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Respiratory Modulation of Olfactory Neurons in the Rodent Brain

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Abstract

In this review we report data from freely breathing animals in an attempt to show how respiratory dynamics can influence bulbar and cortical activity. Relying on *in vivo* data as well as *in vitro* observations, we try to emphasize the multiple mechanisms that underlie this modulation, its multiple origins, and its possible functional role.

Key words: mitral cell, olfactory bulb, phase-locking, respiration

Introduction

The olfactory sense is intimately related to respiration. Their close relationship occurs because in mammals the respiration cycle itself provides the mechanism for sampling of odor stimuli. This repetitive and periodic sampling inevitably has strong effects on olfactory dynamics. For example, the rate of nasal airflow affects the odorant detection threshold (Le Magnen, 1945; Rehn, 1978; Laing, 1983; Youngentob *et al.*, 1986, 1987). Indeed, intensity being constant, the detection threshold is lowered by increasing rhythm and flow rate. Through sniff control, humans demonstrate compensatory mechanisms to maintain perception: when airflow rate is reduced, sniff duration increases (Sobel *et al.*, 2000).

The relationship between olfaction and respiration appears to be reciprocal. This has been nicely shown by a series of experiments where rats were trained to discriminate different sites of electrical stimulation in the mitral cell layer (MCL) as cues for predicting the nature of incoming reinforcement. Identification of such stimuli induces a discriminative respiratory response (Monod *et al.*, 1989). Thus, mimicking odorant stimulation, electrical stimulation of the olfactory bulb (OB) is capable of triggering sniffing (Monod, 1983). Moreover, it has been shown in the rabbit that synchrony between neuronal activity of the respiratory centers and respiratory movements was lost at the start of sniffing, indicating an action of the olfactory system on the respiratory generating mechanisms (du Pont, 1987). This tight reciprocal influence of both systems obviously has important outcomes for physiological olfactory mechanisms, and one may ask to what extent it could be part of olfactory processing. Noam Sobel, in this issue, deals with the possible role of olfactomotor sniff control in olfactory processing.

The first consequence of periodic sampling during nasal inhalation is revealed at the peripheral level where it induces rhythmical activity in olfactory receptor neurons. This is seen in electroolfactogram recordings showing changes in potential that are coupled to respiration (Chaput, 2000). Obviously, such rhythmic activation of olfactory receptor neurons could provide an oscillatory drive controlling the timing of mitral/tufted (M/T) and cortical cell activity. Besides this peripheral influence, bulbar and cortical activities are probably also indirectly subject to centrifugal controls from respiratory centers (Vibert *et al.*, 1979).

In this review, we will first describe the different expressions of respiratory influence onto mass and unitary activities in the OB and cortex. Then, we will attempt to review the various possible mechanisms that could underlie such a modulation. Finally, we will discuss the possible functional role of respiratory modulation and will propose a synthetic view of the supposed implicated mechanisms.

Respiratory influence on mass activities

Since the earliest electrophysiological studies of the olfactory system, the powerful influence of breathing has been noticed in the OB and cortex. Adrian (1942, 1950) had pointed out that the velocity of the air through the nose was the principal factor in determining what he called ''induced waves'' in the OB and cortex. He observed that oscillatory activity appeared at each inspiration. Then followed the ''Freeman years'', during which he and his colleagues devoted a series of experiments to studying electroencephalogram (EEG) and then local field potential (LFP) signals, particularly in the OB and cortex of awake rabbit (Boudreau and Freeman,

1963; Freeman, 1978, 1983; Freeman and Schneider, 1982). EEG signals were characterized by the alternation between a surface-negative slow wave during inspiration and a surface-positive wave during expiration. As for LFP signals, they were described as powerfully determined by respiration. Particularly, the odorant-induced bursts in the γ frequency range observed in the OB and cortex were correlated to respiratory activity, beginning near the crest of inspiration and extending into the expiratory period. The authors also showed that such induced activity disappeared if the nostrils were pinched shut to force mouth breathing (Freeman and Schneider, 1982). More recently, γ bursts were evidenced at each respiratory cycle in waking rats (Martin *et al.*, 2004). However, the aim of all these experiments was not the study of the temporal relationships *per se* between respiratory and LFP signals. Recently, we examined such relationships in the anesthetized rat (Buonviso *et al.*, 2003). We showed that odor stimulation induced oscillatory bursts in bulbar LFPs modulated by high-amplitude respiratory waves. Using wavelet analysis methods, we observed two oscillatory epochs: one in the γ range (40–80 Hz), the other in the β range (15–35 Hz). As shown in Figure 1, these two epochs appeared related to breathing with a striking alternation during the respiratory cycle: while γ oscillations almost exclusively occur around the transition period between inspiration and expiration (I/E), β waves occur primarily during a period going from exhalation to the maximal inhalation epoch.

Apart from the work of Freeman (1978, 1983), only a few data are available regarding respiratory modulation of EEG or LFP signals in the olfactory cortex, and these data remain mainly descriptive. The most accurate study showed that bursts of γ activity were initiated by inspiration and that correlation between bulbar and cortical activities was higher during this period (Bressler, 1987). In a recent study in the anesthetized rat, Fontanini *et al.* (2003) evidenced the occurrence of slow oscillations (<1.5 Hz) in the LFP of OB and cortex whose timing is correlated to the ongoing periodic input resulting from respiration.

The fact that the appearance of β and γ OB bursts depends on the respiratory phase might indicate that the function and/or the target of the information conveyed by bulbar output neurons changes according to the epoch of the respiratory cycle during which the stimulus is delivered. As we hypothesized (Buonviso *et al.*, 2003), the I/E γ period could be devoted to information transmission and communication towards cortex, while β epoch would be devoted to feedback controls. However, a second hypothesis might be advanced that the respiratory modulation itself could carry some information about the odor. Data from voltage-sensitive dye imaging can support this hypothesis. Indeed, odorant stimulation has been shown to induce a respiration-synchronized activation at the glomerular level of the rat and mouse OB (Spors and Grinvald, 2002). It is noteworthy that the temporal component of this modulation is odor specific: amplitude, phase, and spatial distribution of respiratory synchronization are modulated in an odor-specific manner. If such features do not simply reflect the dynamic recruitment of the different types of receptor cells by odor molecules, they could underlie olfactory coding.

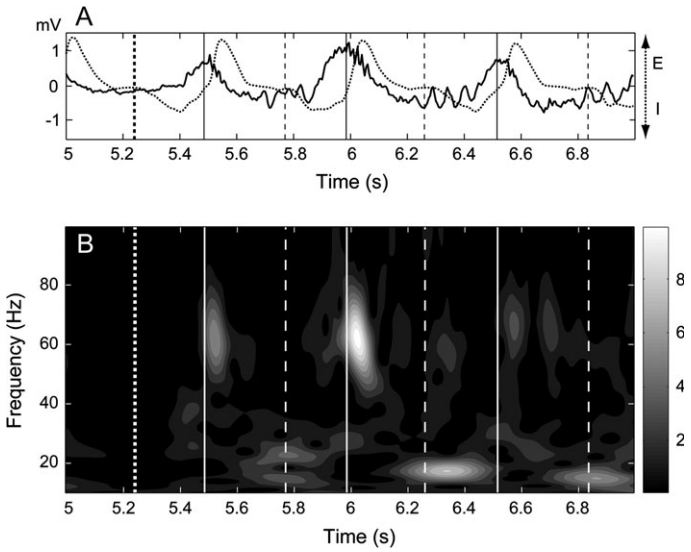

Figure 1 Time–frequency representation of an LFP bulbar signal. **(A)** LFP raw signal (0–208 Hz) and respiratory signal (dotted line). E = expiration, I = inspiration. **(B)** Corresponding scalogram. *x*-axis, time in seconds; *y*-axis, frequency from 10 to 100 Hz. The first dotted vertical bar signals stimulus onset. The gray scale codes signal power, which is defined relative to the maximal energy of the signal.

Respiratory influence on unitary activities

Respiratory synchronization of unitary activity has been extensively reported in the OB; conversely, it has been only recently described in the olfactory cortex. Among all the works conducted *in vivo*, a great number have been carried out using a protocol of artificial sniff. Since such a design may provide data very different from those obtained in the freely breathing animal, these works have been cited in this review only in the case where such a protocol has been purposefully used with the intention to study its effects on respiratory modulation.

Respiratory influence defines temporal patterns of firings

Spontaneous activity of M/T cells has been shown to be synchronized with breathing by a number of studies in both the anesthetized (Walsh, 1956; Macrides and Chorover, 1972; Onoda and Mori, 1980) and awake preparations (Chaput and Holley, 1979; Pager, 1980, 1981). This synchronization could appear difficult to explain in absence of any odor stimulation. Some studies attempted to resolve this question, but the results are still contradictory. On the one hand, Pager (1980) showed that the modulation of M/T multiunit activity by the respiratory cycle persisted when animals were tracheotomized. On the other hand, Onoda

and Mori (1980) reported that temporal firing patterns of OB units were correlated with the inhalation phase during spontaneous respiration, but they observed that this correlation was lost in most cells when airflow at the nostril was bypassed. In such cases, the cause of activation in the absence of odor might be due to chemical contamination of deodorized air or mechanical stimulation of receptor cells by airflow.

During odorant stimulation, the respiratory synchronization of M/T cells becomes quite obvious, as already pointed out even in the earliest reports (Walsh, 1956). For the first time, Macrides and Chorover (1972) claimed that a change in the overall firing frequency of M/T cell discharges was not a sufficient measure of responsiveness. Indeed, odor-evoked M/T cell responses are better characterized by temporal reorganization of their discharges into respiration-related bursts of spikes. In order to characterize this synchronization, a number of analytical measures have been employed, such as inhalation cycle histograms (Macrides and Chorover, 1972), inhalation-synchronized histograms (Potter and Chorover, 1976), complex functions—F(J) and G(J)—sensitive to both mean frequency and temporal pattern (Scott, 1977), and inspiration and expiration profiles (Chaput and Holley, 1980). All of them confirmed that M/T cells showed a definite temporal pattern of firing with respect to particular phases of the respiratory cycle. Chaput and Holley (1980) provided the evidence that an accurate evaluation of the cell responses required the separate processing of the inspiration- and expiration-related activity. In fact, the separate processing made it possible to clear up the confusion between cycle-response profiles induced by different odorants (Chaput, 1986). Then, several authors attempted to classify response patterns as a function of the respiratory cycle. Pager (1985) described, in the unrestrained rat, six typical groups of units based on the respiratory cycle epoch during which they preferentially discharged, ranging from early inspiration to expiration. In the anesthetized rat, we described nine temporal patterns based on the variation of discharge along the respiratory cycle (Buonviso *et al.*, 1992; Chaput *et al.*, 1992). Figure 2 summarizes the different types of temporal patterns among those displaying a respiratory synchronization.

"Excitatory simple synchronized patterns" have a single increase in firing activity at some point in the respiratory cycle, "suppressive simple synchronized patterns" display a single decrease or cessation of firing activity at some point in the respiratory cycle, and "complex synchronized patterns" exhibit multiple firing frequency changes along the respiratory cycle. In most cases, the bursting period (for excitatory patterns) or burst-suppression period (for suppressive patterns) occurred at the transition inspiration/expiration (I/E) epoch. And this period has been shown to coincide with the time of occurrence of the γ waves (Buonviso *et al.*, 2003).

At that time, no intracellular data were available which could help us in interpreting those types of responses. Today,

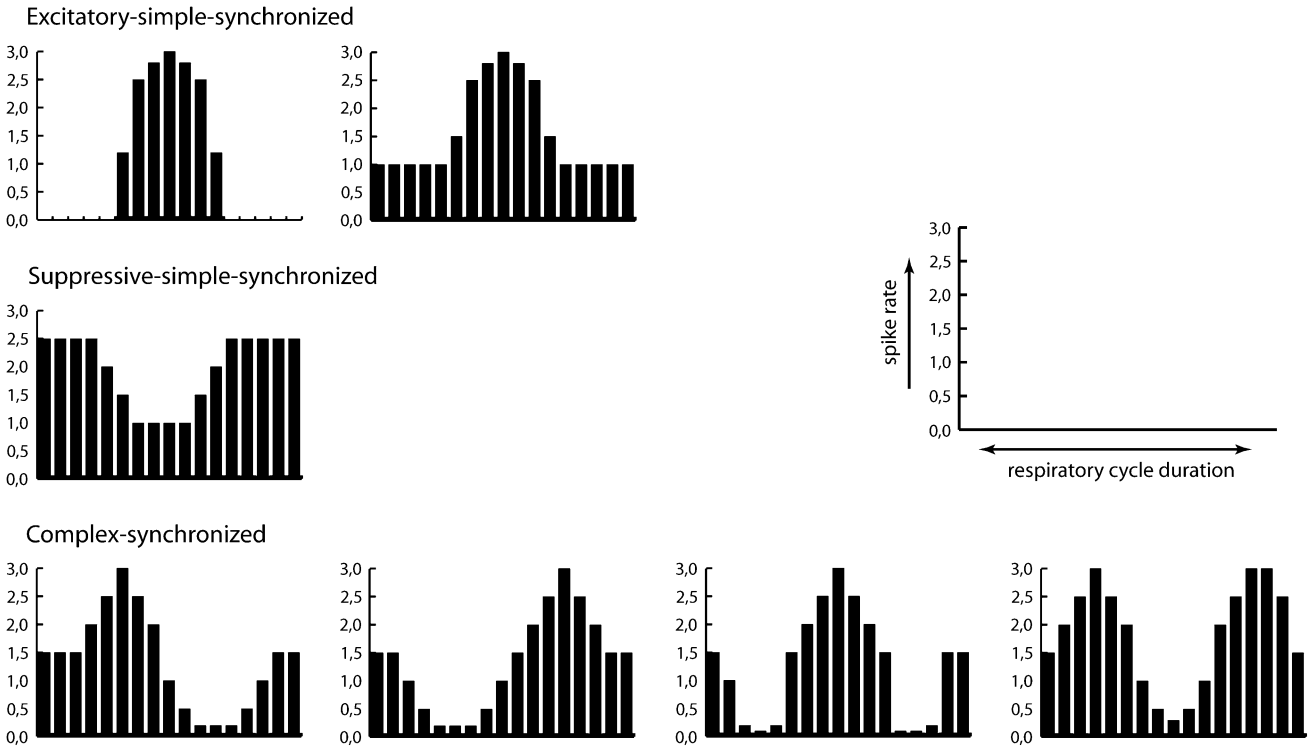

Figure 2 Illustration of respiration-based temporal patterns represented as cycle-triggered histograms. Only types displaying a respiratory synchronization are represented. *x*-axis: duration of a respiratory cycle; *y*-axis: firing frequency.

some intracellular and whole-cell recordings have been performed which shed light on the physiological bases of such patterns. Thus, it has been shown that mitral cell excitatory odor responses were characterized by synaptic depolarization phase locked to the respiratory cycle and upon which are superimposed bursts of action potentials, as illustrated in Figure 3A (Charpak *et al.*, 2001; Cang and Isaacson, 2003, Margrie and Schaefer, 2003). When hyperpolarizing current was injected into M/T cells that responded to odor stimulation with bursts of spikes, these cells displayed subthreshold membrane potential depolarizations, and such depolarizations were clearly correlated with the respiration cycle (Cang and Isaacson, 2003).

The authors proposed that these excitatory responses reflect the summation of Excitatory Post-Synaptic Potential (EPSPs) from olfactory receptor neurons. Thus, even if the mean frequency is not increased during excitatory simple synchronized patterns, such patterns are true excitatory responses. Similarly, inhibitory responses have been characterized by Inhibitory Post-Synaptic Potential (IPSPs) that were also coupled to the respiratory rhythm (Cang and Isaacson, 2003; Margrie and Schaefer, 2003) as shown in Figure 3B. Thus, what we called ''suppressive simple synchronized'' patterns are probably true inhibitory responses. These IPSPs might be attributable to lateral inhibition initiated by other M/T cells that were themselves excited by the odor. Complex types are probably a mixing of depolarization and hyperpolarization sequences. Indeed, Charpak *et al.* (2001) observed mixed excitatory/inhibitory responses in their intracellular recordings.

By comparison to M/T cells, more superficial cells such as external tufted (ET) and periglomerular (PG) cells have been little studied. Recording cells at different depth in the OB, Onoda and Mori (1980) noticed that in the anesthetized rabbit superficial units (in glomerular and external plexiform layers) displayed discharge rates that changed consistently in relation to air-intake cycles and were more strongly influenced by the pattern of olfactory nerve inputs. Compared to M/T cells, they presented simpler temporal patterns. This strong relation with input was reported again by intracellular recordings of PG cells (Wellis and Scott, 1990), showing that odor stimulation produced simple bursts of action potentials. Recently, we reported the existence of two cellular types in the glomerular layer (GL1 and GL2 types), both exhibiting odor-evoked activities synchronized with the respiratory cycle (Buonviso *et al.*, 2003). GL1 cells, which were probably ET cells, exhibited such simple patterns: they responded exclusively by excitatory simple synchronized patterns, whatever the odor used, and were phase locked to the early inhalation period. By contrast, GL2 cells discharge more likely around the I/E epoch, as do M/T cells.

Figure 4 depicts phase relationships relative to respiration for cells recorded in the different OB layers. It illustrates the fact that GL2, as EPL and MCL cell discharges, are locked to the transition I/E epoch. Note that the mean phase of γ oscillatory bursts coincides with the same period. Conversely, GL1 cells are phase locked to the early inhalation period. The diagram of mean vectors shows that GL1 cell discharges precede the γ burst. Obviously, temporal patterning of superficial cells seems to be simpler and is more strongly influenced by breathing than deeper M/T cells.

Surprisingly, the deepest granule cells have been also characterized by simple temporal firing patterns by Onoda and Mori (1980). Their responses have been described as spiking or nonspiking and nonhabituating (response to every sniff) or habituating (response to the first sniff only), but in all cases rhythmic depolarizations were coupled to the

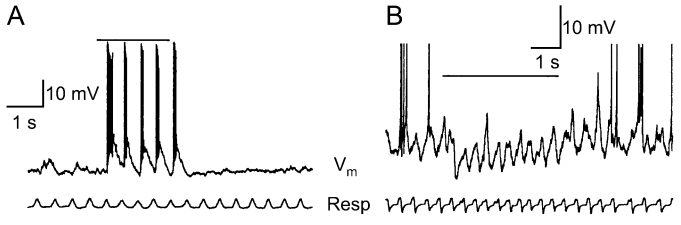

Figure 3 Odor-evoked activity in two M/T cells. **(A)** Excitatory response (bursts of action potentials) evoked by heptanal. **(B)** Inhibitory response evoked by amyl acetate. V_m: membrane potential; Resp: respiratory signal. Horizontal bars indicate the duration of odor application. (With permission, from Cang and Isaacson, 2003.)

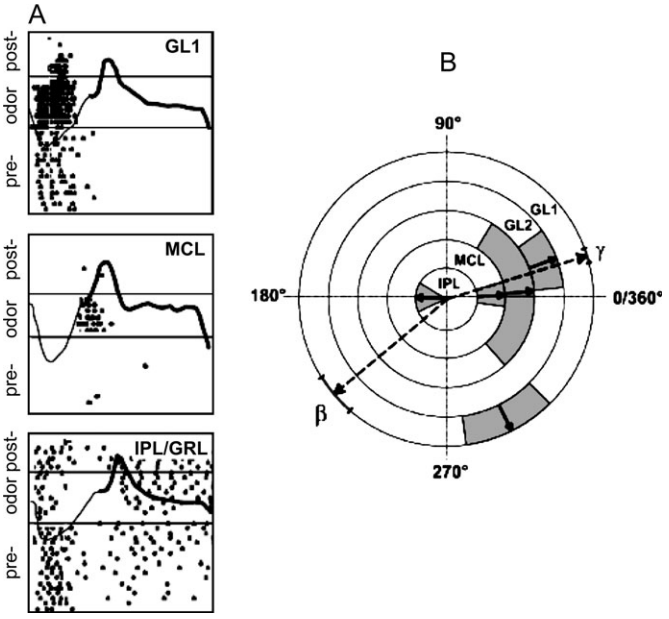

Figure 4 **(A)** Examples of cycle-triggered raster plots obtained from three typical activities of GL1, MCL, and IPL/GRL cells. Spikes are represented as a function of the respiratory cycle. Consecutive respiratory cycles are arranged vertically from bottom to top. The mean respiratory signal is superimposed on each raster plot (inhalation, thin line; exhalation, thick line). **(B)** Mean vector plots of bulbar unit activities (solid arrows), along with β and γ waves (dotted arrows) mean phase as a function of respiratory cycle. Gray boxes represent 2SD. The transition I/E point is roughly located around the γ vector. (With permission, from Buonviso *et al.*, 2003.)

respiration rhythm (Wellis and Scott, 1990; Cang and Isaacson, 2003). We recorded cells located deeply in the internal plexiform layer they characteristically discharged late, during β oscillations of exhalation epoch, as seen in the bottom raster plot and in the mean vector diagram of Figure 4. Due to the location of their somata, these neurons could be horizontal cells described by Schneider and Macrides (1978) and thus might be interneurons.

As already pointed out, there are very few data available about cortical cell responses to odors. Respiratory synchronization of unitary activities has been only recently observed in the olfactory cortex of anesthetized rats (Wilson, 1998; Bouret and Sara, 2002; Litaudon *et al.*, 2003). The piriform cortex (PC) is a functionally heterogeneous structure that could be divided into anterior PC (aPC) and posterior PC (pPC) part (Litaudon *et al.*, 1997a,b). Cortical cells in the aPC share several features with M/T cells. Spontaneous activity of aPC cells is lower than in M/T cells but has been shown to be largely synchronized with breathing. This synchronized spontaneous activity is clearly phase locked with early exhalation (Wilson, 1998; Litaudon *et al.*, 2003). As observed in the OB, aPC cell responses are mainly characterized by temporal reorganization of their discharges into respiration-related bursts of spikes. Litaudon *et al.* (2003) reported that the temporal pattern of odor-evoked responses in the aPC is less complex than in the OB and could be classified as excitatory simple synchronized patterns (Figure 5), spikes being phase locked with the inhalation/exhalation transition (I/E) epoch.

However, intracellular recordings (Wilson, 1998) indicate that odor-induced postsynaptic potentials are more complex since they display a hyperpolarizing–depolarizing or a depolarizing–hyperpolarizing sequence over a respiratory cycle. These temporal patterns, at least in the aPC, may be more dynamic than observed in the OB. Indeed, during prolonged odor stimulation, although OB activity remained relatively phase locked to a particular phase of respiration, aPC activity did not, and the odor-induced phase-shifts habituated rapidly (Wilson, 1998). In the pPC, cell phase locking is more varied (Litaudon *et al.*, 2003). Mean vector representation allowed us to classify pPC cell responses as "late synchronized responses" (phase locked on exhalation) and "early synchronized responses" (phase locked on inhalation) (Figure 5B).

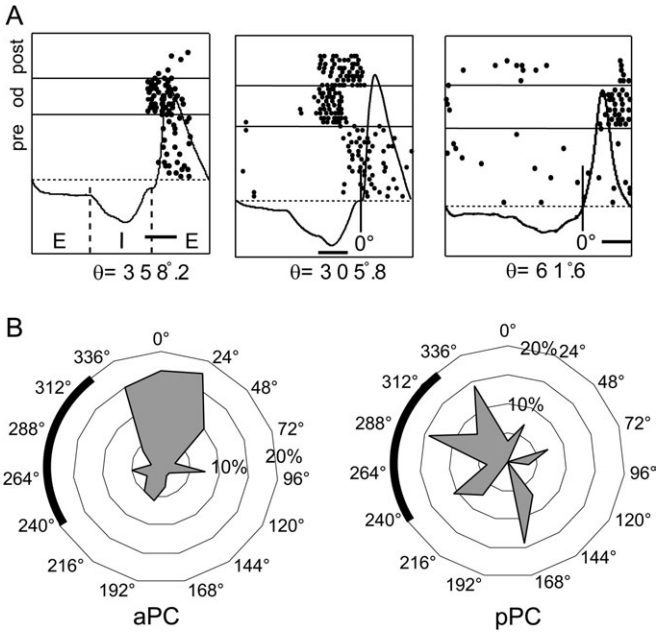

Figure 5 Temporal patterns of PC cell responses. **(A)** Respiratory cycle–triggered raster plots illustrating three examples of synchronized responses (from left to right: I/E response, early response, and late response). Activity is represented from bottom to top before (pre), during (od), and after odor presentation (post). The thick horizontal line indicates the position of the spike burst during odor presentation. Respiratory cycle trace is superimposed on each raster. I = inhalation. E = exhalation. Mean vector phase relative to respiratory cycle (θ), calculated during odor presentation, is indicated below each diagram (0° is defined as the transition between inhalation and exhalation). **(B)** Phase distribution of synchronized excitatory responses in the PC. From unit activity of each synchronized response, we calculate the mean vector whose direction indicates the mean phase of discharge relative to the respiratory cycle. All vectors in each PC area are classified in 24° bins, and their distribution (shade area) is represented on a circular frequency histogram. The thick arc indicates the inhalation epoch. (With permission, from Litaudon *et al.*, 2003.)

Respiratory influence emphasizes the unitary functioning of the glomerulus

Given the idea that each glomerulus might function as a unit for processing sensory input (Jourdan *et al.*, 1980; Buonviso and Chaput, 1990; Spors and Grinvald, 2002), it was fundamental to investigate to what extent M/T cells from a single glomerulus could be similarly phased with regard to respiratory cycle in response to odor. Simultaneous recordings of cell pairs in the *in vivo* rat (Buonviso *et al.*, 1992) have provided the first demonstration. In this study, recorded cells were separated by <50 μm in order to have a great chance to be related to the same glomerulus. The remarkable result was that such nearby cells presented a probability higher than chance to exhibit similar temporal patterns of firing. Furthermore, they tended to burst synchronously according to the respiratory cycle as exemplified in Figure 6.

Such synchronization between M/T cells of a single glomerulus might originate in at least three concomitant mechanisms:

1) A common afferent input.
2) A similar control by granular cells: neighboring M/T cells receive granular-induced inhibition with similar latencies and durations with a probability much higher than independent cells (Buonviso *et al.*, 1996).
3) A common glomerulus-originating slow oscillatory activity: recent patch-clamp studies in OB slices revealed that mitral cells associated with the same glomerulus

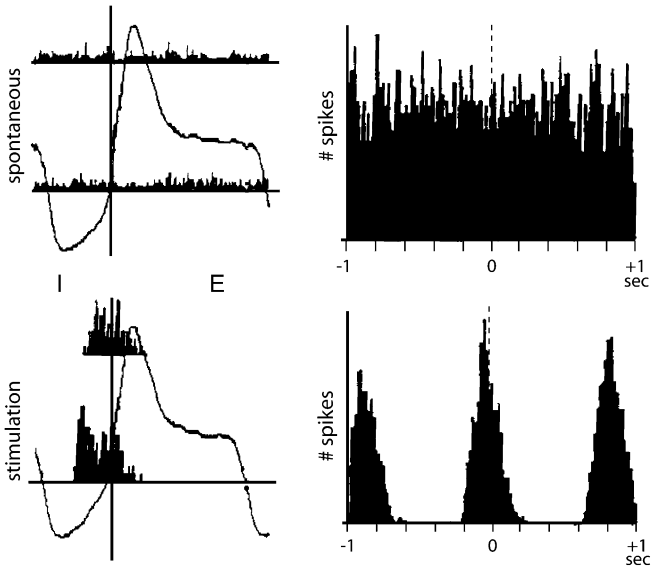

Figure 6 Synchronization between single-glomerulus connected cells. On the left are shown the cycle-triggered histograms corresponding to the spontaneous and odor-evoked activities of two neighbor M/T cells (separated by less than 50 μm), along with the form of the mean respiratory cycle. I = inspiration, E = expiration. On the right, the cross-correlograms show the temporal correlation between the activities of these cells before (top) and during (bottom) stimulation. Both cycle-triggered histograms and cross-correlograms reveal that during odorant stimulation the two cells are similarly phase locked with the transition I/E epoch. (With permission, from Buonviso *et al.*, 1992.)

exhibit synchronous long-lasting depolarizations that have been interpreted as a sustained recurrent excitatory synaptic activity of glomerular origin (Carlson *et al.*, 2000). Furthermore, electrical stimulation of afferents elicits slow (2 Hz) oscillations that are highly synchronized for mitral cells projecting to the same glomerulus (Schoppa and Westbrook, 2001).

All these phenomena could endow the M/T cells of a single glomerulus with the synchronized patterns we observed. Such a common synchronization has also been observed for superficial cells by Hayar *et al.* (2004b), who found that spontaneous bursting was highly correlated among ET cells of the same glomerulus.

Respiratory influence is a robust mechanism

Respiratory phase locking of mitral cell activities seems to be a very powerful and robust mechanism given that it can be affected only in very few cases. One of the rare cases where temporal patterning of M/T cells may disappear is when the respiratory frequency reaches the sniffing rate both in spontaneous and odor-evoked activity (Pager, 1981). Bhalla and Bower (1997) reported that rapid sniffing greatly increased response variability and weakened the general spatial organization of responses. This could be simply due to the fact that the rapidity of sniffing (8–12 Hz) does not permit one to observe a pause in spike discharge between two excitatory inhalation events. But another explanation can be found in the interesting observation made by Schoppa and Westbrook (2001) in OB slices. They showed that rhythmical slow depolarizations were induced in M/T cells by a single olfactory nerve shock; such oscillations can be maintained when trains are delivered to olfactory nerve in the extent that the frequency of shocks does not exceed 8 Hz. The interpretation of such phenomenon could be that M/T cells would be spontaneously locked to a ~2 Hz rhythm; they would be entrained to breathing rhythm when the peripheral input is delivered in a frequency range close to that rhythm (<8 Hz) but would loose this property with an afferent rhythm of higher frequency. This hypothesis needs to be verified in the awake animal during sniffing, which is the most biologically relevant situation.

The second manipulation that could affect M/T cell respiratory locking is OB isolation (Potter and Chorover, 1976; Chaput, 1983). The respiration-related synchronization of neurons recorded in isolated bulbs is markedly reduced compared to that in intact animals. Particularly, although cells continued to show inhalation-related synchronization, it was much less salient since units displayed only a weak period of excitation and no period of strong inhibition.

Except these rare cases, respiratory synchronization seems to be very significant and robust. Thus, it appears very early in rat pups where mitral cell activity responses are time locked to the inhalation cycle (Mair and Gesteland, 1982), even if there is a decrease in the number of cells exhibiting correlation (during spontaneous activity) with age (Philpot *et al.*, 1997). Moreover respiratory synchronization seems to be maintained in spite of several different manipulations. First, M/T cell discharges maintained a similar respiratory phase locking with increasing odor intensity (Chalansonnet and Chaput, 1998). Second, patterning of OB single-unit activity was observed even when odor was applied as a series of "inspirations" without intervening expirations (Sobel and Tank, 1993).

Origin of the respiratory modulation

Several groups attempted to investigate the origin of respiratory synchronization. The most evident possibility is a peripheral origin since the phasic nature of the inputs may easily determine the phasic response of bulbar cells. Second, the bulbar activity may be phasically modulated by centrifugal projections which are themselves synchronized to breathing. Third, local mechanisms and intrinsic circuitry may play an important role. Of course, these three possibilities are likely not exclusive.

Several observations favor a central origin of respiratory modulation. The most convincing arguments come from experiments in which OB has been disconnected from the brain or where the nasal airflow has been manipulated. Indeed, the synchronization of units with the inhalation

cycle has been shown to be markedly reduced in animals with isolated OB as compared with normal preparations (Potter and Chorover, 1976; Chaput, 1983). Similarly, while the relation between respiration and neuronal activities is maintained in tracheotomized animals, such synchronization is lost if the olfactory peduncle is sectioned (Pager, 1980). Ravel and coworkers have explored in more detail the olfacto-respiratory relations in a series of experiments where animals were either intact or tracheotomized, or alternatively both (Ravel *et al.*, 1987; Ravel and Pager, 1990). Respiratory patterns of unit activity were still observed in more than one-third of cells even in the absence of intranasal airflow. Also, the authors concluded that respiratory modulation of M/T cell activity involved a central component.

Other data favor the idea of a peripheral origin. Indeed, whereas OB units showed temporal firing patterns correlated with the inspiratory phase during spontaneous respiration, such periodic activity disappeared when airflow at the nostril was bypassed (Macrides and Chorover, 1972; Onoda and Mori, 1980). Similarly, the peak at 2 Hz observed in the OB with voltage-sensitive dye methods was suppressed with tracheal breathing (Spors and Grinvald, 2002). Hence, the periodic activity seems to be mainly due to periodic activation of olfactory receptor cells. The observation that the patterning of OB activity was synchronized with the time course of the nasal stimulation was later confirmed by Sobel and Tank (1993), who gave the nicest demonstration that the respiratory patterning of M/T cells was not directly dependent on centrifugal inputs synchronized to respiration. They used the double tracheal cannulation technique in order to uncouple stimulation of the olfactory epithelium from respiration. This protocol allowed the odor stimulation to be applied at different points in the ongoing respiratory cycle. The authors showed that the patterning of OB units was unchanged when odors arrived at different phases of the ongoing respiratory cycle. Moreover, patterning of OB single-unit activity was also observed when odor was applied as a series of inspirations without intervening expirations. The authors concluded that the observed respiration-related patterns of M/T cell discharge reflected the phasic stimulation of the olfactory receptors with each inspiration.

Pharmacologically, respiratory oscillations of M/T cells can be reduced by OB superfusion of sodium channel blockers Tetrodotoxin or glutamate receptor antagonists NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline) plus D-APV (2-amino-5-phosphonovalerate) (Margrie and Schaefer, 2003). After intraperitoneal injection of an NMDA (N-methyl-D-aspartate) receptor antagonist (MK-801), respiration-related mitral cell activity became asynchronous (Philpot *et al.*, 1998), while it did not have a systematic effect on the firing rate of M/T cells. All these data confirm the peripheral origin of respiratory M/T cell modulation and show furthermore that such modulation is mediated by glutamatergic activation. Besides, Spors and Grinvald (2002) showed that the fluorescence changes they observed at 2 Hz, reflecting at least partially changes in bulbar neurons membrane potential, are blocked by TTX and significantly reduced by the glutamate antagonist. Therefore, this slow rhythm is dependent on nasal airflow, requires action potential generation/propagation, and glutamatergic transmission within the OB.

Until a few years ago, central and peripheral origins were only the two possibilities considered to explain M/T cell respiratory synchronicity, and only little importance was attached to local and/or intrinsic bulbar circuitry mechanisms. The debate has not been simplified when patch-clamp recording techniques in OB slices raised the possibility of a glomerular and/or intrinsic origin of slow oscillations. Of course, it seems difficult to refer to "respiration" in slice preparations. However, several groups have evidenced slow oscillations in such preparations, in a frequency range close to that of sniffing. Indeed, Schoppa and Westbrook (2001) reported that electrical stimulation of ON fibers elicited slow (2 Hz), synchronized oscillations in mitral cells along with an underlying persistent depolarization. Bath application of NMDA induced similar oscillatory activity. Since only mitral cells projecting to the same glomerulus showed highly synchronized oscillatory activity, they concluded that synchrony originated in glomeruli. Using a battery of pharmacological devices, they showed that the persistent depolarization was generated by glutamate activation of dendritic autoreceptors, while the slow frequency was determined primarily by the duration of regenerative glutamate release. This was the first demonstration of a locally induced rhythm, whose frequency has a timescale similar to that of breathing.

Several other groups have recorded bulbar cell oscillations in slices and postulated that these cells could oscillate spontaneously. McQuiston and Katz (2001) recorded from glomerular interneurons and found a category of bursting neurons (most of them being PG or ET cells) which produced a calcium channel–dependent low-threshold spike when depolarized. These bursting neurons also could oscillate spontaneously in the delta range (1.7 ± 0.2 Hz). Similarly, while recording ET cells, Hayar *et al.* (2004a) pointed out that a significant population of ET cells spontaneously generates rhythmic spike bursts at frequencies associated with rodent sniffing. Rhythmical bursting was not affected by receptor blockers; on the contrary, elimination of synaptic input increased the regularity of bursting. They concluded that rhythmical bursting might be an intrinsic property of those cells. ET cell bursts were entrained on olfactory nerve stimuli delivered on the range of sniffing frequency. They showed that persistent sodium channels were essential for such spontaneous bursting. Moreover, this spontaneous bursting was highly correlated among ET cells of the same glomerulus (Hayar *et al.*, 2004b), hence reinforcing the concept of the glomerular unit. Similarly, mitral cells have been shown to generate intermittent, irregularly timed spike clusters at slow theta frequency (Balu *et al.*, 2004). The main finding of this experiment is that simple step depolarization

induces highly variable, unreliable spiking of mitral cells; on the contrary, mitral cells can respond reproducibly when phasic stimuli are delivered in the theta frequency range, a rhythm which has been shown to be related to sniffing (Komisaruk, 1970). These properties enable mitral cells to act as high-pass filters, responding selectively to stimuli repeated at >1 Hz and ignoring single simulated EPSP events.

The origin of respiratory modulation in the olfactory cortex is not known. Fontanini *et al.* (2003) noticed a correlation between bulbar and cortical LFPs and membrane potential of pyramidal cells. Moreover, the periodic behavior of membrane potential is not dependent on breathing in tracheotomized animals, and the correlation is returned with air-puff stimulation. Hence, it can be hypothesized that the temporal pattern of cortical cells in the aPC was mainly driven by bulbar M/T cells. Such a hypothesis is in agreement with the functional coupling between OB and aPC as previously observed (Chabaud *et al.*, 1999).

Conclusions: a hypothesis for functional role of respiratory modulation

Respiratory modulation of bulbar and cortical activities has multiple origins, and it is probably the combination of all of them that makes it's influence so robust. First of all, the peripheral rhythmic effect of sampling drives bulbar activity. Then, glomeruli and bulbar cells seem to be intrinsically pretuned to a frequency close to respiratory frequency; this intrinsic frequency can be entrained by the natural rhythmic sensory input. Finally, centrifugal controls originating both in olfactory cortices and respiratory centers could themselves act phasically. All those different mechanisms probably overlap and operate together.

However, the essential information about an odor does not seem to be contained in the respiratory temporal patterns of cells since no study has ever demonstrated specificity in such patterns. Thus, we never found a temporal pattern which predominates in response to a particular odor (Buonviso *et al.*, 1992; Chaput *et al.*, 1992). Similarly, Cang and Isaacson (2003) observed that the excitatory responses of individual M/T cells to different types of odors could be remarkably similar. So, bulbar respiratory modulation *per se* does not seem to be part of odor coding.

Conversely, respiratory modulation could be implicated in other mechanisms such as information routing or communication between areas in the network. We hypothesized that the temporal window of a respiratory cycle could be split into three phases, each related to a particular function (Buonviso *et al.*, 2003): 1) by triggering the activity into the bulbar network, the early inspiratory patterns of glomerular cells could be dedicated to a priming function; 2) mitral and tufted cell discharges occurring during the γ episodes around transition I/E epoch would serve a gating function, for which relative synchrony between bulbar and cortical γ oscillations would make signal transfer possible; and 3) the late and slow expiratory patterns of deeper cells could serve a tuning function, modulating the bulbar message during β oscillatory episodes. Such late and slow patterns are probably due to central controls which might function as permissive filters onto mitral cells during the incoming of sensory signals.

In such a hypothesis, the different epochs of a respiratory cycle would not be equivalent in function. However, these assumptions are mainly drawn from results acquired in the anesthetized animal. Now, the crucial point is to know to what extent those assumptions might hold during sniffing in the awake animal. Monod (1983) provided a first clue when she showed that the animal receiving an electrical stimulation in the MCL gave a different respiratory response if it was delivered during inhalation or exhalation. The most spectacular effect was observed when stimulation was delivered at the end of the inhalation phase: in this case, the effect was an important shortening of inhalation phases extending to several cycles and a shortening of the exhalation in the first cycle. These results tend to indicate that the respiratory phase could represent an important parameter in olfactory processing in the waking animal.

Besides its phase, the frequency of respiratory cycles could also have an important role. One can hypothesize that an ''optimal'' frequency might exist which could, on the one hand, facilitate an odor molecule's access to the nasal mucosa and, on the other hand, tune optimally the duration of the temporal unit for information processing. Macrides *et al.* (1982) studied the temporal relationship between sniffing and the limbic θ rhythm in rats during odor-discrimination learning. They demonstrated a preferred latency relationship between the onset of each sniff cycle and a particular phase of the hippocampal θ rhythm driven by the medial septum–diagonal band complex. Since both rhythms change frequency together, their phase relation was maintained constant. Moreover, a strong correlation between bulbar neuron bursting activity and limbic θ cycles was also observed (Macrides *et al.*, 1979). Taken together, these results could mean that the θ rhythm might function as a coherent time base for central olfactory and limbic structures: during the periods of stimulus sampling, the sniffs would be optimally timed to maintain a preferred latency relationship with the pacemaker activity driving the θ rhythm so that information and/or memorization/extraction processes would be facilitated during epochs of phase matching between olfactory and limbic structures. However, the nature of such a ''supervisor'' center, capable of synchronizing respiratory, olfactory, and limbic structures, is a fascinating issue which still needs to be elucidated.

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