Anesthetic regimes modulate the temporal dynamics of local field potential in the mouse olfactory bulb

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Chery R, Gurden H, Martin C. Anesthetic regimes modulate the temporal dynamics of local field potential in the mouse olfactory bulb. J Neurophysiol 111: 908–917, 2014. First published November 27, 2013; doi:10.1152/jn.00261.2013.—Anesthetized preparations have been widely used to study odor-induced temporal dynamics in the olfactory bulb. Although numerous recent data of single-cell recording or imaging in the olfactory bulb have employed ketamine cocktails, their effects on networks activities are still poorly understood, and odor-induced oscillations of the local field potential have not been characterized under these anesthetics. Our study aimed at describing the impact of two ketamine cocktails on oscillations and comparing them to awake condition. Anesthesia was induced by injection of a cocktail of ketamine, an antagonist of the N-methyl-D-aspartate receptors, combined with one agonist of α2-adrenergic receptors, xylazine (low affinity) or medetomidine (high affinity). Spontaneous and odor-induced activities were examined in anesthetized and awake conditions, in the same mice chronically implanted with an electrode in the main olfactory bulb. The overall dynamic pattern of oscillations under the two ketamine cocktails resembles that of the awake state. Ongoing activity is characterized by gamma bursts (>60 Hz) locked on respiration and beta (15–40 Hz) power increases during odor stimulation. However, anesthesia decreases local field potential power and leads to a strong frequency shift of gamma oscillations from 60–90 Hz to 100–130 Hz. We conclude that similarities between oscillations in anesthetized and awake states make cocktails of ketamine with one α2-agonist suitable for the recordings of local field potential to study processing in the early stages of the olfactory system.

Sensory systems provide accurate information about the external world to the brain. In behaving animals, sensory networks are critically modulated by changes of state that can be induced by cognitive processes, including attention, arousal, emotion, or experience (Engel et al. 2001; Fontanini and Katz 2008). Many experiments on animals require sedation. Anesthesia is induced by drugs which target neuromodulatory systems and influence cortical processing. Thus it is crucial to better understand the impact of the most widely employed drugs on network activity.

In the main olfactory bulb (MOB), each odor triggers a specific pattern of glomerular activation (Rubin and Katz 1999). This organized input is then transferred to deeper layers of the structure where mitral and tufted cell (M/TC) activity is tuned by inhibitory granule cells (GC) through dendrodendritic reciprocal synapses. M/TC-GC functional loop is a key element for the generation of local field potential (LFP) oscillations which reflect transient coordinated activity of neuron assemblies (Rall and Shepherd 1968; Rojas-Líbano and Kay 2008). Among them, odor-induced beta (15–40 Hz) and gamma oscillations (60–90 Hz) have been linked to odor perception and learning (Beshel et al. 2007; Chapuis et al. 2009; Kay et al. 2009; Martin et al. 2004).

Most of the studies examining the characteristics of neuronal activities in the rodent MOB have been conducted under urethane anesthesia (Buonviso et al. 2003; Cenier et al. 2008; Chapuis and Wilson 2012; Fletcher and Wilson 2003; Neville and Haberly 2003). However, this drug produces drastic differences in the pattern of neuronal oscillations, in the absence or in the presence of odorant, compared with the awake condition. Notably, under urethane, gamma activity in the absence of odor is strongly diminished and does not display bursts locked to the respiratory modulation (Aylwin et al. 2009; Li et al. 2012). Alternatively, numerous recent data of single-cell recording or imaging in the MOB were obtained using the anesthetic cocktail composed of ketamine and xylazine (XX) (Carey and Wachowiak 2011; Fukunaga et al. 2012; Rinberg et al. 2006). However, odor-induced oscillations have not been characterized under these anesthetics. Both M/TC and GC express N-methyl-D-aspartate (NMDA) receptors. Ketamine, a noncompetitive antagonist of NMDA receptors, has been reported to alter M/TC inhibition driven by GC in the MOB (Wilson et al. 1996). Xylazine, a widely used α2-agonist, stimulates the noradrenergic system to induce sedation (Wixon and Smiler 1997). More recently, medetomidine has been used as a replacement for xylazine (Roughan et al. 1999) as its α2/α1-receptor selectivity binding ratio is 10-fold higher (Virtanen 1989), leading to fewer side effects. Moreover, ketamine/medetomidine (KM) cocktail makes possible the use of atipamezole, a drug that reverses sedative and analgesic effects of medetomidine and rapidly wakes the animals (Virtanen 1989). Noradrenaline (NA), released from the locus coeruleus, is a major neuromodulatory system involved in arousal and emotional states (Berridge and Waterhouse 2003). During sensory processing, NA alters network properties to sharpen stimulus representations (Manunta and Edeline 2009; Kay et al. 2009; Martin et al. 2004). In the MOB, NA predominantly modulates the M/TC-GC dendrodendritic synapses (Mouly et al. 1995; Trombly and Shepherd 1992).

Our study aimed at characterizing the impact of two ketamine cocktails on chronically recorded LFP oscillations in mice MOB, both in the absence and in the presence of odor, and comparing them to the awake condition. We found that, although both KX and KM impact temporal dynamics in the...
MOB, the oscillatory pattern recorded under anesthesia shows similarities to that seen in awake mice.

MATERIALS AND METHODS

Subjects

The subjects were 7 male C57BL6 mice (~20 g, 7 wk old) obtained from Janvier Laboratories (Le Genest-St-Ise, France). They were kept in a temperature (22 ± 0.5°C) and humidity (50 ± 5%) controlled room, and maintained on a 12:12-h reversed light dark cycle (lights on at 8:00 PM). All experimental sessions were performed during the dark portion of the cycle. Animals were housed in group until the surgical procedure, and housed individually afterward. Food and water were available ad libitum, except during the behavioral procedure when access to water was restricted and given once a day (1 ml per mouse in their home cage at 5:00 PM) in addition to the water received in the experimental chamber. All experiments were performed in accordance with the European Directive 86/609/EEC regarding the care and use of laboratory animals. The experimental protocol was controlled and approved by the French veterinary authorities (DDPP authorization B91471101).

Electrodes Implantation

Anesthesia was induced by inhalation of isoflurane (5%) and maintained by an intraperitoneal (ip) injection of a cocktail of ketamine (100 mg/kg) and xylazine (10 mg/kg). Adequate level of anesthesia was confirmed by toe pinch and absence of ocular reflex. The electrode (diameter 125 μm, stainless steel, Plastic One) was positioned stereotaxically in the MOB (4.5 mm anterior, 1 mm lateral and 0.8–1.1 mm ventral relative to bregma) at the level of the GC layer using electrophysiological monitoring of the signal characteristics, i.e., high-power activity in the gamma band (60–130 Hz) and sharp respiratory modulation. The reference electrode was connected to a skull screw located above the posterior portion of the contralateral cortical hemisphere. The connector of the electrode was fixed onto the mouse’s head with dental acrylic. A recovery period of 2 wk followed surgical procedure before resuming of behavioral experiment.

Histology

Following the end of all experiments, the animals were killed by a lethal dose of pentobarbital, and an electrocoagulation (5 repetitions, 10 mA, 3 s) was performed through the electrode. Brains were removed and frozen. Forty-micrometer-thick coronal slices were sectioned with a cryomicrotome, and Nissl staining was performed for subsequent histological examination.

Electrophysiological Recordings and Odor Delivery

LFP signals were recorded from the same animals that underwent different protocols, in anesthetized or awake conditions, with or without odor stimulation. Time course of the procedure is represented in Fig. 1.

For all the recording sessions, mice were connected to the recording device by a tether plugged into the implanted connector. Monopolar activity was acquired using a custom DasyLab (IOTECH) script driving an XCellAmp 64 amplifier (Dispi) coupled with a DaqBoard 3000 USB system (IOTECH). Signal was sampled at 2,000 Hz, amplified (×2,500), and digital filters were set at 0.1–300 Hz. Odors were delivered using an automated olfactometer (ValveBank II, AutoMate Scientific) that controlled the duration and the flow rate of the stimulation. A piece of filter paper, loaded with 50 μl of a solution of pure odorant diluted in mineral oil at the desired concentration was used to odorize the flow. For clean air stimulation, no odor was applied on the filter paper. All the experiments took place in a grounded Faraday cage.

Recordings in anesthetized animals. Following electrode implantation under KX cocktail, LFP in the MOB was recorded under four conditions: spontaneous activity in the absence of airflow, activity under airflow and response to odor stimulations [hexanal (5%) or butanal (5%)]. Each condition was repeated 10 times and consisted in 5 s of stimulation, preceded and followed by 30 and 25 s of deodorized airflow, respectively. The same protocol was repeated during the second recording session under anesthesia induced by ip injection of a cocktail of ketamine (75 mg/kg) and medetomidine (0.425 mg/kg) (see Fig. 1 for timeline of experiments).

Recordings in awake, freely moving animals. Recordings were performed for two separate conditions: during a protocol of active odor exposure, and during pharmacological assessment of xylazine and medetomidine effect.

Active odor exposure. Mice were engaged in a behavioral task in which odor sampling was motivated by water delivery. The experimental cage (Habitest, Coulbourn Instruments) is a box containing one wall equipped with two ports: 1) a self-delivery drinking system consisting of a liquid dipper; and 2) a separated stimulation port connected to the olfactometer. Both ports were equipped with beam detectors. Clean air constantly flowed through the stimulation port. Detection of a mouse nose poke in this port triggered odor delivery for 2 s. After odor sampling, a nose poke in the liquid dipper triggered distribution of 20 μl of water, which remained accessible for 10 s. The whole system was controlled by Graphic State (Coulbourn Instruments). The behavioral task was preceded by a 24-h water restriction. For the preoperative shaping, each mouse was placed in the experimental chamber for 30 min/day. During the first session, every nose poke in the odor port automatically triggered water distribution, while in the following sessions, mice had to nose poke in the dipper to receive the water reward. The odor used was limonene (+) diluted at 80% in mineral oil. Shaping ended when the animals performed at least 20 trials in one session. During postoperative sessions, the LFP was recorded in the MOB for every trial. Mice had to follow the same rules as during the shaping phase, but the new odorant was used: hexanal (5%) and butanal (5%).

NA pharmacology. Influence of each α2-agonist on the MOB spontaneous activity was assessed in the same animals while they were freely moving. This protocol was conducted in three consecutive daily sessions, 24 h apart. The first day was separated from the KX anesthesia used for electrodes implantation by 1 wk of rest. Animals were injected ip with xylazine (10 mg/kg) immediately before being

**Fig. 1.** Experimental timeline of local field potential (LFP) recordings in the main olfactory bulb (MOB) during different brain states. LFP was acquired for the same chronically implanted animals in the 6 following conditions: anesthetized with ketamine + xylazine (KX) during electrode implantation, awake freely moving, anesthetized with ketamine + medetomidine (KM) and awake injected with xylazine, medetomidine and saline successively. Mice are anesthetized during states represented by light gray boxes. They are awake during states in dark gray. wd, Water deprivation.
placed in the experimental chamber to which they were habituated. During the second and third sessions, saline (0.3 ml ip) and medetomidine (0.425 mg/kg ip) were injected, respectively. For each session, LFP was recorded during 30 s every minute for 20 min.

Data Analysis

All electrophysiological data were exported and stored in a MySQL database for subsequent analysis. Data were processed using OpenElectrophy software [http://packages.python.org/OpenElectrophy (Garcia and Fourcaud-Trocmé 2009)]. The signal was first visually controlled to discard artifacts.

Because the signal recorded in the MOB is highly modulated in time and characterized by transient changes in frequency, we chose to use wavelet analysis, a particularly powerful tool for studying transient phenomena without any prior knowledge of frequency bands of interest (Tallon-Baudry and Bertrand 1999). A continuous Morlet wavelet transform was then applied between 5 and 160 Hz, resulting in an estimate of oscillatory power for each time and frequency values. We obtained time frequency power maps, where each level of energy was represented for each time and frequency value. Analyses in the frequency bands of interest are conducted separately.

For each trial, we set a preperiod, before odor stimulation, and an odor period, during odor stimulation. Time windows for the analysis were defined differently according to the behavioral condition to adapt to the shorter odor sampling duration in the awake state. In KX and KM conditions, pre- and odor periods lasted for 2 s, ranging from 2.5 to 0.5 s before odor onset for preperiod and from 0 to 2 s after odor onset for odor period. In awake condition, pre- and odor periods were reduced to 1 s, ranging from 2.9 to 1.9 s before odor onset for preperiod and from 0 to 1 s after odor onset for odor period. Analyses of spontaneous activity in the presence or in the absence of airflow were performed on the whole recorded period (10 s).

LFP averaged power was calculated for both pre- and odor periods for each frequency band. A power threshold (mean + 3 SD) was calculated in the time-frequency window corresponding to the prep-period. A wavelet ridge extraction was applied for the pre- and odor period time windows. The method has been detailed elsewhere (Cézier et al. 2008; Roux et al. 2007). Briefly, it consists of detecting local maximum energy points above threshold to compute the wavelet ridge, i.e., the path of lowest energy decrease. The instantaneous frequency and phase of the signal is then extracted from the ridge. Only the bursts presenting at least three cycles were kept for subsequent analysis. For each trial, the number of bursts above threshold and the mean frequency value of detected bursts were obtained.

Power spectra analysis was performed offline using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Power spectra were estimated by applying a 1,024-point Hanning taper to data windows for the pre- and odor period time segments of every single trial. They were then averaged for pre- and odor periods across the 20 consecutive trials corresponding to a given condition and across the seven animals.

Statistical Analysis

Statistical analyses were performed using nonparametric tests on the power and frequency values, and on the number of bursts above threshold. Two independent factors were tested: the state (KX or KM anesthesia, awake and injected with α2-agonists) and the odor (hexanal vs. butanal) using Mann-Whitney test. One paired factor, the period factor (pre, odor), was tested using Wilcoxon paired-samples tests.

Drugs and Odors

Pure odorants were purchased from Sigma Aldrich; they were diluted in mineral oil. Ketamine (Imalgene 500, Merial), xylazine (Rompun, Bayer) and medetomidine (Domitor, Pfizer) were diluted in saline.

RESULTS

For each mouse chronically implanted with an electrode in the MOB (n = 7), LFP oscillatory activities were recorded in six conditions (Fig. 1): anesthetized with a cocktail of KX or KM, awake not injected, and awake with an ip injection of either xylazine, medetomidine or saline.

Examples of signals recorded in the same mouse before and during odor stimulation (hexanal 5%) in each condition are presented in Fig. 2. As expected, respiratory modulation is impacted by anesthesia, under which more regular and ample deflections of the LFP are observed. Qualitative comparison of the signal between the conditions revealed a similar pattern: odor elicited power increase in the 15- to 40-Hz band (beta band) associated with an overall power decrease in the 60- to 130-Hz range (gamma band). Under ketamine cocktails, however, the maximal power in the gamma band in the absence of odor stimulation is found in higher frequencies (100–130 Hz) compared with the awake condition.

Odor Presentation Increases Beta Band (15–40 Hz)

Oscillatory Power Both in Awake and Ketamine Cocktail-Anesthetized Mice

To study odor responses for each condition and evaluate the putative effect on the signal of KX and KM compared with the awake state, we extracted quantitative data from the wavelet analysis. Mean power is reported for time-frequency windows corresponding to the pre- and odor period for the 15- to 40-Hz frequency band (Fig. 3A). For the three conditions (KX, KM and awake), odor presentation is associated with a significant increase in power compared with preperiod (P < 0.01 Wilcoxon paired samples test). Even in the absence of odor (preperiod), beta power is impacted by anesthetics: it is diminished under both KM and KX compared with the awake state (P < 0.01, Mann-Whitney). Power is even lower under KM than under KX (P < 0.01, Mann-Whitney). A differential beta response according to the stimulus is observed specifically in anesthetized conditions: beta power is significantly higher during hexanal (5%) than during butanal (5%) presentation (P < 0.01 Mann-Whitney). This effect is not found in awake.

We further compared the frequency of beta oscillations between the different states. For each burst of oscillation detected above a threshold calculated from the preperiod, we extracted the frequency of the point of maximum energy. Average across animals reveals a slight decrease in frequency under anesthesia: the frequency of the bursts extracted in KX and KM is significantly lower compared with the awake condition (P < 0.01, Mann-Whitney). Except for butanal in KX condition, beta bursts frequency is under 25 Hz in anesthetized and around 30 Hz in awake animals (Fig. 3B).

Anesthetics Change Gamma Frequency Independently of the Type of α2-Noradrenergic Agonists

To characterize changes in gamma band due to anesthe-sia, we first averaged power spectra for all the animals in the three recorded conditions (Fig. 4A). Within the broad gamma range (60–130 Hz), the peak of gamma is clearly shifted from low frequencies (60–90 Hz) in the awake
condition to high frequencies (100–130 Hz) under anesthesia. For further analysis we chose to split gamma frequencies into two bands, referred as high (100–130 Hz) and low (60–90 Hz) gamma bands.

As for the beta band, overall power in gamma range is reduced under anesthesia. In the awake condition, odor stimulation leads to gamma power decrease in the two frequency bands 60–90 Hz and 100–130 Hz (Fig. 4B) ($P < 0.01$; Wilcoxon paired samples test). On the contrary, in both KX and KM conditions, odor stimulation leads to a power increase in the 60- to 90-Hz band ($P < 0.01$, except for hexanal in KM condition, $P > 0.05$; Wilcoxon paired samples test). In the 100- to 130-Hz range, power significantly decreased under KX ($P < 0.01$; Wilcoxon paired samples test); the decrease does not reach significance under KM. In addition to the mean LFP power, we focused our analysis on the oscillatory activity within the LFP by extracting bursts whose power is above a threshold calculated from the preperiod (Fig. 4C). Results show that, even though the decrease in the mean power is slight in the 100- to 130-Hz range, the number of bursts is dramatically reduced during sampling of the two odors for the two ketamine cocktails ($P < 0.01$, Mann-Whitney). However, no impact of odor presentation is seen in the 60- to 90-Hz frequency band. No difference was observed in the number of bursts.
bursts between KX and KM (Mann-Whitney, P > 0.05 for all comparison).

Taken together, these results show that the episodes of beta and gamma rhythm recorded in awake rodents MOB during odor presentation are maintained under KX and KM. Still, ketamine cocktails modulate the two rhythms, both in the presence and in the absence of odor stimulation. These changes impact both power and frequency of the LFP activity. The most noticeable effect is a dramatic shift in the frequency of gamma bursts in the absence of odor presentation. The choice of the \( \alpha_2 \)-agonist has only a limited impact on the oscillations.

**Effect of the Anesthetics on Oscillatory Activities in the Absence or in the Presence of Airflow**

To decipher the possible modulation exerted by the choice of the \( \alpha_2 \)-agonists on the MOB network, we examined LFP power and oscillatory bursts in the absence of odor. We focused our analysis on the broad gamma band (60–130 Hz), where ongoing activity occurs.

To dissociate the effect of odorant binding from the mechanical stimulation applied to olfactory receptors by airflow, LFP were recorded in two conditions (Fig. 5): either in the absence of airflow, or with the same constant deodorized airflow that was present during the preperiod in the odor stimulation protocol. For both KX and KM, airflow dramatically reduces by almost twofold high gamma power (47% power decrease for KX, 35% power decrease for KM, both significant \( P < 0.01 \), Mann-Whitney): this effect is specific of the 100- to 130-Hz frequency band and is not observed in the 60- to 90-Hz gamma range (Fig. 5A). However, if we now analyze the mean number of bursts per trial, the oscillatory activity decrease is significant in the two frequency ranges (\( P < 0.01 \); Mann-Whitney). In the absence of airflow the number of bursts per trial is lower with KM (\( P < 0.01 \); Mann-Whitney); this difference is abolished after the introduction of airflow (Fig. 5B). Overall, the airflow-induced decrease in gamma power is stronger under KX than under KM.

**Impact of \( \alpha_2 \)-Noradrenergic Agonists Alone on the Awake Ongoing Activity**

To determine to what extent effects observed in anesthetized conditions were driven by ketamine, and to study the specific action of the two \( \alpha_2 \)-agonists on oscillatory activities, we injected ip either xylazine alone, medetomidine alone or saline to the same mice used in the previous experiments. LFP was recorded in the absence of odor stimulation while mice explored the behavioral cage where they were previously recorded in the awake condition (Fig. 6). During the recording session, animals injected with one of the \( \alpha_2 \)-agonists showed reduced mobility but kept consciousness.

\( \alpha_2 \)-Agonists differentially modulated power and frequency of detected oscillations compared with saline. Power spectra displayed in Fig. 6A shows that injection of either xylazine or medetomidine alone abolished the peak of gamma power centered on 70 Hz in the control animals (saline). Injections also abolished the peak of gamma observed under KX and KM (shown in details in Fig. 4A). In contrast with results under KX and KM (Fig. 4), power in the 60- to 90-Hz band was twice as high as power in the 100- to 130-Hz band (xylazine: \( P < 0.005 \); medetomidine: \( P < 0.005 \); saline: \( P < 0.005 \); Mann-Whitney). LFP power was higher under xylazine than medetomidine (\( P < 0.05 \); Mann-Whitney), but for both \( \alpha_2 \)-agonists it was strongly decreased compared with saline (\( P < 0.05 \); Mann-Whitney).

In conclusion, the two \( \alpha_2 \)-noradrenergic agonists, xylazine and medetomidine, strongly reduce low and high gamma power. Injected alone, they fail to enhance high gamma power (100–130 Hz) observed under ketamine cocktails anesthesia.

**DISCUSSION**

We studied the impact, on oscillatory activities in the mouse MOB, of anesthesia induced by cocktails of ketamine, an NMDA receptor antagonist, mixed with one of the two commonly used \( \alpha_2 \)-receptor agonist, xylazine or medetomidine.
The overall oscillatory pattern under this anesthesia regime has numerous similarities with the awake state: it is modulated by respiration, gamma bursts are prominent in the absence of odor, and beta power increases during odor stimulation. We also observed differences between anesthetized and awake conditions on both spontaneous and odor-induced activities. An overall power decrease is noticed under anesthesia and a robust frequency shift impacts gamma activity. Using ip injection of xylazine or medetomidine alone, we isolated the specific effect of the \( \alpha_2 \)-agonists from the perturbation induced by ketamine, and revealed a global decrease in gamma power rather than a frequency shift. Finally, oscillatory activities are slightly more impacted under KM than KX.

**The Profile of the Odor-Response Is Similar Under Ketamine Cocktails Anesthesia and Awake States**

Although ketamine cocktail anesthesia is often used for functional imaging (Gurden et al. 2006; Lecoq et al. 2009; Soucy et al. 2009), to our knowledge, its impact on oscillatory dynamics in the MOB, with and without odor stimulation, has not been characterized so far. In the MOB, few studies have stressed the impact of anesthetized state on electrophysiological activities compared with awake condition (Davison and Katz 2007; Fontanini et al. 2003; Li et al. 2012; Rinberg et al. 2006). Many reports on oscillatory activities under anesthesia used urethane (Aylwin et al. 2009; Buonviso et al. 2003;
Cenier et al. 2008; Li et al. 2012; Neville and Haberly 2003). Under this drug, spontaneous activity is characterized by a respiration-related rhythm (Courtiol et al. 2011), but, in contrast to what is obtained during the waking state, the LFP signal lacks gamma frequency (60–90 Hz) bursts (Aylwin et al. 2009; Li et al. 2012). Interestingly, while under both urethane and ketamine cocktails animals reached the same level of analgesia and sedation, our results show striking differences in the expression of oscillatory activities compared with what has been observed in the literature under urethane. In line with the study of Fontanini et al. (2003), we found that, under KX or KM, spontaneous activity is dominated by bursts at gamma frequencies superimposed on the respiratory modulation, as described in awake rats (Eeckman and Freeman 1990; Martin et al. 2004; Ravel et al. 2003) and mice (Lepousez et al. 2010; Nusser et al. 2001). However, our results differ from Fontanini’s study, since we found that ongoing gamma bursts have a higher frequency range (100–130 Hz) under ketamine cocktail anesthesia.

With either KM or KX, the response during odor presentation is similar to awake condition, characterized by an increase in oscillatory power in the beta band (15–40 Hz) and a corresponding decrease in the high frequencies (100–130 Hz). Interestingly, while LFP power is almost unchanged under KM, bursting activity is strongly abolished by odor stimulation, in the same way for the two anesthetics (Fig. 4), suggesting a modification in the network dynamics. This profile differs from the broad frequency range increase (12–90 Hz) recorded under chloral hydrate or pentobarbital (Li et al. 2011) or the mixed beta and gamma oscillatory bursts elicited by odorant stimulation under urethane anesthesia (Buonviso et al. 2003; Neville and Haberly 2003). In addition, beta oscillations power discriminates the two odors, as it is reported in the awake rat (Martin et al. 2007) and under urethane (Cenier et al. 2008).

### Gamma Band Oscillatory Activity Is Shifted in Frequency Under Ketamine Cocktail Anesthesia

Gamma bursts are the most prominent features in the MOB (Eeckman and Freeman 1990; Kay 2003; Martin et al. 2004) and are under the dependence of the reciprocal effects of excitatory MT/C and inhibitory GC (Rojas-Líbano and Kay 2008). As opposed to other anesthetics, ketamine cocktails do not abolish gamma bursts; on the contrary, bursting activity is dominant in the 100- to 130-Hz band under anesthesia. However, ketamine cocktails strongly impact gamma frequency. Association of ketamine with a noradrenergic agent is mandatory to maintain an adequate level of respiratory rate and heartbeats (Wixon and Smiler 1997). To rule out an effect of α2-agonists independently of ketamine effects on NMDA receptors under anesthesia, we assessed the specific effects of xylazine and medetomidine alone. Our results reveal a differential influence of α2-agonists and point out that the power increase in the high gamma (100–130 Hz) under anesthesia is due to the action of ketamine.

We found that blocking NMDA currents using ketamine induce a faster oscillatory regime in the MOB [already mentioned in (Neville and Haberly 2003) for rats], as it was observed in the hippocampus (Lazarewicz et al. 2010; Ma and Leung 2007) and in cortical electroencephalogram (Hong et al. 2010; Pinault 2008). Changes in the gamma band in the MOB are likely to reflect a modification of the excitation-inhibition profile.
balance at the M/TC-GC synapse. Both local and distant mechanisms could be involved in the modulation of this balance. Locally, NMDA receptors have a critical role in dendrodendritic activation at the M/TC-GC reciprocal synapse (Schoppa et al. 1998). GC is also the cell type receiving the strongest cortical feedback within the MOB, and impairment of NMDA activation by ketamine could modulate the cortical feedback received by GC (Fig. 7) (Wilson et al. 1996).

Our results show a differential modulation of the MOB oscillations by KX and KM. Under KM, ongoing beta power is more impacted, and the relative change in beta and gamma power due to odor stimulation and airflow is lowered. The MOB receives strong and widespread noradrenergic inputs from the locus coeruleus, and both M/TC and GC express noradrenergic receptors subtypes α1 and α2 (Fig. 7) (Ennis et al. 2007; Linster et al. 2011). α2-Receptors have a higher affinity for NA than α1-receptors (Virtanen 1989). Consequently, a lower concentration of NA would mainly recruit α2-receptors, while higher concentrations would activate both α1- and α2-receptors. As medetomidine is a hundred times more specific to α2 than xylazine, it might have less nonspecific interactions with α1-receptors than xylazine. Interestingly, in vitro studies revealed opposing effects on the MOB according to the concentration of NA. Low doses of NA or α2-agonist decreased GABAergic transmission from GC to M/TC, while higher doses of NA or α1-agonists increased M/TC inhibition (Nai et al. 2010). Even if we cannot rule out an effect of the antagonists at the glomerular level, α1-α2-receptors are far less represented in this layer (Fig. 7) (Ennis et al. 2007). Microdialysis analysis showed that NA concentration in the MOB is about 1 nM (El-Etri et al. 1999); however, the actual concentration of NA under physiological conditions at the site of NA receptors is not known. To isolate the impact of the α2-agonists without ketamine from the anesthetized condition, it was necessary to use the same concentration of agonist for injections in awake as in the anesthetic cocktail. But as this is high concentration, even xylazine could affect both α1- and α2-receptors (Linster et al. 2011). Overall, the power difference that we observe comparing injections of xylazine and medetomidine support the critical role of inhibition in MOB oscillations generation and the impact of NA at the MT/C-GC reciprocal synapse.

**The Olfactory Bulb Activity Is Modulated by Airflow Under Ketamine Cocktail Anesthesia**

Because it determines the signal-to-noise ratio (Economou and White 2012; Engel et al. 2001), ongoing activity level can modulate the dynamic range of odor-induced responses in the MOB. The source of ongoing activity in the MOB and the relative influence of sensory neurons input or centrifugal fibers is unclear (Joseph et al. 2012; Stakic et al. 2011). Gamma oscillations in the MOB are modulated by the centrifugal feedback (Gray and Skinner 1988; Martin et al. 2006). However, olfactory receptor neurons have been shown to be mechanical sensors that provide a peripheral driving force to synchronize MOB activity with breathing cycles (Grosmaître et al. 2007). We observe that, even in the absence of odor, airflow alters gamma oscillation power. If we cannot directly compare this experimental situation to a modulation of breathing, it shows that population activity is also under the dependence of olfactory receptor neuron activation. Respiratory cycle has been shown to influence MOB oscillations both under urethane anesthesia (Buonviso et al. 2003; Cenier et al. 2009; Fourcaud-Trocme et al. 2011) and in awake animals (Fuentes et al. 2008; Rosero and Aylwin 2011). The frequency shift induced by the anesthetics in the gamma band is too dramatic to be attributed to breathing characteristics. However, since the brain state modulates respiratory rhythm, we cannot rule out that some effects on power modulation originate from breathing parameters. In our study, the frequency of respiratory cycles is reduced, and their amplitude is larger under KX and KM compared with awake (Fig. 2). Since gamma bursts are locked to inspirations, a decrease in frequency leads to a reduction in the number of bursts, as it was described when awake rats slow down their respiratory frequency (Rosero and Aylwin 2011). Interestingly, in their study the amplitude of each burst was unchanged. A decrease in the number of bursts has necessarily an impact on the LFP power, but since the maximum of gamma activity is not at the same frequency for the anesthetized and awake conditions, we cannot directly compare them. On the contrary, Courtiol et al. (2011) reported that under urethane, an experimental increase of the nasal inspiration rate enhanced gamma bursts power. Even though in our experiment gamma bursts were actually enhanced, they are still dramatically reduced compared with awake condition.

In conclusion, as for other anesthetics, several neurotransmitter systems are altered under anesthesia induced and maintained by KX or KM. One has to take these specific effects into account.

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Fig. 7. Diagram showing the distribution of N-methyl-d-aspartate (NMDA) and noradrenaline (NA) α1- and α2-receptors. NMDA receptors are found in every layer of MOB except the internal plexiform layer. They are present on mitral and tufted cell (M/TC) apical dendrites and on juxtaglomerular cells, which receive glutamatergic input from the olfactory receptor neurons. At the granule cell (GC)-to-M/TC synapse, in addition to NMDA receptors colocalized with AMPA receptors on GC dendrites, NMDA autoreceptors are present on M/TC and could be activated by spillover. α1-Receptors are predominantly located in the external plexiform layer, and on M/TC and GC bodies; α2-receptors are present in M/TC bodies and to a higher extend on GC. α1- and α2-receptors are only sparsely distributed at the level of the glomerular layer. α2-Receptors may be present presynaptically on centrifugal inputs to the GC. [Based on a concept represented by Ennis et al. 2007.]
account while interpreting neuronal responses. However, in opposition to other anesthetics, the similarities in oscillations between anesthetized vs. awake states make these cocktails suitable for electrophysiological recordings of LFP to decipher odor processing in the MOB.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.C. and C.M. conception and design of research; R.C. performed experiments; R.C. and C.M. analyzed data; R.C., H.G., and C.M. interpreted results of experiments; R.C. and C.M. prepared figures; R.C. and C.M. edited and revised manuscript; R.C., H.G., and C.M. approved final version of manuscript.

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