Variable Coupling Between Olfactory System Activity and Respiration in Ketamine/Xylazine Anesthetized Rats

Alfredo Fontanini1 and James M. Bower2

1Division of Biology, California Institute of Technology, Pasadena, California; and 2Research Imaging Center, University of Texas Health Science Center at San Antonio and Cajal Neuroscience Center, University of Texas San Antonio, San Antonio, Texas

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Fontanini, Alfredo and James M. Bower. Variable coupling between olfactory system activity and respiration in ketamine/xylazine anesthetized rats. J Neurophysiol 93: 3573–3581, 2005. First published February 2, 2005; doi:10.1152/jn.01320.2004. In this study, we have characterized slow and fast oscillations at several stages of olfactory processing under light and deep ketamine/xylazine anesthesia in the albino rat. While monitoring the animal’s respiration, we also obtained field potentials from the olfactory bulb and piriform (olfactory) cortex and simultaneously recorded membrane potentials in piriform cortex pyramidal cells. Our results demonstrate that oscillations are generally found at higher frequencies under lighter and lower frequencies under deeper anesthesia. In previous studies of cerebral cortex, similar results in ketamine/xylazine anesthetized animals have been interpreted to correspond with the higher frequencies found during waking and lower frequencies found in the sleep state. Correlation and coherence analysis between data obtained in the bulb and cortex reveals a clear difference in coupling depending on the anesthetic state of the animal. Specifically, activity recorded in the whole system is highly correlated with respiration during deep anesthesia, whereas only the olfactory bulb, and not the cortex, is correlated with respiration during light anesthesia. These data suggest that global activity in the piriform cortex is actually more directly tied to peripheral slow respiratory input during slow wave than fast wave states and that the coupling between olfactory structures can be dynamically modulated by the level of anesthesia and therefore presumably by different brain states as well.

INTRODUCTION

The study of the relationship between cerebral cortical oscillations and behavioral states has been a major focus of neuroscience since the first electroencephalographic (EEG) recordings were obtained several decades ago (Adrian 1942; Bremer 1958). Perhaps the most enduring relationships found have been the association of increased cortical activity (e.g., waking and rapid-eye-movement sleep) with high-frequency (15–100 Hz), low-amplitude oscillations (“fast” oscillations), and of presumed periods of cortical inactivity [e.g., slow wave sleep (SWS) or deep states of anesthesia] with low-frequency (0.5–1.5 Hz), high-amplitude oscillations (“slow” oscillations) (Destexhe et al. 1999; Gray and Singer 1989; Gray et al. 1989; Steriade et al. 1996a, 1993b,c). Several recent studies have gone beyond this simple association to suggest that these two states specifically correspond to different degrees of intracortical and thalamo-cortical coherence: slow oscillations are characterized by high levels of spatiotemporal coherence, whereas fast oscillatory states display restricted synchrony and are more local in nature (Destexhe et al. 1999; Steriade et al. 1996b), reflecting an intense intra-cortical activity. Additionally, these two states have been proposed to differ markedly in cortical responses to sensory stimuli (Contreras and Llinás 2001; Petersen et al. 2003; Sachdev et al. 2004; Timofeev et al. 1996). In general, these changes have been attributed to dynamical modifications of functional relationships between the neocortex and the thalamus with the thalamo-cortical loop switching between an active sensory processing state (during fast oscillations) and a passive neocortical resting state (during slow oscillations) (Destexhe and Sejnowski 2001; Steriade 2000).

Against this background, the dynamical and functional relationships within the olfactory system are interesting because the thalamus is not interposed between the olfactory periphery and the piriform cortex. Yet, like neocortex, the piriform cortex has been shown to alternate between fast and slow oscillatory states (for a review, see Lledo et al. 2005) and to vary the levels of system-wide coherence depending on the behavioral state of the animal (Bressler and Freeman 1980; Chabaud et al. 1999; Kay and Freeman 1998). Further, unlike neocortex where it has been proposed that the slow state is specifically associated with sensory deafferentation (Steriade 2000), the high degree of intracortical and bulbo-cortical coherence, found during slow oscillations, is directly related to the respiratory input and therefore to activity at the sensory periphery (Fontanini et al. 2003). This observation raises the question as to whether the less-synchronized activity recorded during fast oscillations might be less coupled to respiration and whether this decoupling occurs in the olfactory bulb or in the piriform cortex. These results may provide a different perspective on the relatively simple picture emerging from neocortical studies where slow wave states have less association with the sensory periphery than fast wave states.

The specific aim of the present report therefore was to compare and analyze the degree of system-wide (respiration, bulb and cortex) coupling during slow and fast oscillations in the same anesthetized preparation. To do so, we monitored the animal’s respiration while simultaneously recording field potentials from the piriform cortex and bulb and membrane potentials from individual cortical pyramidal cells. We report here that deep anesthesia is associated with a prevalence of slow oscillations, whereas there is a prevalence of fast oscillations during lighter anesthetic conditions. Our data further...
demonstrate that the pattern of oscillations within and between the bulb and cortex changes under each condition with a reduction in global coupling at the respiratory (afferential) frequency between bulb and cortex during light anesthesia. These results suggest that an important and perhaps more fundamental distinction between active (e.g., awake) and brain states considered to be more passive (e.g., SWS) may be in the degree of system wide coupling and therefore differences in the global versus local nature of cortical processing rather than in the presence or absence of available information from the sensory periphery, per se.

**Methods**

**Animal surgery**

All animal procedures were approved in advance by the Animal Use Committee of the California Institute of Technology. Adult Sprague-Dawley rats (250–300 g) were anesthetized with intraperitoneal ketamine/xylazine (100 mg/kg, 5 mg/kg) and mounted on a stereotaxic frame (Kopf Instruments, Tujunga, CA). Heart rate and body temperature were monitored throughout the experiment; body temperature was maintained constant (36 ± 1°C) using a custom-designed biofeedback system. The general level of anesthesia was maintained so that hind limb pinching produced no reflex movement. When necessary, an additional dose (30% of initial dosage) of ketamine/xylazine was injected intraperitoneally. Xylocaine was applied topically at the edge of all incisions and at pressure points to minimize pain. After exposure of the skull, burr holes were drilled in its dorsal part above the olfactory bulb, lateral olfactory tract, and anterior piriform cortex; the dura mater was carefully removed, and the brain was covered with mineral oil to prevent drying. The cisterna magna was widely incised, and the cerebrospinal fluid was drained to minimize brain pulsations. Respiration was monitored by recording chest wall movements using a piezoelectric device.

**Extracellular field potential recordings and electrical stimulation**

Extracellular field potential recordings and electrical stimulation were both accomplished using unipolar tungsten electrodes (Micro-Probe, Potomac, MD) with resistance of 1 MΩ. Stimulating and extracellular recording electrodes were placed in the olfactory bulb, lateral olfactory tract, and anterior piriform cortex; the dura mater was carefully removed, and the brain was covered with mineral oil to prevent drying. The cisterna magna was widely incised, and the cerebrospinal fluid was drained to minimize brain pulsations. Respiration was monitored by recording chest wall movements using a piezoelectric device.

**Intracellular recordings**

Intracellular recordings in piriform cortex were obtained using sharp electrodes pulled with a horizontal puller (P87: Sutter Instruments, Novato, CA) from borosilicate glass (1 mm OD, 0.58 mm ID, A-M Systems) and positioned ~1–2 mm from the extracellular electrode. Recording electrodes with impedance ranging between 40 and 70 MΩ were filled with 3 M potassium acetate. Electrodes were slowly (~100 μm/min) lowered dorsally with a hydraulic micropositioner (Kopf Instruments) using field potential responses to lateral olfactory tract stimulation to determine when the electrode tip had reached layers II/III of piriform cortex (usually ~6 mm ventral from the brain surface). Recordings were performed in bridge balance mode using an Axoclamp 2-A intracellular amplifier (Axon Instruments, Union City, CA). Neurons included in this study were required to maintain spontaneous membrane potentials more negative than ~60 mV with regenerative action potentials the amplitude of which exceeded 50 mV. In addition, all recorded neurons responded to stimulation of the lateral olfactory tract with the biphasic response characteristic of cells identified as pyramidal type with intracellular labeling techniques as reported in previous studies (Fontanini et al. 2003; Haberly and Bower 1984). Respiratory activity and intracellular and extracellular data were simultaneously acquired at 20 kHz with a Digidata 1200 board (Axon Instruments) connected to a PC running Clampex 8 acquisition software (Axon Instruments).

**Data analysis**

Thirty- or 60-s-long traces were used for analysis. Sixty-second-long traces were used for spectral analysis, to achieve sub-hertz resolution. After preliminary verification that the length of the segment did not influence the result, the rest of the analysis was performed on 30-s segments of data for computational efficiency. To reduce the influence of different amplitudes of the signals, all the raw recordings (membrane potential and field potentials) were normalized by subtracting the mean and dividing by the SD before performing correlation analysis.

**Histograms of membrane potentials**

Membrane potential histograms were constructed for each cell in the following manner: values of spontaneous membrane potential recorded over 30 s were collected at each time point, grouped in 0.25-mV bins and plotted as a frequency histogram. This plot provides a representation of the proportion of time spent by each neuron at a specific membrane potential. The experimental histogram was then fitted with a Lavenberg-Marquard algorithm using either a single Gaussian, in the case of the unimodal distribution observed during light anesthesia or a sum of two Gaussians in the case of the bimodal distribution observed during deep anesthesia. Parameters such as mean and SD of the Gaussians were extracted and used to describe the mean and SD of membrane potential fluctuations.

**Cross-covariance and coherence analysis**

The correlations between membrane potential of the piriform cortex pyramidal cell and local field potentials in layer I of the piriform cortex, or local field potentials in granular layer of the olfactory bulb, were obtained by computing the cross-covariance (cross-correlation normalized by the mean) between 30-s recordings of intracellular neuronal membrane potentials and local extracellular field potentials (Fig. 4, A–C). The absolute value of the peak of cross-covariance was computed within ±0.5-s lags. The coherence function was estimated using a ~2-s Hanning window, with a 1-s overlap; then the mean of the coherence between 0.5 and 1.5 Hz was computed and used as a parameter for estimating the coherence in the respiratory frequency (Fig. 4, D–F).

**Respiratory-triggered average**

The correlation between membrane potential and respiratory activity was also investigated by estimating the respiratory-wave-triggered average (Fig. 5). A breathing cycle (from the beginning of one inspiration to the beginning of the next) was divided into 16 bins, and the average membrane potential was computed within each bin. We repeated this operation for every breathing cycle in a ~30-s recording,
and the average membrane potential for each of the 16 bins was then averaged across all the respiratory cycles. Data presented in Fig. 5 are the average of all the experiments in the different conditions.

Spectral analysis

Power spectral density was estimated using Welch’s averaged periodogram method. Traces were divided into ~30-s Hanning windows, with a 50% overlap, and analyzed with a 0.128-ms resolution FFT. The relative power of a frequency band was computed by dividing the power in each band by the total power of all the bands and multiplying the result by 100. The bands analyzed were: respiratory (from 0.5 to 1.5 Hz), theta (4–9 Hz), beta (15–40 Hz), and gamma band (45–100 Hz). Power spectral density and relative power were computed for each signal in every recording condition and averaged to obtain the final averaged power spectral density and relative power shown in Fig. 6.

Filtering and envelope

Simultaneously recorded signals were filtered, as shown in Fig. 7, A–F, with a FFT-based algorithm and different bands (respiratory, theta, beta, and gamma as mentioned in the preceding text) were extracted. The envelope of beta and gamma bands was computed, as in Hartmann and Bower (2001) by removing the positive frequency components in the FFT of the data, and then taking the inverse FFT of the remaining spectrum. The power spectrum of the resulting envelope was used to compute the ratio between low respiratory frequency and the other frequency bands (Fig. 7, G–I).

Data analysis was performed using Matlab (The Mathworks). Data are expressed as means ± SD.

Histology

On the termination of the experiment an electrolytic lesion (100 μA for 5–10 s) was made to verify the proper positioning of all recording and stimulation electrodes. The rat was then deeply anesthetized (using 1 ml ip Nembutal) and transcardiatically perfused with saline and stimulation electrodes. The rat was then deeply anesthetized for 5–10 s) was made to verify the proper positioning of all recording electrode (from 0.5 to 1.5 Hz), theta (4–9 Hz), beta (15–40 Hz), and gamma band (45–100 Hz). Power spectral density and relative power were computed for each signal in every recording condition and averaged to obtain the final averaged power spectral density and relative power shown in Fig. 6.

RESULTS

Single-cell activity during ketamine/xylazine anesthesia

The results presented here are based on 27 neurons recorded in the piriform cortex of 16 rats. Twenty five of these recordings revealed a predominant high-frequency (>15 Hz), low-amplitude oscillatory pattern, 20 of these same cells also showed slow (0.5–1.5 Hz), high-voltage, respiratory-related, membrane potential oscillations. Two of the reported neurons showed only slow oscillations. As described in the following text, the presence of one or another form of oscillation was clearly related to the depth of anesthesia as revealed by behavioral signs and as correlated with the interval between recordings and a previous supplemental dose of anesthetic.

Figure 1 shows a representative cell displaying the two behaviors described in the preceding text. The fast pattern of membrane potential oscillations (Fig. 1A) typically occurred at an interval >40 min from the last injection of anesthetic (30% of induction dose of ketamine/xylazine) and was often correlated with the appearance of behavioral signs of light anesthesia (small amplitude vibrissae whisking and the presence of a forepaw pinching reflex). The histogram of the distribution of membrane potentials approximated a unimodal Gaussian distribution (Fig. 1B) centered around the mean of 67.49 ± 7.04 mV, n = 25 and with a mean SD of 2.85 ± 0.83 mV, n = 25.

In contrast, Fig. 1, C and D, shows typical neuronal membrane potentials characterized by high-amplitude fluctuations and established during deeper states of anesthesia—within 5–30 min of a supplemental anesthetic dose. Unlike the unimodal distribution of membrane potential characteristic of light anesthesia, deep anesthetic recordings are characterized by a bimodal distribution (Fig. 1D) of basal membrane potentials centered around ~88.40 ± 6.38 and ~76.13 ± 4.81 mV, n = 20. The more depolarized of the two membrane potentials was clearly more variable (mean SD 4.88 ± 2.40 mV, n = 20) than was the more hyperpolarized potential (mean SD 1.45 ± 0.59 mV, n = 20).

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measures of piriform cortex activity and activity in the olfactory bulb are relatively stable. Also the relationship between both measures has smaller amplitude fluctuations as compared with the light state (gray), with the absolute value of cross-covariance between membrane potential and cortical field potentials being 0.52 ± 0.19, n = 19 during deep versus 0.27 ± 0.13, n = 24 during light anesthesia. Similarly, the cross-covariance between membrane potential and bulbar field potentials decreases from 0.45 ± 0.11, n = 19 to 0.34 ± 0.10, n = 24 with anesthesia lightening. All differences are statistically significant (P < 0.005).

Visual inspection of the traces in Fig. 4, A–C, suggests that the reduction of the cross-correlation occurs in the temporal scale of the respiratory frequency. To further confirm this result, the coherence in the respiratory range (0.5–1.5 Hz) was computed (Fig. 4, D–F). As shown in Fig. 4D, the mean coherence in the respiratory range between field potentials in the cortex and in the bulb significantly (P < 0.005) decreases from 0.47 ± 0.23, n = 19 during deep (dark bar) to 0.19 ± 0.13, n = 24 during light anesthesia (gray bar). A similar and significant (P < 0.005) reduction was observed in the coherence between membrane potential and local field potentials in the olfactory bulb during deep anesthesia (Fig. 4, E–F).

System-wide activity and patterns of correlation during deep and light anesthesia

In addition to recording membrane potentials from neurons in piriform cortex, we also monitored the general physiological state of the olfactory system by simultaneously recording local field potentials in the superficial layer of the piriform cortex and in the granule cell layer of the olfactory bulb. Additionally, ongoing respiratory patterns were recorded by monitoring chest wall movements through a piezo-electric device (see Methods).

During deep anesthesia (Fig. 3B), both intracellular membrane potentials and extracellular field potentials show large amplitude variations; on the contrary, under light anesthesia (Fig. 3A), both measures have smaller amplitude fluctuations and are relatively stable. Also the relationship between both measures of piriform cortex activity and activity in the olfactory bulb differs between the two anesthetic states. While the olfactory bulb mirrors the strong oscillatory pattern of cortical activity under deeper anesthesia, in the lighter state, it maintains a more oscillatory pattern than is seen in either cortical measure of activity. Comparing these changing patterns with the ongoing basal pattern of respiration, the representative data in Fig. 3 suggest that all measures are correlated under deep anesthesia, whereas only activity in the olfactory bulb is clearly correlated with respiration in the lighter anesthetic state.

This general observation is quantified by computing the average cross-covariance and coherence between the different signals (Fig. 4). Figure 4A shows a strong change in the extent of the correlation between piriform cortex and olfactory bulb field potentials related to the depth of anesthesia: the absolute value of cross-covariance decreases from 0.5 ± 0.17, n = 19 during deep anesthesia (black trace) to 0.29 ± 0.17, n = 24 during light anesthesia (gray trace). Correlations between the membrane potentials and field potentials in both the piriform cortex (Fig. 4B) and the olfactory bulb (Fig. 4C) are generally larger in amplitude for the deep anesthetic state (black) as compared with the light state (gray), with the absolute value of cross-covariance between membrane potential and cortical field potentials being 0.52 ± 0.19, n = 19 during deep versus 0.27 ± 0.13, n = 24 during light anesthesia. Similarly, the cross-covariance between membrane potential and bulbar field potentials decreases from 0.45 ± 0.11, n = 19 to 0.34 ± 0.10, n = 24 with anesthesia lightening. All differences are statistically significant (P < 0.005).

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Anesthetic effects on oscillatory frequencies

Candies the olfactory bulb is essentially unchanged by anesthetic state in phase of respiration and the amplitude of field potentials in the (Fig. 5). In contrast, however, the relationship between the and gamma from 0.51 ± 0.51 to 5.67 ± 4.37%, with anesthesia lightening. All the differences are statistically significant (deep anesthesia, n = 19; light, n = 24; P < 0.001).

An overall similar variation is observed in the piriform cortex field potentials with respiratory band varying from 75.5 ± 13.31 to 59.20 ± 14.19%, theta from 19.48 ± 10.90 to 21.57 ± 11.20%, beta from 4.66 ± 2.42 to 16.82 ± 8.11%, and gamma from 0.35 ± 0.28 to 2.39 ± 1.99%. Differences for all the bands but theta were statistically significant (deep, n = 19; light, n = 24; P < 0.001).

In contrast, the OB shows qualitatively different changes, with lighter anesthesia associated with a small but significant (deep, n = 19; light, n = 24; P < 0.01) increase in respiration-related frequencies—from 74.34 ± 8.29 to 81.87 ± 8.38%—and a significant (deep, n = 19; light, n = 24; P < 0.001) shift downward in the theta band—from 21.40 ± 7.20 to 11.82 ± 7.32%. Furthermore, high frequencies increase with anesthesia lightening: a small nonsignificant increase for beta, from 3.81 ± 1.44 to 4.46 ± 1.95% and a significant (deep, n = 19; light, n = 24; P < 0.001) gamma band increase from 0.43 ± 0.34 to 1.83 ± 1.11%.

The overall power spectral analysis shown in Fig. 6 does not, of course, provide any information on possible temporal variations in these frequencies. Accordingly, Fig. 7 compares the recorded neural activity when filtered for different intrinsic frequencies in the case of light (A–C) and deep (D–F) anes-

either the piriform cortex (Fig. 4E; from 0.50 ± 0.26, n = 19 to 0.16 ± 0.11, n = 24) or bulb (Fig. 4F; from 0.47 ± 0.13, n = 19 to 0.34 ± 0.15, n = 24).

Figure 5, A–C, examines the relationship between all three physiological brain measures and the phase of respiration in both light (gray) and deep (black) anesthetic conditions. There is no clear phase relationship between piriform cell membrane potential and respiration in the case of light anesthesia, whereas there is a clear relationship during deeper states. This finding is paralleled in the piriform cortex field potential data (Fig. 5B); in contrast, however, the relationship between the phase of respiration and the amplitude of field potentials in the olfactory bulb is essentially unchanged by anesthetic state (Fig. 5C).

Anesthetic effects on oscillatory frequencies

Figure 6 compares the overall frequency distributions of activity in membrane potential (Fig. 6A), piriform cortex field potential (Fig. 6B), and olfactory bulb (Fig. 6C) data. As in previous figures, data recorded during light anesthesia is shown in gray, whereas data obtained during deep anesthesia is shown in black. As expected from our previous analysis, these power spectral densities indicate that deeper anesthesia is associated with increased activity in the low-frequency ranges, and all three types of recordings have larger gamma band (40–100 Hz) frequency components in the lighter than deeper anesthetic state.

These effects are quantified in the histograms of Fig. 6, D–F, which represents relative power data obtained at four different frequencies—the respiratory band (0.5–1.5 Hz), theta (4–9 Hz), beta (15–40 Hz), and gamma (45–100 Hz)—for the three different neural recordings (membrane potential, Fig. 6D; piriform cortex field potential, E; olfactory bulb field potential, F).

In the case of membrane potential, the respiratory band varies from 86.75 ± 7.20 to 52.38 ± 16.92%, theta from 9.94 ± 6.15 to 22.94 ± 8.24%, beta from 2.78 ± 1.35 to 18.99 ± 10.21%, and gamma from 0.51 ± 0.51 to 5.67 ± 4.37%, with anesthesia lightening. All the differences are statistically significant (deep anesthesia, n = 19; light, n = 24; P < 0.001).

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Fig. 6. Power spectral analysis of olfactory system activity. A: average power spectral density of $V_m$; B: local field potential in the PC; C: local field potential in the OB. Right: relative power of respiratory, theta, beta, and gamma bands for $V_m$ (D), PC (E), and OB (F). In black: data from deep anesthesia; gray: data from light anesthesia.

The temporal patterning of activity at different frequencies changes depending on the anesthetic levels. This is particularly apparent in the case of gamma frequencies, where during deeper anesthetic conditions activity more clearly occurs in respiratory-correlated bursts, whereas, during light anesthesia there is much less patterning in the gamma frequency activity in all three types of recordings and a much less clear relationship between the occurrence of bursts and respiration.

To quantify the respiratory related bursting behavior, the envelope of beta and gamma oscillations was extracted (black trace superimposed) and, after a spectral analysis of the envelope, the ratio between the power in the respiratory band and the power at other frequencies was computed. This ratio is significantly larger for signals recorded during deep anesthesia than during light anesthesia indicating a respiratory modulation of beta and gamma oscillations: the ratio for membrane potential is $453.1 \pm 144.27$ during deep and $55.8 \pm 33.06$ during light anesthesia for beta and $148.9 \pm 64.32$ versus $22.86 \pm 16.68$ for gamma. Similarly the ratio for beta and gamma recorded in cortical field potential (Fig. 7G) falls from $494.93 \pm 215.8$ to $104.08 \pm 53.55$ and from $71.20 \pm 36.59$ to $29.37 \pm 12.84$, respectively. Finally in bulbar recordings (Fig. 7I) beta goes from $454.31 \pm 245.69$ to $133.82 \pm 92.46$ and gamma from $148.24 \pm 64.99$ to $79.20 \pm 57.77$. All these differences are statistically significant, $P < 0.001$ ($n = 19$ for deep anesthesia and $n = 24$ for light).

Interestingly, the frequency of respiration does not significantly differ in the two conditions being $0.975 \pm 0.16$ Hz (range from $0.64$ to $1.15$ Hz), $n = 19$ during periods dominated by slow activity and $1.05 \pm 0.17$ Hz (range from $0.77$ to $1.28$ Hz), $n = 24$ in fast oscillations periods; increases in respiratory frequency up to the range of sniffing ($4 – 9$ Hz) were not observed during the recording conditions.

**DISCUSSION**

In the present report, we characterized the relationships between activity in the bulb and cortex under light and deep ketamine/xylazine anesthesia. We also compared these patterns to the animal’s ongoing basal respiration. As has been reported
previously for both piriform cortex (Freeman 1959) and neocortex (Steriade et al. 2001), neuroelectric activity can be generally characterized as being dominated by either low or higher frequency, depending on the levels cortical activation and arousal (i.e., deep sleep and deep anesthesia vs. awake and rapid-eye-movement states). We show here that the system wide slow oscillations, which during deep anesthesia appear to be directly related to the respiratory wave (Fontanini et al. 2003; Macrides and Chorover 1972; Wilson 1998), are present only in the olfactory bulb and not the piriform cortex under light anesthesia. As a result, under light anesthetic conditions, there is little or no coupling between either field potentials or pyramidal cell membrane potentials recorded in the piriform cortex and bulbar field potentials or respiration. We conclude that the level of anesthesia changes the degree of coupling within the olfactory system, and the coupling between baseline respiration and the olfactory bulb on the one hand, and the piriform cortex on the other.

Use of anesthetic to explore brain states

Since the discovery that slow oscillatory patterns are spontaneously present during ketamine/xylazine anesthesia (Steriade et al. 1993a–c), this anesthetic preparation has been increasingly applied as a model for cortico-thalamic slow wave behavior during sleep (for a review, see Amzica and Steriade 1998). Short periods of desynchronized fast activity were described to occasionally interrupt slow waves during deeper anesthesia and have been suggested to be analogous to short periods of cortical activation typical of REM sleep (Steriade and Amzica 1996; Steriade et al. 1996b). To our knowledge, however, no previous investigations have specifically described the relationship between these different patterns of activity and the depth of anesthesia. Moreover while previous reports have shown the occurrence of sustained fast cortical activity after activation of neuromodulatory nuclei (Jones 2003; McCormick and Bal 1997; Moruzzi and Magoun 1995; Steriade et al. 1996a), long and sustained periods of fast activity have never been described as spontaneously occurring in an anesthetized preparation. As reported here, there is a clear positive correlation in the piriform cortex between lightness in anesthesia and the incidence of high-frequency desynchronized activity; these results establish a novel experimental model for studying spontaneously occurring fast activity, the switch between slow and fast activity and, finally, the influence of these different states on sensory evoked responses (Murakami et al. 2004).

Oscillatory bands in the olfactory system

Through the work of numerous previous authors on oscillations in the olfactory system, four oscillatory bands have been described and characterized: respiratory, theta, beta, and gamma (Bressler 1984; Bressler and Freeman 1980; Chapman et al. 1998; Fontanini et al. 2003; Freeman 1960 1959; Kay 2003; Kay and Freeman 1998; Neville and Haberly 2003; Vanderwolf 2000; Zibrowski and Vanderwolf 1997). In our experimental conditions, both the power and temporal profiles...
of these oscillatory bands were found to vary depending on the anesthetic levels. Specifically, while the respiratory band was strongly reflected in field potentials within the olfactory bulb regardless of the level of anesthesia, the influence of this frequency band in the cortical measures was greatly reduced under lighter anesthetic conditions. While more subtle and variable, we also show anesthetic-related changes in theta frequencies (see Fig. 6, D–I, for population average). Previous studies in awake behaving and anesthetized animals have strongly associated theta frequency activity in both the bulb and cortex with sniffing (Bressler 1987) and/or odor stimulation (Margrie and Schaefer 2003; Spors and Grilli 2002). The monitored respiration in our rats revealed no evidence for sniffing, and instead we found quite similar patterns of basal respiration regardless of anesthetic state. This result, in agreement with previous modeling efforts (Wilson and Bower 1992), supports the ability of the olfactory system to intrinsically produce power in the theta band even in the absence of sniffing or odor stimulation.

Our data show that beta and gamma oscillations, which typically occur in sniffing modulated bursts during odor sampling in awake animals (Bressler and Freeman 1980; Vanderwolf 2000; Zibrowski and Vanderwolf 1997), are also highly influenced by anesthetic level in both their overall power and their temporal profile. Both types of oscillations have a higher overall power in the bulb during light anesthesia but are reflected in a burstier temporal pattern under deep anesthetic conditions. Similarly, this increase in beta and gamma oscillations under light anesthesia in the bulb is reflected in recordings in the cortex that also show very little respiratory modulated beta and gamma bursts during light anesthesia.

Variations in apparent respiration-related coupling between the olfactory bulb and cortex

While it is clear from the data presented here that the relationships between the oscillatory properties of the bulb and cortex are complex, several known features of these olfactory structures suggest specific mechanisms that could contribute to regulating the degree of coupling between bulb and cortex. Specifically, excitatory connections in the apical dendrites of piriform cortex pyramidal cells are laminarily organized into two systems: a distal, afferent system arising in the bulb, and a more proximal intrinsic auto-associative system providing recurrent excitation from other pyramidal cells (Haberly 1998). We have previously suggested that the overall physiology (Bower and Haberly 1986; Haberly and Bower 1984; Hasselmo and Bower 1990) and function (Hasselmo and Bower 1993) of the piriform cortex may fundamentally involve the dynamic balance between the afferent (bulbar) and intrinsic influences. It seems worth considering whether the anesthetic related changes in bulb-cortical coupling shown here might also reflect changes in this balance. Specifically, we have previously shown that intrinsic recurrent connections are particularly sensitive to anesthetic (Haberly and Bower 1984), in this case, Nembutal. These excitatory connections are also the principal source of the N-methyl-D-aspartate-associated synapses on pyramidal cells (Haberly 1998) and are presumably therefore specifically affected by ketamine (Hoffman and Haberly 1989; Thomson et al. 1985). If cortical dynamics depend on a balance between afferent and intrinsic synaptic influences, then it seems reasonable to suggest that a ketamine-produced reduction in the strength of intrinsic recursive connections might favor a greater coupling between bulbar input and cortical activity. Under these conditions, the synchronized rhythmic respiratory activity coming from the bulb would drive an “input prone” cortex to follow that rhythm. In contrast, during light anesthesia the intrinsic excitatory system should be less affected, promoting higher levels of intrinsic recurrent activity.

Functional interpretations of anesthetic-related changes

Of course, the fact that the animals studied here were anesthetized limits the functional interpretation of these results. In particular, we found no statistically significant difference in the rate or temporal patterns of breathing in light and deep anesthetic states. In contrast, it has previously been shown that awake behaving animals have a clear bimodal distribution in breathing rate with peaks centered around 1.5 and ~7 Hz (Bhalla and Bower 1997), corresponding to quiet respiration and active sniffing, respectively. These changes in respiration rate are associated with clear changes in the relationships between activity of multiple bulbar neurons simultaneously recorded (Bhalla and Bower 1997). In particular, bulbar neurons are much more variable in their response to peripheral stimuli during periods of sniffing than during periods of ongoing baseline respiration, supporting the view that active acquisition of olfactory sensory data changes the dynamical state of the olfactory system—as also demonstrated by Kay and Freeman (Kay and Freeman 1998; Kay et al. 1996). The results presented here further demonstrate that the relationship between neurons and networks in the olfactory system is in general strongly dependent on the state of the system as a whole.

Beyond this general statement, our and other groups’ data (Achermann and Borbely 1998; Destexhe et al. 1999) would support the general idea that awake states (and sniffing states in our particular case) are specifically characterized by a decoupling of global network activity in favor of the more independent activity of individual neurons. In this way, slow-wave sleep is associated with a more global type of computation, as for example is presumably necessary for functions like memory consolidation, now believed to be associated with this state of sleep (Lee and Wilson 2002). In contrast, higher frequency patterns of activity would appear to be associated with less global and therefore more local types of processing, whether driven by afferent input (awake state), or centrally generated streams of activity (rapid-eye-movement sleep state). Thus we propose that it is not the presence or absence of afferent input that is key to cortical processing strategies but instead changes in the dynamics of the circuits themselves which either favor global or local types of processing. In any event, we believe that further study of the olfactory system, from which cerebral cortical networks are likely to have first evolved (Bower 1995), are likely to continue to provide a unique and fundamental perspective on the different states of cerebral cortical computation.

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