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(RESEARCH ARTICLE)

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Olfactory deafferentation generates gamma activity in the hippocampus during the wake-sleep cycle in the armadillo (*Chaetophractus villosus*)

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Abstract

Background: There are recent evidence of sleep-dependent changes in olfactory system structure and function which contribute to odor memory and perception.

Objective: The aim of the present study is to study the effect of the elimination of the olfactory mucosa on the electrical activity of the hippocampus, olfactory bulb, olfactory tubercle, pyriform cortex and frontal cortex during wakefulness and sleep.

Methodology: Fifteen adult armadillos chronically prepared for electrographic recordings were employed in this study. Some animals were subjected to peripheral olfactory deafferentation by removal of the olfactory mucosa. They were studied during wakefulness and sleep phases.

Results: The lack of peripheral olfactory input causes invasion in all the structures mentioned by very conspicuous and almost continuous bursts of high amplitude gamma activity (32 Hz) during slow wave sleep and paradoxical sleep.

Conclusions: The hippocampus, together with some olfactory structures participates in an outstanding phenomenon produced by the suppression of olfactory receptors. The appearance of continuous bursts of gamma activity is in sharp contrast to other published studies because it is entirely independent from olfactory input. The relevance of this phenomenon is discussed considering the so called non olfactory functions of the olfactory bulb and the participation of the hippocampus in emotion, learning and memory. The importance of studying the effects of peripheral olfactory deafferentation during sleep is emphasized in view of possible derivations of interest for the interpretation of some pathological conditions. The significance of these findings is discussed.

Keywords: Hippocampal activity; Olfactory deafferentation; Gamma activity; Slow wave sleep; Paradoxical sleep; Armadillo

1. Introduction

There are important relations between the olfactory system and hippocampal formation. Anatomically, olfactory bulb (OB) neurons project to the lateral entorhinal cortex in rodents [1,2] from where afferents reach the dentate gyrus [3]. The mammalian olfactory-hippocampus system expresses a variety of neuronal oscillations that provide a temporal framework for coordinated networks activity [4]. Functional connectivity between the OB and olfactory cortex [5], and between these primary olfactory structures and hippocampus (HC) [6] and orbitofrontal cortex [7] also were descript.

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Experiments on rats have shown that olfactory stimulation elicits bursts of rhythmical 15-30 Hz in the dentate gyrus [8]. On the other hand, olfactory stimuli activate the medial entorhinal cortex via the HC [9].

Gamma oscillations (30-100 Hz), originally seen in the OB, have long been a defining characteristic of sensory coding in the olfactory system. The relations between olfactory input, behavior and the presence of gamma activity in the pyriform cortex (PC) were studied in freely moving rats [10].

The induced gamma activity is suppressed by the elimination of the olfactory receptors in rabbits [11]. The interruption of nasal airflow by tracheotomy prevents that activity in cats [12] and in rats [13]. According to Peñaloza-Rojas and Alcocer-Cuarón [14] tracheotomy does not abolish the activity in cats provided that they remain awake. These studies were performed while the animals were awake. In contrast, the aim of this paper is to investigate the effects of peripheral olfactory deprivation on the HC and some regions of the olfactory system not only during wakefulness but also during slow wave sleep (SWS) and paradoxical sleep (PS) of the armadillo. The armadillo has been used as a non-traditional animal model in neurobiological studies [15-18]. In OB, very peculiar electrical oscillations (8-12 Hz) are recording only during wakefulness. These oscillations were termed “rhino-central rhythm” (RCR) because they require both peripheral and central influences in order to appear [15-17]. There are recent evidence of sleep-dependent changes in olfactory system structure and function which contribute to odor memory and perception in mammals [19].

2. Material and methods

2.1. Animals

Fifteen male armadillos of the species *Chaetophractus villosus* (Xenarthra, Dasypodidae) weighing 3.4-4.2 kg were used. Data on geographical distribution, morphological features and behavior of this species will be found in Iodice and Cervino [19,20].

The animals were treated following the code of ethics outlined by the Canadian Council on Animal Care [21] and also according to the Argentine law. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Implantation of the Electrodes

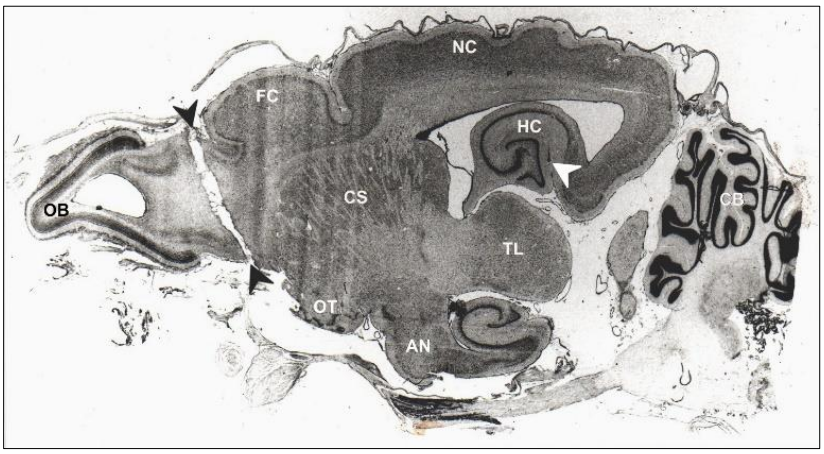

Figure 1 Parasagittal histological preparation of the brain of *Chaetophractus villosus*, 6 days after the complete cross section of both olfactory peduncles (black arrows). Histological verification of the deep electrode placement (white arrow) in the hippocampus is indicated. Note the preservation of the laminar structure of the olfactory bulb. AN, amygdala nuclei; CB, cerebellum; CS, corpus striatum; FC, frontal cortex; HC, hippocampus; NC, neocortex; OB, olfactory bulb; OT, olfactory tubercles; TL, thalamus. (Nissl staining method, 10x)

It was performed under ketamine hydrochloride (40 $mg.kg^{-1}$, i.m.) and sodium pentobarbital (35 $mg.kg^{-1}$, i.p.). The skull bones were exposed by removing a piece of the carapace with a saw. The bones were then drilled over the OB and the dorsal neocortex of both brain hemispheres. Very thin bipolar electrodes made of stainless steel pins and insulated except at the tip were placed on the dorsal surface of the OB and neocortex (frontal cortex, FC) [16]. Additionally the same type of electrodes was inserted through the brain and placed into the olfactory tubercles (OT), PC and cornu ammonis proper very near the dentate gyrus (HC) (Figure 1). The insertion was made by means of a stereotaxic

instrument (David Kopf, New York) specially built for armadillos. Electrodes were soldered to DB connector and the whole set was covered with dental acrylic. The electrical activity was bipolar recorded. ECG and EMG electrodes -last into one muscle of the hind limbs- were implanted in order to aid the diagnosis of PS.

2.3. Technique for the elimination of the Olfactory Mucosa

For the study of the effect of olfactory mucosa (OM) elimination on the electrical activity (OMX animals), in nine armadillos previously implanted, both nasal cavities were perfused with a 5 % solution of $ZnSO_4$. This was done under the same anesthetic protocol that electrodes implantation. Then, a curved thin tube ending in a hollow hook was introduced into the mouth in such a way as to insert it into each one of the choanae [16]. Ten milliliters of zinc solution for each nasal cavity were perfused slowly while the animals were in dorsal decubitus position. Later, was washed with saline solution.

2.4. Section of the Olfactory Peduncles

In five armadillos the olfactory peduncles were sectioned rostrally to the anterior olfactory nucleus, under anesthesia, by means of a blade introduced stereotaxically (OPX animals) (Figure 1).

2.5. Control Experiments

Control experiments consisted of sham nasal perfusion with saline instead of zinc sulphate (n= 3) or sham OPX (n= 3).

2.6. Analysis of the Electrical Activity of Olfactory System

The EEG signals were recorded and digitized with a sampling frequency of 256 Hz. They were filtered through a bandpass 1.6-110 Hz (notch-filter at 50 Hz). Under visual inspection of the records there was selected periods of 30-40 seconds in which the Fast Fourier Transform (FFT) was computed. This computation was performed on adjacent epochs of 512 points corresponding to a duration of two seconds for the sampling rate of 256 Hz. Previously, the adopted software subtracts from all points the mean value of each epoch. The data were then tapered with a cosine window occupying 40% of the epoch. This computation resulted in a 0.50 Hz frequency resolution with components up to 128 Hz. The power spectrum was obtained according to the following procedure: the periodogram was computed for each epoch. For each Fourier coefficient the components were squared and summed. The periodograms of all the epochs selected were then averaged. Thus, power spectrum and their absolute and relative powers were obtained for each channel (OB, olfactory bulb; FC, front cortex; OT, olfactory tubercle; PC, pyriform cortex; HC, hippocampal cortex) and frequency band. The latter were defined as follows: delta (δ) 1.6-3.9 Hz, theta (θ) 4.0-7.9 Hz, alpha (α) 8.0-13.0 Hz, beta (β) 13.1-20.0 Hz and gamma (γ) 20.1-80.0 Hz. The boundary between beta and gamma activity was determined at 20 Hz according to previous studies conducted in this animal model [16,17].

The EEG recordings and analysis were made by means of Harmonie and Sensa Software respectively (Stellate Systems Quebec, Canada).

2.7. Ambient Conditions for the Recordings and the Observation of the Animals

The animals were placed in individual cages placed within a Faraday cage maintained at 22±2º C, under a light-darkness schedule of 12 h with lights on at 7 AM. The animals were observed through a closed circuit TV system. The EEG recordings and the behavioral observations were made continuously during the light period (these are nocturnal animals) when they were asleep most of the time. However, some records were made during the dark period with an array of 48 infra-red LEDs that was placed in the roof of the cage at a distance of 50 cm from the animals.

2.8. Experimental Schedule

After the implantation of the electrodes and of all the experimental procedures a period of 96 h was left for recovery.

The experiments were performed in two consecutive stages performed in the same animals (n= 9). The electrical activity of the above mentioned structures was studied during wakefulness, SWS and PS. The stages were as follows:

Stage 1: after the implantation of the electrodes, the electrical activities were studied during five days.

Stage 2: when stage 1 was finished, the same animals were submitted to olfactory deafferentation (OMX). From the first day after the deafferentation the electrical activity of the OB and other structures was studied during seven days.

Later stage 2, five OMX animals were submitted to section of the olfactory peduncles (OPX).

After the experiments, the armadillos were euthanized and were perfused with formaldehyde 10% in saline. The sacrificed procedure was under anesthesia suggested in the AVMA Guidelines on Euthanasia for mammals reported by the American Veterinary Medical Association [22]. The brains were removed and paraffin sections were stained with the Nissl method in order to verify the placement of the electrodes (Figure 1).

2.9. Statistical Analysis

In general, the spectral values of the variables studied in the quantified EEG (qEEG) do not follow a normal distribution. To meet this assumption of parametric statistical analysis it was necessary to perform transformations to obtain normality in the data. In the case of total power (TP), the log_{10} transformation was used. For the values of absolute and relative powers of the different frequency bands selected, the transformation *log (r/(1 - r))* was used, where *r* was the value obtained from spectral analysis [23].

Two-Way Repeated Measures ANOVA were performed to determine the significance of the differences observed between treatments OM vs OMX and phase of the sleep-wake cycle (SWC), for each CNS region studied. Tukey test was additionally performed to determine the differences between treatments. In all cases, a probability value of $p<0.05$ was used. For the statistical analysis of the data obtained, the software SigmaStat for Windows 3.5 (Systat Software, 2006) was used.

3. Results

3.1. The Electrical Activity of Olfactory System in OM and OMX Animals

3.1.1. During wakefulness

- Before OMX. The classical Adrian's induced waves (induced gamma activity, 23-24 Hz), Ottoson's waves and a peculiar 8-12 Hz-RCR were observed in the OB. Adrian´s waves were clearly seen FC (Figure 2, OM). When the animals were in relaxed wakefulness, the RCR dominated the tracings. Generally, the more this rhythm was observed the less Adrian's waves were seen. The power spectra of the induced gamma activity showed maximal power at 24 Hz in the OT and PC (Figure 2, OM). The HC appeared rather synchronized exhibiting 4-8 Hz waves and some 15-20 bursts.
- After OMX. The global electric activity is strongly diminished. The Adrian's waves were completely absent in the OB. The same happened with Ottoson's waves and with the RCR. The theta rhythm (~5 Hz) in the OB characteristic of peripheral olfactory deafferentation was clearly seen. The induced gamma activity of OT and PC disappeared. The HC bursts disappeared but not the 4-8 Hz waves (Figure 2, OMX).

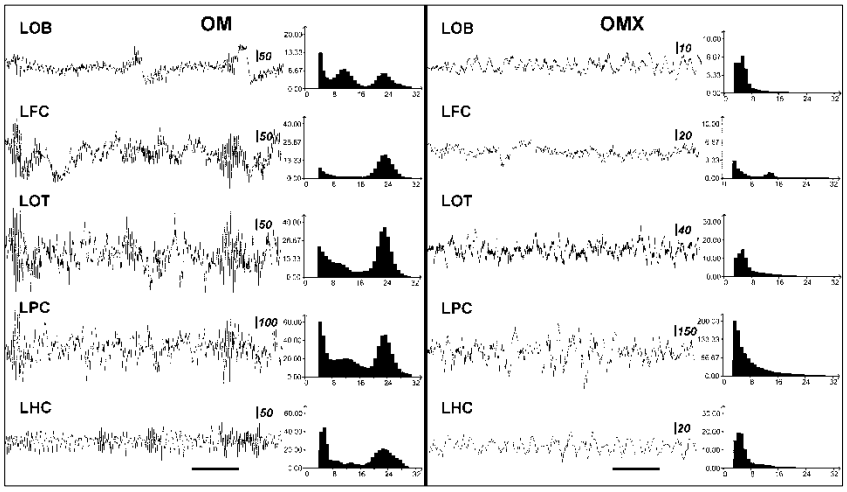

Figure 2 Electrical activity and power spectra before (OM) and after (OMX) the elimination of the olfactory mucosa during wakefulness. LOB, left olfactory bulb; LFC, left frontal cortex; LOT, left olfactory tubercle; LPC, left pyriform cortex; LHC, left hippocampal cortex. Horizontal bar, 1 second. Vertical bars indicate the amplitude in microvolts

3.1.2. During SWS

- Before OMX. The Adrian's waves were reduced or absent in the OB. The same happened with Ottoson's waves. The RCR was absent. In the five tracings the power spectra showed that there were abundant slow waves. Sleep spindles with maximal power at 14-16 Hz were seen in FC (Figure 3, OM). No 4-8 Hz waves were seen in HC.
- After OMX. Bursts of very conspicuous high amplitude gamma activity were observed throughout this phase of sleep in all the structures including the HC. A clear modulation of their amplitude was also observed. The power spectra showed maximal power at 32 Hz (Figure 3, OMX). When these animals were submitted to the section of the olfactory peduncles the high amplitude gamma activity disappeared from all the structures.

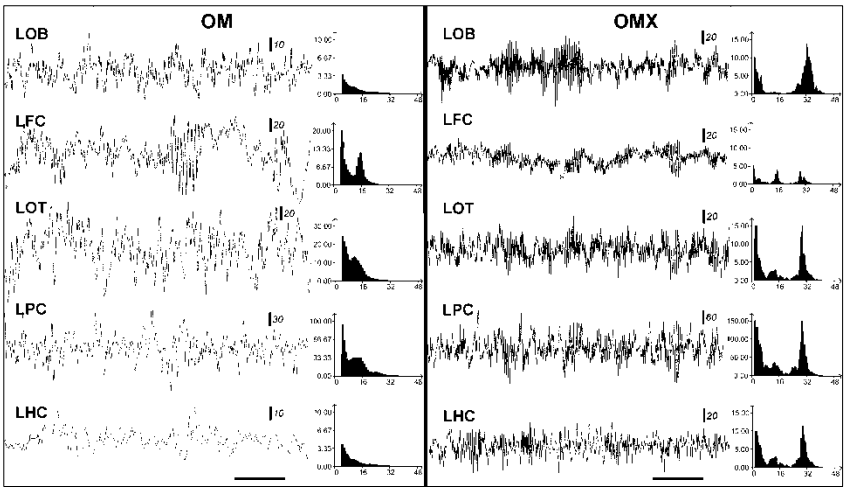

Figure 3 Electrical activity and power spectra before (OM) and after (OMX) the elimination of the olfactory mucosa during slow wave sleep. LOB, left olfactory bulb; LFC, left frontal cortex; LOT, left olfactory tubercle; LPC, left pyriform cortex; LHC, left hippocampal cortex. Horizontal bar, 1 second. Vertical bars indicate the amplitude in microvolts

3.1.3. *During PS*

- Before OMX. The Adrian's waves were also greatly reduced or absent. The same happened with Ottoson's waves. The RCR was completely absent (Figure 4, OM). The power spectra show maximal power at 4-8 Hz (theta rhythm).

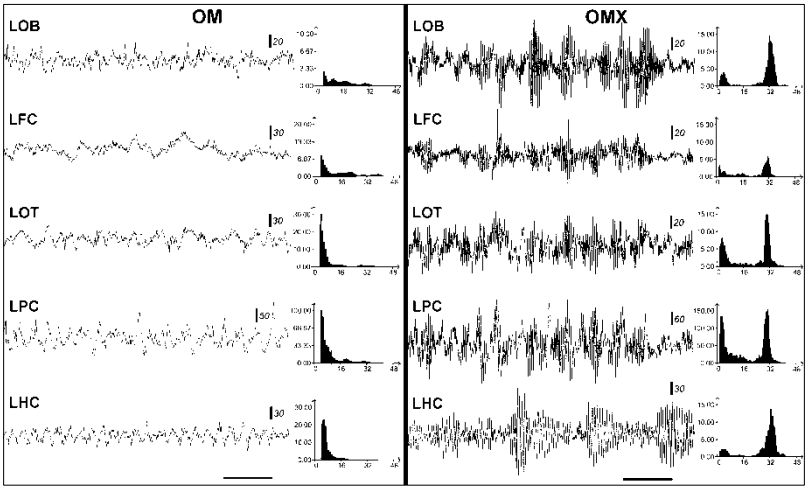

Figure 4 Electrical activity and power spectra before (OM) and after (OMX) the elimination of the olfactory mucosa during paradoxical sleep. LOB, left olfactory bulb; LFC, left frontal cortex; LOT, left olfactory tubercle; LPC, left pyriform cortex; LHC, left hippocampal cortex. Horizontal bar, 1 second. Vertical bars indicate the amplitude in microvolts

- After OMX. Bursts of very conspicuous gamma activity were observed throughout this phase of sleep. A clear modulation of their amplitude was also observed. The power spectra showed maximal power at 32 Hz (Figure

4, OMX). Generally, the gamma activity occupied more time of the tracings and exhibited greater amplitude than during SWS. There was a significant difference (P<0.01, Paired t-test) in frequency between induced Adrian´s waves and the gamma activity of sleep in the peripherally deafferented animals. When these animals were submitted to the section of the olfactory peduncles the high amplitude gamma activity disappeared from all the structures (Figure 5, OMX after OPX).

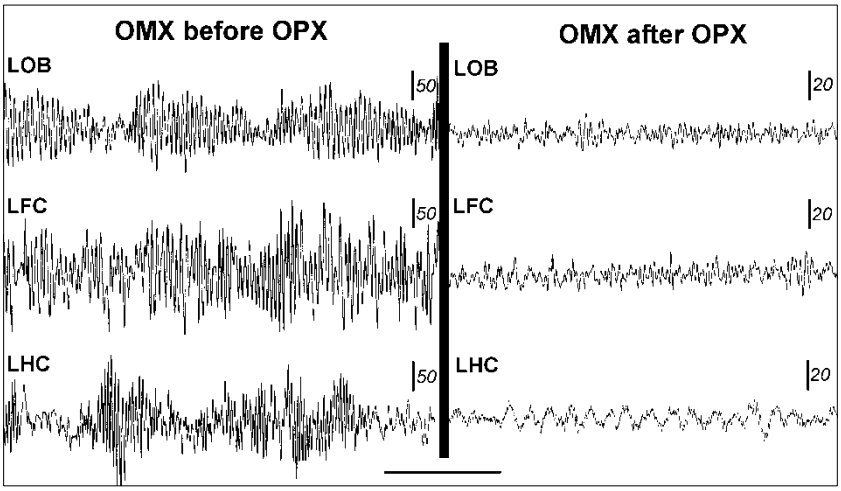

Figure 5 Electrical activity in OMX animals before and after the section of the olfactory peduncles (OPX) during paradoxical sleep. The bioelectrical activity of isolated BO and HC was characterized by a rhythmic activity of 4.5 to 6.0 Hz (~25 μV amplitude). LOB, left olfactory bulb; LFC, left frontal cortex; LHC, left hippocampal cortex. Horizontal bar, 1 second. Vertical bars indicate the amplitude in microvolts

3.1.4. During Arousing Stimuli in OMX Animals

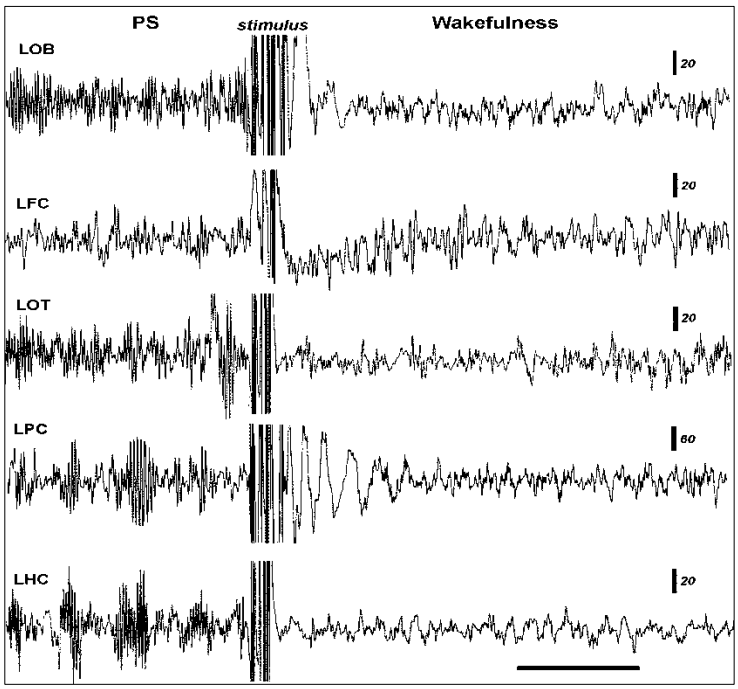

Figure 6 Arousal from paradoxical sleep (PS) in the peripherally deafferented armadillo. The artifact indicates application of an auditory stimulus. Note the theta rhythm characteristic of peripheral olfactory deafferentation in LOB and LPC. LOB, left olfactory bulb; LFC, left frontal cortex; LOT, left olfactory tubercle; LPC, left pyriform cortex; LHC, left hippocampal cortex. Horizontal bar, 1 second. Vertical bars indicate the amplitude in microvolts

When arousing stimuli (auditory, tactile, etc.) were applied, in SWS and PS, there was an immediate behavioral arousal with abrupt interruption of the gamma activity in all the structures. The latter phenomenon was completely similar in

both sleep stage. After the application of the stimuli the theta rhythm characteristic of deafferented animals during wakefulness was also immediately seen in the OB and HC (Figure 6).

3.2. After the Sham Elimination of the OM

The electrophysiological patterns of these animals were similar to the intact animals.

3.3. Quantification of Cerebral Bioelectrical Activity (qEEG) before and after Bilateral Peripheral Olfactory Deafferentation

Figure 7 shows the variation throughout the sleep-wake cycle (SWC) of different qEEG parameters for the OB and FC, in nine armadillos before and 48 hs after peripheral olfactory deafferentation.

EEG total power (TP): SWC phase, OM-OMX condition, and the interaction have statistically significant differences ($p<0.001$), in all structures studied. After peripheral olfactory deafferentation, TP declines during quiet wakefulness (QW) and SWS. In all structures studied, except FC, during PS the TP is increased due to the presence of conspicuous gamma activity. In deafferented animals, TP in OB during QW decrease due to the absence of Ottoson's waves and Adrian's gamma activity, which have great amplitude in animals with OM intact. In OM animals, the TP in FC during SWS increases due to the presence of delta waves and sleep spindles.

Relative delta (%δ): both SWC phase and OM-OMX condition, showed significant differences ($p<0.001$), in OB, OT, PC and HC. The interaction was not significant. Multiple contrasts showed that the decrease in %δ during the SWC was significant between sleep phases ($p<0.05$). In FC throughout the SWC, the abundance of delta waves showed significant differences ($p<0.001$). Multiple contrasts showed significant increase in %δ during SWS ($P<0.05$). Instead, the OM-OMX condition and interaction were not significant in the FC.

Relative theta (%θ): SWC phase, OM-OMX condition and interaction varied significantly ($p<0.001$) in OB, FC, OT, PC and HC. In animals with peripheral olfactory deafferentation, during QW, a 400% increase of %θ was observed compared to animals with OM intact, especially in OB, due to presence of "θ of deafferentation". During QW and PS the abundance of theta is evident in the hippocampus ($P<0.05$).

Relative alpha (%α): in all recorded brain regions the factors SWC phase and OM-OMX condition show statistically significant differences ($p<0.001$); the interaction was significant ($p<0.011$ for OB, OT and PC; $p<0.028$ for NC and HC). Multiple pairwise comparisons show that during QW in animals with intact MO, %α is higher compared to OMX animals ($P<0.05$). EEG traces of the OB show that the RCR rhythm -within the α-frequency- present in awake animals with OM is not found in OMX armadillos, where the %α is due to waves present in the EEG, but without forming a true rhythm.

Relative beta (%β): SWC phase and OM-OMX condition, as well as the interaction have statistically significant differences ($p<0.001$), in OB, OT, PC and HC. In OMX animals, the incidence of the band beta in OB is increased during QW ($p<0.05$), and is diminished during PS ($p<0.05$). In FC, the SWC phase ($P<0.001$) and OM-OMX condition ($P=0.002$), as well as the interaction ($P=0.012$) shown statistically significant differences. During QW, %β is markedly increased after peripheral olfactory deafferentation, in this last region.

Relative gamma (%γ): SWC phase and OM-OMX condition, as well as the interaction have statistically significant differences ($p<0.001$), in OB, FC, OT, PC and HC. In OB, during QW, when analyzing the incidence of the gamma activity -corresponding to Adrian's induced sinusoidal activity (22 Hz)- there are significant differences ($p<0.05$), since during sleep this activity is absent. In OMX animals, the %γ during SWS it doubled ($p<0.05$) and during PS it tripled ($p<0.05$), approximately, with respect to QW, due to the presence of conspicuous rhythmic gamma activity around 32 Hz. Something similar happens in OT, PC and HC. In FC, during QW, %γ is diminished after peripheral olfactory deafferentation, but during SWS and PS gamma activity is significantly higher ($P<0.05$).

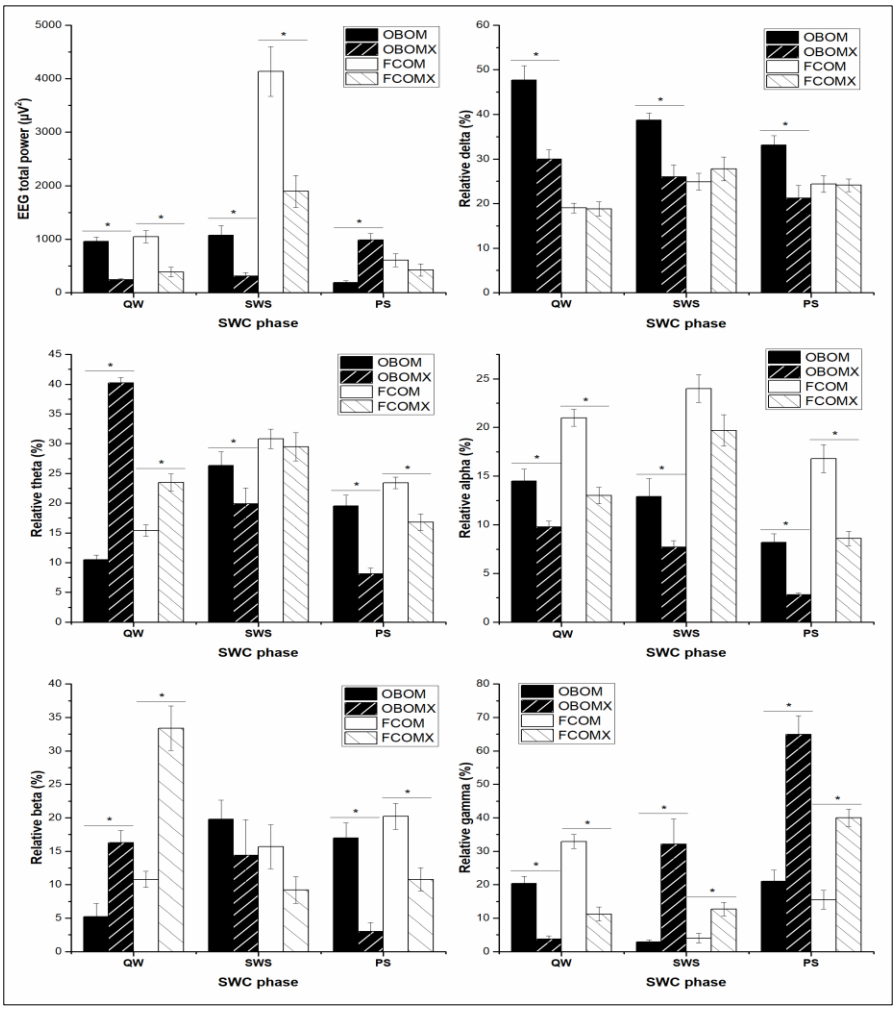

Figure 7 Variation of the qEEG parameters of the olfactory bulbs (OB) and frontal neocortex (FC) during quiet wakefulness (QW), slow wave sleep (SWS) and paradoxical sleep (PS) of animals with intact olfactory mucosa (OM) and peripheral olfactory deafferentation (OMX). Error bars represent the mean ± SE (N= 9). Asterisk indicates to significant differences (Tukey test, $p<0.05$) between OM vs OMX condition

4. Discussion

These results show that the elimination of the OM produce striking changes in the electrical activity of all the structures studied. The overall picture varies whether the animals are awake or asleep. During wakefulness, the most salient change is the disappearance of the 8-12 Hz-RCR. During both phases of sleep an almost continuous high amplitude gamma activity invades the OB, the OT, the PC, the FC and the HC in spite of the absence of olfactory receptor neurons.

Consistent with these findings, previous work demonstrated that OB rhythms disappear after bypassing nasal airflow through tracheotomy in rat [13] and in armadillo [15], which shows that they are generated by peripheral stimuli as opposed to central respiratory networks.

The most surprising phenomenon appears during sleep. It is represented by the appearance of gamma activity invading the HC and the olfactory structures during SWS and PS in spite of the absence of OM. The functional role that gamma oscillations may play in olfaction and in sensory perception is still under debate, as is the nature of processing in the OB [24]. The presence of gamma activity in the FC is particularly interesting. In fact, neocortical projections of the olfactory cortex have been described [25]. Apparently, armadillos have a powerful frontal representation of smell. The olfactory structures here studied represent approximately two thirds of the brain surface of armadillos [26]. A noteworthy fact is the spreading of gamma activity to the HC providing additional proof of its participation in olfactory mechanisms. Considering that this structure is also involved, although not exclusively, in memory, cognition, emotion and learning [27], the relevance of this sleep phenomenon appears evident.

During sleep, while the electrical activity of olfactory system in intact animals is reduced, the activity shifts to gamma activity in OMX animals, reminiscent of that observed in the OB during wakefulness. Obviously, in deafferented animals the gamma activity was not produced by the peripheral input to the OB because the OM had been eliminated. The latter fact was demonstrated by histological controls and by the complete absence of Adrian´s induced activity following odorous stimuli during wakefulness. The OM absence and the disappearance of the gamma activity after the section of the olfactory peduncles suggest that the centrifugal input to the OB is responsible for the generation of gamma activity in this olfactory structure. For contrary, if the lateral olfactory tract –axons of a subset of cells from the OB that project to the PC [28]– is disrupted in animals with OM and awake, gamma oscillations in the OB persist [29], but gamma no longer occurs in PC [28]. This suggests, under these conditions, that the mechanism involved in producing gamma oscillations resides within the OB.

It is not surprising that the appearance of gamma activity in the OB has different origins. In fact, gamma oscillations are not of a single type associated with odor coding in the OB. According to Kay [30], they are of at least two types of gamma activity, distinguishable by their differential relationships to behavioral state and the sniffing cycle and by their frequency characteristics.

There are many brain structures from which centrifugal fibers arise: anterior olfactory nucleus, locus coeruleus, raphe nuclei, pyriform cortex, lateral entorhinal cortex, some amygdaloid nuclei [2]. Although the generator of this gamma activity in unknown, one hypothesis is that they are driven by the highly auto-excitatory endopiriform nucleus, which has broad excitatory connections throughout PC [31]. The experiments performed do not permit to determine from what nucleus or nuclei the influence is originated, but the results strongly suggest that whatever the nucleus or nuclei might be they are active during both phases of sleep.

Why are they active? Here, the lack of stimulation of the olfactory receptors undoubtedly plays a major role. This is an experimental condition, which is present after OMX. Also, the electrical activity during tracheal breathing was quite similar to that observed after OMX [15]. Therefore, the sequence of events appears to be as follows: during sleep, the nasal airflow impinges upon the intact OM initiating an action, which impedes the generation of gamma activity in the OB and all the other structures. In this way, the role of nasal breathing for the maintenance of the normal electrophysiological patterns of sleep appears fundamental. In the absence of nasal airflow or when the OM is eliminated the impediment represented by the activation of the olfactory receptors is removed and consequently the gamma activity reappears. I do not know how this is accomplished. It is probable that the nasal airflow initiates a direct action on the OB, which blocks its response to the centrifugal input. Another possibility is that the influence initiated by the nasal airflow blocks the centrifugal impulses at their site of origin. Only future experiments can elucidate this point.

Since Adrian´s induced waves diminish or disappear during sleep one might think that the olfactory system remains "closed" during sleep. However, it is evident that during this state some kind of influence originated in the OM continues to arrive at the brain. As an outcome of this arrival, no gamma activity can be seen. I suggest that bulbar and pontine respiratory centers being partially responsible for nasal airflow indirectly participate in the regulation of brain electrical activity.

From the fact that the gamma activity is immediately interrupted after the application of arousing stimuli, it is suggested that the brain systems responsible for the arousal reaction directly or indirectly block the influence of the centrifugal system.

Respiration-entrained oscillations in the OB have first been described by Adrian [32]. Gault and Leaton [33], in cats, studied the relationship between respiration and the sinusoidal induced waves. They observed, first, that there was a clear correspondence between Ottoson slow waves and ventilation, to the point of serving as an indicator of it. On the other hand, they showed that the burst of Adrian's induced waves appeared coincidentally with the passage of air through the nose and were not recorded when the nostrils are closed. Gault and Coustan [12] in cats, showed the absence of Adrian's induced waves in tracheostomized animals. Pagano [34], in acute experiments on curarized cats, studied the OB electric activity after stimulating the mesencephalic reticular formation, bulbar reticular formation and mesencephalic central gray matter. In all cases it was observed an increase in the amplitude of sinusoidal induced waves that accompanied the ventilation movements. The section of the olfactory peduncle prevented the increase and stimulation when it was not accompanied by passage of air through the nose, did not appear sinusoidal induced activity.

On the other hand, based on recordings from urethane-anesthetized mice, Yanovsky *et al.* [35] demonstrate that nasal respiration causes prominent oscillation at near-θ frequencies (2-4 Hz) in the HC. The rhythm is highly coherent with nasal respiration and with rhythmic field potentials in the OB. These results suggest that respiration-induced

oscillations have a role in information processing in hippocampal networks, providing a long-range synchronizing signal between olfactory and hippocampal structures.

As OMX determine the appearance of gamma activity it is suggested that the centrifugal centers are very active during sleep. Sleep, therefore, might represent a valuable research "tool" for revealing new feedback relations between centrifugal fibers and the olfactory structures. Recent work has begun exploring the role of sleep in olfactory memory [19]. Olfaction is interesting in this regard given the unusual anatomy of the olfactory pathway compared to all other sensory systems, most notably the relatively limited involvement of a thalamic nucleus prior to the primary sensory cortex.

In direct relation with these results, studies in waking rats [10] showed that the generation of gamma activity in the PC is entirely dependent on olfactory input. The results shown in this paper with armadillos are in opposition to those findings. However, Vanderwolf made his study during wakefulness whereas the phenomenon descript here was found during sleep. Of course, species differences cannot be discarded. Yet, these results provide evidence of the existence of gamma activity independent of olfactory input. As far as I know, this is the first report communicating the existence of gamma activity in the olfactory system and HC being entirely independent from peripheral olfactory input.

What is the physiological significance of gamma activity independent of the olfactory input during sleep? A rather simplistic interpretation of these results, probably wrong, could be that one of the functions of the olfactory input is to avoid the invasion of the olfactory structures by gamma activity during sleep.

In fact, when the OM is intact no gamma activity is seen. However, caution is needed in this respect. The abnormalities of the electrical activity induced by ablation procedures may not be understood simply in terms of the absence of a putative function that is normally mediated by the ablated tissue. Rather, the abnormal electrical activity may reflect the normal dynamic interactions of the OB with other interconnected structures.

I wish to suggest that these experiments on the effects of the sole elimination of the peripheral olfactory input contribute to differentiate this procedure from other procedures producing anosmia. I think that this is a very important point. In fact, olfaction is very important in many aspects of mammalian life. It is therefore not surprising that numerous investigators tried to assess the physiological and behavioral effects of smell suppression. Unfortunately, in past years, this was done by bilateral ablation of the OB. They obtained a variety of interesting results, which in many cases were simply attributed to the absence of olfaction. However, when the effects of peripheral olfactory damage are compared to those of bulbectomy certain general modulatory functions of the OB are strongly suggested. There is a vast amount of data regarding the effects of bulbectomy which cannot be attributed to anosmia per se [36,37]. It is therefore, of the outmost importance to distinguish between bulbectomy and peripheral olfactory deprivation. The latter occurs in several experimental conditions: 1) suppression of nasal airflow (closure of the nostrils, tracheal breathing); 2) decrease in the number of olfactory receptors by elimination of the OM; 3) exposure to odor-free environments. Several papers were published describing changes in morphology, neurochemistry and metabolism of the olfactory system after those procedures (review in Maruniak [38]). However, studies regarding the effects on the electrical activity of the olfactory system and other brain structures are scanty. Regarding sleep, those studies are missing. To provide data on this problem was one of the aims of the present paper.

Last but not least, I do not know if the findings presented here can be transferred to microsmatic animals like humans. The human olfactory system also appears similarly depressed during SWS [39]. In this sense, I think that research concerning the latter point is badly needed because in humans there are pathological conditions in which serious olfactory deficits and lesions in olfactory structures are observed. Olfactory deficits are associated with a variety of disorders including, but not limited to Alzheimer's Disease [40,41], Parkinson's disease [42,43], Huntington's disease [44], schizophrenia [45], major depression [46], including malfunctions of the olfactory epithelium due to infections, such as in COVID-19 [47].

Does this affect the quality of sleep? Of course, this might have far-reaching consequences for studies on brain development and brain health. All the above mentioned disorders are also associated with sleep disturbances such as insomnia and sleep fragmentation [48]. Thus, I speculate that a contributing factor to the widespread occurrence of sleep disorders, across diverse pathologies may be related to underlying olfactory disorders. Perhaps, the most important lesson of this research on armadillos is that it is advantageous to be patient enough as to wait for the onset and maintenance of sleep.

5. Conclusion

The HC, together with some olfactory structures, participates in an outstanding phenomenon produced by the suppression of olfactory receptors. The appearance of continuous bursts of gamma activity is in sharp contrast to other published studies because it is entirely independent from olfactory input. The relevance of this phenomenon can be associated with the so-called non-olfactory functions of the OB and the participation of the HC in emotion, learning and memory. It is concluded that it is important to study the effects of peripheral olfactory deafferentation during sleep in view of possible derivations of interest for the interpretation of some neuropathological conditions in humans.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares to have no conflict of interest.

Statement of ethical approval

Ethical approval was obtained from ethical committee. The animals were treated following the code of ethics outlined by the Canadian Council on Animal Care and also according to the Argentine law. All efforts were made to minimize animal suffering and to reduce the number of animals used.

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