactory pathways. Phase analysis concluded that this activity propagates in a caudal direction, from the OB to the EC (Chapman *et al.*, 1998). Finally, in the context of an olfactory conditioning task, the 12–35 Hz signal was found to propagate in a bidirectional way and the preferential direction depended on the behaviour. For instance, for a short period preceding odour sampling, coherence and phase analysis showed a transmission from the EC to the OB (Kay and Freeman, 1998).

A systemic injection of a low dose of scopolamine does not significantly modify spontaneous power spectra in the OB. Insensitivity of spontaneous bulbar LFP signals to scopolamine likely results from the fact that OB resting activity is predominantly determined by the intrinsic circuitry. In contrast, in the APC, PPC and EC scopolamine increases energy in the 15–30 Hz frequency band and shifts the median value towards low frequencies. In line with this observation, topical application of a cholinergic agonist (carbachol) on the PC was found to enhance fast oscillations (Biedenbach, 1966). These opposite effects of agonist and antagonist applications led us to conclude that in non-treated, awake, quiet rats basal release of acetylcholine likely modulates ongoing electrical activity in the PC and EC in favour of the expression of high frequencies.

Nutritional modulation and its sensitivity to scopolamine

In odour-trained rats (Kay and Freeman, 1998) and cats (Boeijinga and Lopes Da Silva, 1989) odour presentation reduced power in the γ band. This effect may be training-dependent since in the non-trained animals used in the present study odour presentation was not associated with a reduction in power in the γ band. In a general way, the results show that responses were dependent on the relation between the odourant and the internal state. At the OB level the food-deprived state increased the rate of response to FO during the first presentation and suppressed the habituation process. Such nutritional modulation of OB responsiveness has been previously described (Pager *et al.*, 1972; Gervais and Pager, 1983). The effect of nutritional modulation has not yet been studied in other olfactory areas. Here, we show that differential habituation to FO was also expressed in the EC and PPC, but not in the APC. In the food-deprived state there is maintenance of the response in the EC concurrent with a progressive increase in response rate in the PPC. This suggests that a simple form of short-term olfactory memory could be expressed differentially across olfactory areas.

Nutritional modulation of OB responsiveness has been shown to be dependent on the action of centrifugal nerve fibres reaching the OB through the medial part of the olfactory peduncle (Pager, 1974, 1978). Neurochemical systems mediating this feedback control do not seem to primarily involve the serotoninergic projections originating from the raphe (Gervais *et al.,* 1984) nor the noradrenergic fibres originating from the locus coeruleus (Gervais and Pager, 1983). In the present experiment scopolamine administered to food-deprived animals suppressed the differential OB response between FO and ISO for the first presentation and prevented maintained OB responsiveness to repeated presentations of FO in the food-deprived condition. In both cases scopolamine reduced OB responsiveness to FO. Thus, although we have seen that basal acetylcholine release during spontaneous activity did not influence resting OB activity, release following presentation of a meaningful odour could contribute to the maintenance of high OB reactivity. This could be done through a reduction in local

inhibition (Elaagouby *et al.*, 1991). In a more general way, such an interpretation is compatible with the suspected role of the cholinergic basal forebrain system in selective attention (Everitt and Robbins, 1997). However, the action of other neuromodulatory systems can also maintain a high level of OB reactivity. In particular, at the OB level the action of noradrenaline has also been shown to contribute to the maintenance of high responsiveness, as detected as changes in power in the γ band (Gray *et al.*, 1986). Since both acetylcholine and noradrenaline can be released simultaneously in the OB in response to behaviourally meaningful odours (Kendrick *et al.*, 1992), this data set supports the hypothesis of a combined cholinergic and noradrenergic modulation of OB responsiveness to behaviourally relevant odours.

Functional characteristics of the anterior and posterior parts of the piriform cortex

With LFP recordings, a recent study showed a functional dissociation along the antero-posterior axis of the rat PC. Indeed, coherence analysis on spontaneous activity revealed a clear hierarchy in the strength of coupling within the OB, APC, PPC and EC complex. For instance, there was dissociation within the PC: the PPC was more tightly coupled with the EC than with the APC (Chabaud *et al.*, 1999). The present paper has revealed three other functional differences between the two parts of the PC. First, spontaneous oscillatory activities in both structures differed greatly. Second, odour responsiveness was affected differentially following scopolamine treatment. Indeed, for the first odour presentation the scopolamine treatment did not affect APC responsiveness, while it induced a differential rate of PPC responsiveness between the two odours. This selective effect of scopolamine on the PPC could be related to the higher density of cholinergic fibres reaching the PPC compared with the APC (Lysakowski *et al.*, 1989). Nevertheless, very little data provide insights on how acetylcholine modulates oscillatory activities in the PC of intact rats (Biedenbach, 1966). However, slice recordings together with neural modelling suggest that the level of extracellular acetylcholine plays a critical role in whether the PC expresses either low or high oscillatory frequencies. This modulation is exerted through the time constant of neural adaptation and the intervention of intrinsic associative fibres (Lijenström and Hasselmo, 1995). Third, expression of habituation was very different between the APC and PPC. While responses in the APC were stable to repeated FO in the food-deprived condition, responses in the PPC increased.

Odour-related β band oscillations in the olfactory system

In this study changes in power associated with odour presentations were in most cases not selective or restrained to one frequency band, in contrast to what was observed in trained cats (Boeijinga and Lopes da Silva, 1989) and rabbits (Freeman and Schneider, 1982). This suggests that odour presentation can induce general increases in signal power. However, we also found that ISO and FO could be associated with significant changes restricted to the β band (15–30 Hz). Within this range, maximum power peaked near 20 Hz in both the OB and EC. Using LFP recordings, Chapman and co-workers (Chapman *et al.*, 1998) also showed that a strong olfactory stimulus induced oscillations centred on 19 Hz in olfactory structures. In our experiment this phenomenon appeared in response to the first presentation and during maintenance of high OB reactivity to a behaviourally meaningful odour. Thus, power amplification of bulbar β band activity could be related to centrifugal modulation related to high attentional and motivational levels. However, induction of β band activity also seems tightly associated with odourant input. In lightly restrained and non-reinforced awake rabbits presentation of isoamyl acetate and clove oil increased the amplitude of 15–25 Hz oscillations (Gray and Skinner, 1988b). In anaesthetized or awake rats bursts of β frequency (15–35 Hz) oscillations occur in the OB following stimulation with a strong noxious stimulus (toluene) (Chapman *et al.*, 1998) or non-noxious olfactory stimuli (Zibrowski and Vanderwolf, 1997). Finally, in cats engaged in a discrimination task, sniffing of conspecific urine significantly increased power in the 15–20 Hz frequency band (Boeijinga *et al.*, 1989). It appears that the significance of β band oscillatory activity in olfactory processing (Kay and Freeman, 1998) deserves further investigation.

References

Adrian, E.D. (1950) *The electrical activity of the mammalian olfactory bulb*. EEG Clin. Neurophysiol., 2, 377–388.

Becker, C.J. and **Freeman, W.J.** (1968) *Prepyriform electrical activity after loss of peripheral or central input, or both*. Physiol. Behav., 3, 597–599.

Biedenbach, M.A. (1966) *Effects of anesthetics and cholinergic drugs on prepyriform electrical activity in cats*. Exp. Neurol., 16, 464–479.

Boeijinga, P.H. and **Lopes da Silva, F.H.** (1989) *Modulations of EEG activity in the entorhinal cortex and forebrain olfactory areas during odour sampling*. Brain Res., 478, 257–268.

Boudreau, J.C. (1964) *Computer analysis of electrical activity in the olfactory system of the cat*. Nature, 201, 155–158.

Boudreau, J.C. and **Freeman, W.J.** (1963) *Spectral analysis of electrical activity in the prepyriform cortex of the cat*. Exp. Neurol., 8, 423–439.

Bressler, S.L. (1984) *Spatial organization of EEGs from olfactory bulb and cortex*. EEG Clin. Neurophysiol., 57, 270–276.

Bressler, S.L. and **Freeman, W.J.** (1980) *Frequency analysis of olfactory system EEG in cat, rabbit, and rat*. EEG Clin. Neurophysiol., 50, 19–24.

Buzsaki, G. and **Gage, F.H.** (1989) *The cholinergic nucleus basalis: a key structure in neocortical arousal*. EXS, 57, 159–171.

Cape, E.G. and **Jones, B.E.** (1998) *Differential modulation of high-frequency γ-electroencephalogram activity and sleep-wake by noradrenaline and serotonin microinjections into the region of cholinergic basalis neurons*. J. Neurosci., 18, 2653–2666.

Chabaud, P., Ravel, N., Wilson, D.A. and **Gervais, R.** (1999) *Functional coupling in rat central olfactory pathways: a coherence analysis*. Neurosci. Lett., 273, 1–4.

Chapman, C.A., Xu, Y., Haykin, S. and **Racine, R.J.** (1998) *Beta-frequency (15–35 Hz) electroencephalogram activities elicited by toluene and electrical stimulation in the behaving rat*. Neuroscience, 86, 1307–1319.

Chaput, M. and **Holley, A.** (1976) *Olfactory bulb responsiveness to food odour during stomach distension in the rat*. Chem. Senses, 2, 189–201.

Elaagouby, A., Ravel, N. and **Gervais, R.** (1991) *Cholinergic modulation of excitability in the rat olfactory bulb: effect of local application of cholinergic agents on evoked field potentials*. Neuroscience, 45, 653–662.

Everitt, B.J. and **Robbins, T.W.** (1997) *Central cholinergic systems and cognition*. Annu. Rev. Psychol., 48, 649–684.

Freeman, W.J. (1960) *Correlation of electrical activity of prepyriform cortex and behavior in cat*. J. Neurophysiol., 23, 111–131.

Freeman, W.J. (1975) Mass Action in the Nervous System. Academic Press, New York.

Freeman, W.J. and **Schneider, W.** (1982) *Changes in spatial patterns of rabbit olfactory EEG with conditioning to odors*. Psychophysiology, 19, 44–56.

Gervais, R. and **Pager, J.** (1983) *Olfactory bulb excitability selectively modified in behaving rats after local 6-hydroxydopamine treatment*. Behav. Brain Res., 9, 165–179.

Gervais, R., Araneda, S. and **Pujol, J.F.** (1984) *Effect of local 5,6-dihydroxytryptamine on the rat olfactory bulb responsiveness during wakefulness and sleep*. EEG Clin. Neurophysiol., 57, 462–472.

Gray, C.M. and **Skinner, J.E.** (1988a) *Centrifugal regulation of neuronal activity in the olfactory bulb of the waking rabbit as revealed by reversible cryogenic blockade*. Exp. Brain Res., 69, 378–386.

Gray, C.M. and **Skinner, J.E.** (1988b) *Field potential response changes in rabbit olfactory bulb accompany behavioral habituation during the repeated presentation of unreinforced odors*. Exp. Brain Res., 73, 189–197.

Gray, C.M., Freeman, W.J. and **Skinner, J.E.** (1986) *Chemical dependencies of learning in the rabbit olfactory bulb: acquisition of the transient spatial pattern change depends on norepinephrine*. Behav. Neurosci., 100, 585–596.

Haberly, L.B. (1985) *Neuronal circuitry in olfactory cortex: anatomy and functional implications*. Chem. Senses, 10, 219–238.

Hunter, A.J. and **Murray, T.K.** (1989) *Cholinergic mechanisms in a simple test of olfactory learning in the rat*. Psychopharmacology, 99, 270–275.

Jones, B.E. (1993) *The organization of central cholinergic systems and their functional importance in sleep–waking states*. In Cuello, A.C. (ed.), Progress in Brain Research: Cholinergic Function and Dysfunction. Elsevier, Amsterdam, The Netherlands, pp. 61–71.

Kay, L.M. and **Freeman, W.J.** (1998) *Bidirectional processing in the olfactory-limbic axis during olfactory behavior*. Behav. Neurosci., 112, 541–553.

Kendrick, M.K., Lévy, F. and **Keverne, E.B.** (1992) *Changes in the sensory processing of olfactory signals induced by birth in sheep*. Science, 256, 833–836.

Kerr, D.I.B. and **Dennis, B.J.** (1972) *Collateral projection of the lateral olfactory tract to entorhinal areas in the cat*. Brain Res., 36, 399–403.

Liljenström, H. and **Hasselmo, M.E.** (1995) *Cholinergic modulation of cortical oscillatory dynamics*. J. Neurophysiol., 74, 288–297.

Litaudon, P. and **Cattarelli, M.** (1995) *Piriform cortex late activity revealed functional spatial heterogeneity*. Neuroreport, 6, 1249–1252.

Litaudon, P., Mouly, A.M., Sullivan, R., Gervais, R. and **Cattarelli, M.** (1997) *Learning-induced changes in rat piriform cortex activity mapped using multisite recording with voltage sensitive dye*. Eur. J. Neurosci., 9, 1593–1602.

Lopes da Silva, F. (1987) *EEG analysis: theory and practice*. In Niedermeyer, E. and Lopes da Silva, F. (eds), Electroencephalography: Basic Principles, Clinical Applications and Related Fields. Urban and Schwarzenberg, Baltimore, MD, pp. 871–897.

Luiten, P.G.M., Gaykema, R.P.A, Traber, J. and **Spencer, D.G. Jr** (1987) *Cortical projections patterns of magnocellular basal nucleus subdivisions as revealed by anterograded transported* Phaseolus vulgaris *leuco-agglutinin*. Brain Res., 413, 229–250.

Lysakowski, A., Wayner, B.H., Bruce, G. and **Hersh, L.B.** (1989) *An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex*. Neuroscience, 28, 291–336.

Marple, S.L. (1987) *Classical spectral estimation*. In Digital Spectral Analysis with Applications. Prentice-Hall, Englewood Cliffs, NJ, pp. 130–171.

Mouly, A.M., Litaudon, P., Chabaud, P., Ravel, N. and **Gervais, R.** (1998) *Spatiotemporal distribution of a late synchronized activity in olfactory pathways following stimulation of the olfactory bulb in rats*. Eur. J. Neurosci., 10, 1128–1135.

Pager, J. (1974) *A selective modulation of olfactory input suppressed by lesions of the anterior limb of the anterior commissure*. Physiol. Behav., 13, 523–526.

Pager, J. (1978) *Ascending olfactory information and centrifugal influxes contributing to a nutritional modulation of the rat mitral cell responses*. Brain Res., 140, 251–269.

Pager, J., Giachetti, I., Holley, A. and **Le Magnen, J.** (1972) *A selective control of olfactory bulb electrical activity in relation to food deprivation and satiety in rats*. Physiol. Behav., 9, 573–579.

Paolini, A.G. and **McKenzie, J.S.** (1993) *Effects of lesions in the horizontal diagonal band nucleus on olfactory habituation in the rat*. Neuroscience, 57, 717–724.

Paxinos, G. and **Watson, C.** (1996) The Rat Brain in Stereotaxic Coordinates, 3rd edn. Academic Press, New York.

Rasmusson, D.D., Clow, K. and **Szerb, J.C.** (1994) *Modification of neocortical acetylcholine release and electroencephalogram desynchronization due to brainstem stimulation by drugs applied to the basal forebrain*. Neuroscience, 60, 665–677.

Ravel, N., Vigouroux, M., Elaagouby, A. and **Gervais, R.** (1992) *Scopolamine impairs delayed matching in an olfactory task in rats*. Psychopharmacology, 109, 439–443.

Ravel, N., Elaagouby, A. and **Gervais, R.** (1994) *Scopolamine injection into the olfactory bulb impairs short-term olfactory memory in rats*. Behav. Neurosci., 108, 1–8.

Rosin, J.F., Datiche, F. and **Cattarelli, M.** (1999) *Modulation of the piriform cortex activity by the basal forebrain: an optical recording study in the rat*. Brain Res., 805, 105–111.

Schwob, J.E. and **Price, J.L.** (1978) *The cortical projections of the olfactory bulb: development in fetal and neonatal rats with additional observations in the adult*. Brain Res., 151, 369–374.

Singer, W. (1993) *Synchronization of cortical activity and its putative role in information processing and learning*. Annu. Rev. Physiol., 55, 349–374.

Vertes, R.P. and **Kocsis, B.** (1997) *Brainstem-diencephalo-septo-hippocampal systems controlling the theta rhythm of the hippocampus*. Neuroscience, 81, 893–926.

Vigouroux, M. and **Chaput, M.** (1988) *A simple and flexible device to odorized large stimulation areas*. Chem. Senses, 13, 587–596.

Vigouroux, M. and **Royet, J.P.** (1981) *An olfactometric cage suitable for short duration stimulation of unrestrained small animals*. J. Neurosci. Methods, 4, 189–196.

Vinogradova, O.S., Kitchigina, V.F. and **Zenchenko, C.I.** (1998) *Pacemaker neurons of the forebrain medial septal area and theta rhythm of the hippocampus*. Membr. Cell Biol., 11, 715–725.

Welch, P.D. (1967) *The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short modified periodograms*. IEEE Trans. Audio. Electroacoust., AU-15, 70–73.

Woolf, N.J., Eckenstein, F. and **Butcher, L.L.** (1984) *Cholinergic systems in the rat brain: I. Projections to the limbic telencephalon*. Brain Res. Bull., 13, 751–784.

Zaborszky, L., Carlsen, J., Brashear, H.R. and **Heimer, L.** (1986) *Cholinergic and GABAergic afferents to the olfactory bulb in the rat with special emphasis to the projection neurons in the nucleus of the horizontal limb of the diagonal band*. J. Comp. Neurol., 243, 877–906.

Zibrowski, E.M. and **Vanderwolf, C.H.** (1997) *Oscillatory fast wave activity in the rat pyriform cortex: relations to olfaction and behavior*. Brain Res., 766, 39–49.

Accepted March 31, 2000