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(RESEARCH ARTICLE)

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Bilateral ablation of the vomeronasal organs produces dramatic changes in the EEG of the main olfactory system during paradoxical sleep

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Abstract

Background: The vomeronasal organ (VNO) plays an important role in reproductive physiology, behavior, sexual behavior and aggression.

Objective: The aim is to report a surprising effect of the removal of the VNO on the cortical electrical activity of the olfactory bulb, piriform lobe and frontal cortex exclusively during paradoxical sleep (PS).

Methodology: Seventeen adult armadillos chronically prepared for electrographic recordings were employed. Some animals were subjected to VNO bilateral remove. They were studied during wakefulness and sleep phases.

Results: An outstanding phenomenon occurs during PS of armadillos submitted to bilateral ablation of the VNO. There is a dramatic change in the oscillations of the olfactory bulb electrical activity strictly confined to PS. The change consists of the sudden appearance, as soon as PS initiates, of high amplitude gamma oscillations during the whole duration of each PS episode. They disappear as soon as PS comes to an end. The oscillations are also seen in the paleocortex and the frontal neocortex. They disappear after the transverse section of the olfactory peduncles.

Conclusions: Our results show the absence of VNO deeply modifies the electrophysiological expression of PS in the olfactory bulb and other extensive brain areas. They are elicited by the centrifugal input to the olfactory bulb. From there they propagate to the piriform and frontal neocortex. These results show a new and subtle role for the organs. Some hypotheses related to the effects of bilateral removal of the VNO are presented.

Keywords: Vomeronasal organ; Paradoxical sleep; Gamma activity; Main olfactory system; Accessory olfactory system; *Chaetophractus villosus*

1. Introduction

The accessory olfactory system (AOS) [1, 2, 3, 4] is also termed vomeronasal system. It comprises the vomeronasal organ (VNO), the vomeronasal nerve, the accessory olfactory bulb, some nuclei of the amygdaloid nuclear complex, and some regions of the bed nucleus of the stria terminalis. This system and the main olfactory system (MOS) are morphologically separate and show different functions. However there are connections between them. Investigations show that the functional dichotomy between both systems is not so definitive because both the AOS and MOS have the ability to detect large overlapping sets of chemosensory cues [5, 6, 7, 8].

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The VNO is a tubular organ contained in a bony capsule situated bilaterally along the base of the nasal septum [9]. In most species its lumen is lined by different epithelial cells, sensory and non-sensory [10]. In different species, there is communication either with nasal or oral cavities or both [11]. This organ occurs in most terrestrial vertebrates [9]. It is communicated with the accessory olfactory bulb by means of the vomeronasal nerve. That bulb projects to the amygdaloid complex [4, 12]. Efferent projections from the medial amygdaloid nucleus were shown to terminate in an area which includes parts of the medial preoptic area, anterior hypothalamic nucleus and ventromedial and ventral premammilary hypothalamic nuclei. The intermediate and medial divisions of the bed nucleus of the stria terminalis project to the same preoptic hypothalamic regions [13].

The structures of the AOS are sexually dimorphic [3, 14]. Males show greater values of volume and number of neurons than females [15].

The VNO plays an important role in reproductive physiology, behavior, sexual behavior and aggression [4, 9, 16]. Contrary to prior observations, new anatomical studies indicated that a VNO is present in human adults and reports were published indicating that this system might be functional [17, 18, 19]. The latter observations are still controversial [20].

Several types of experiments were done to investigate the functions of the VNO. Its removal (VNX animals) was performed in different mammal's species. The action of this procedure was studied on detection of predator odor by golden hamsters [21]; perception of alarm pheromone in male rats [22]; lordosis behavior of mice [23]; olfactory sex discrimination or mate recognition in ferrets [24]; sperm motility in male mice [25]; sexual behaviors and intermale aggressive behaviors in prosimian primate [26]; maternal aggression in rats [27]; perception of ephemeral pheromones in mice [28]; reproductive performance and aggression in voles [29]; aggression in female mice [30]; aggression in male mice [31]; lordosis in female hamsters [32]; release of LH in female rats [33]; the role of prolactin in acceleration of puberty in mice [34], and sexual behavior of male hamsters [35].

Studies of the electrical activity of the accessory olfactory bulb were reported [4, 8, 36, 37, 38, 39].

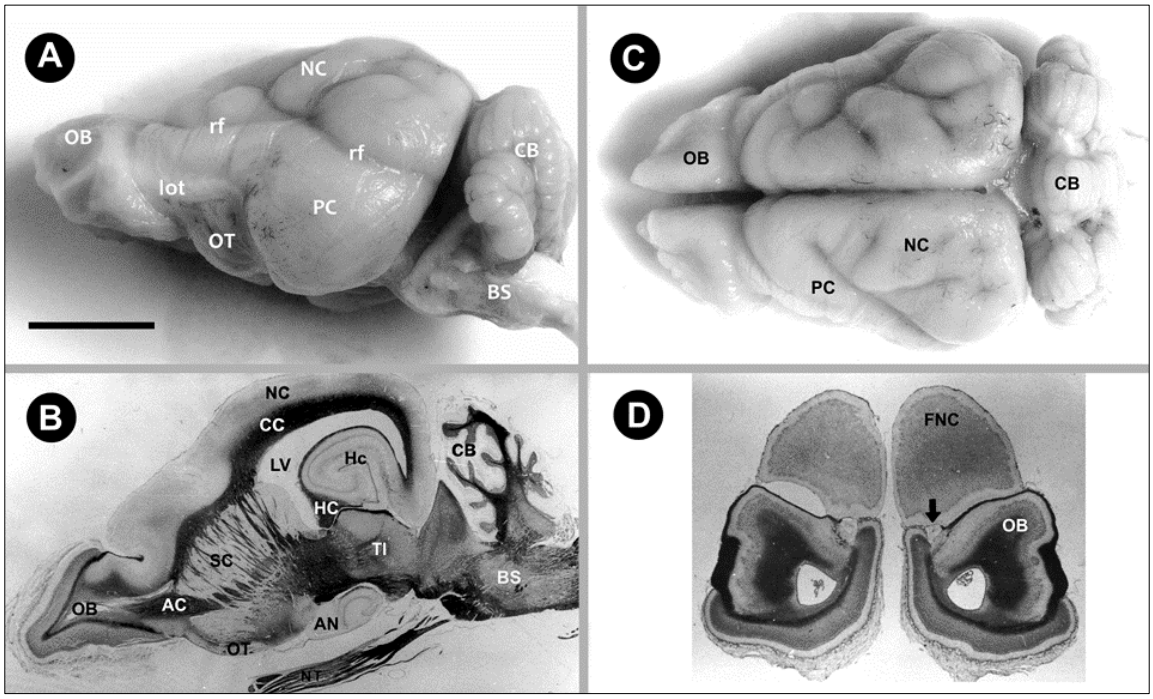

Figure 1 Left lateral (A) and dorsal (C) view of the brain of the armadillo *Chaetophractus villosus*, adopted as experimental model. Parasagittal histology of the brain (B) and coronal histology along the main olfactory bulb (D) of *C. villosus* is also shown. The arrow points to the accessory olfactory bulb. *AC,* anterior commissure; *AN,* amygdala nuclei*; BS*, brainstem; *CB*, cerebellum; *CC*, corpus callosum; *FNC*, frontal neocortex, *Hc*, hippocampus; *HC,* habenular commissure*; lot*, lateral olfactory tract; *LV,* lateral venticle*; NC*, neocortex; *NT,* trigeminal nerve; *OB*, olfactory bulb; *OT*, olfactory tubercle; *PC*, piriform cortex; *rf*, rhinal fissure; *SC*, striate corpus; *Tl,* thalamus

As far as we know there is no study of the effects of removing the VNO on the electrical activity of the MOS. Neither was done in sleeping animals.

In our Laboratory we develop a technique to remove the VNO in the armadillo [40]. The aim of this paper is to report a surprising effect of the removal of the VNO on the cortical electrical activity of the olfactory bulb (OB), piriform cortex (PC) and dorsal surface of the frontal neocortex (FNC) exclusively during paradoxical sleep (PS). The experiments were done on the South American armadillo *Chaetophractus villosus*. We were motivated to perform this research by our previous finding of the dramatic effects of the deafferentation of the MOS on the electrical activity of some brain areas during slow wave sleep (SWS) and PS [41, 42, 43].

The high position of the rhinal fissure on the lateral side of the brain in this armadillo facilitates the study of paleocortical areas (**Fig. 1**). Studies on vomeronasal anatomy, histology and ultrastructure of our experimental animal were done by our research group [44, 45].

2. Material and methods

2.1. Animals

Seventeen male armadillos of the species *Chaetophractus villosus* (Xenarthra, Dasypodidae) weighing 3-4 kg were used. Data on geographical distribution, morphological features and behavior of this species will be found in Iodice and Cervino [46, 47]. Some peculiar functional characteristics will be found in Affanni and Cervino [42] and in Affanni et al. [48].

Ethical approval was obtained from ethical committee of the University of Morón. The animals were treated following the code of ethics outlined by the Canadian Council on Animal Care [49] 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

It was performed under ketamine hydrocloride (KETONAL 50®, Lab. Richmond, 40 mg/kg, *i.m.*) and sodium pentobarbital (PENTHOTAL SODICO®, Lab. Abbott, 35 mg/kg, *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 wires and insulated except at the tip were placed on the dorsal surface of the OB and frontal cortex [43, 50]. Additionally the same type of electrodes was inserted through the brain and placed into the PC by means of an *ad hoc* stereotaxic apparatus (David Kopf, New York). The electrical activity was recorded by means bipolar EEG leads. ECG and EMG were recorded whit bipolar electrodes implanted into one muscle of the hind limbs to facilitate detection of PS.

2.3. Technique for bilateral removal of the VNO

The general histological aspect of both VNO of our animal model is shown in (**Fig. 2**). The animals were anaesthetized with ketamine hydrochloride (KETONAL 50®, Lab. Richmond) at a dose of 50 mg/kg, combined with xylacine (ROMPUM®, Lab. Bayer) at doses of 3 mg/kg, diazepam (DIAZEPAN LAMAR®, Lab. Lamar) at doses of 10 mg/kg and atropine sulfate (BIOQUIM, Lab. Duncan) at doses of 0.1 mg/kg intramuscularly. They were fastened to a stereotaxic apparatus (David Kopff, New York) specially built for armadillos. They were put in dorsal decubitus and with the mouth widely opened an incision was made in palatal mucosa. Then, a slit 15 mm long, 4 mm wide was made in the palatal bone by means of a dental drill. The VNO lying bilaterally at the base of the nasal septum could be clearly seen under a dissecting microscope and excised by means of forceps. The slit in the bone was then closed by means of dental acrylate and the palatal mucosa sutured. The detail of the VNO ablation technique can be found in Iodice et al. [40].

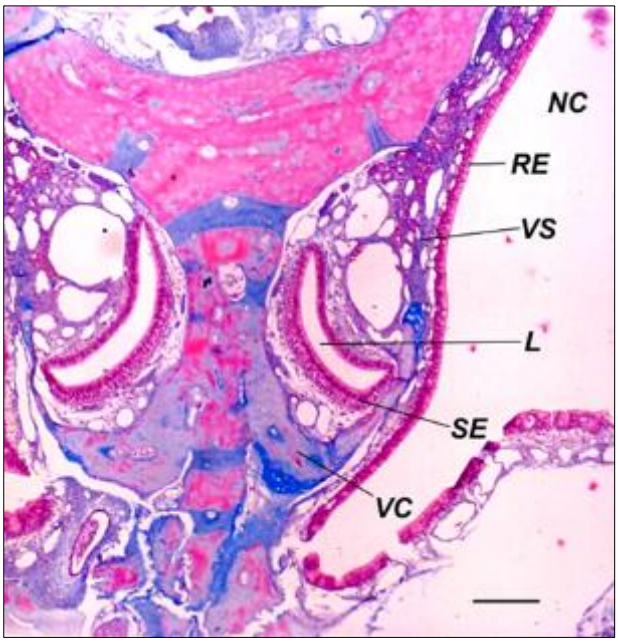

Figure 2 Transverse section of both vomeronasal organs of *Chaetophractus villosus*. *SE*, sensory epithelium; *L*, lumen of VNO; *NC*, nasal cavity; *VC*, vomeronasal cartilage; *VS*, large venous sinus. Hematoxilin-Eosin. Bar, 200 μm

2.4. Section of the Olfactory Peduncles

Under the same type of anesthesia as for VNO removal, the olfactory peduncles were sectioned, rostral to the anterior olfactory nucleus, by means of a blade guided stereotaxically [43, 50].

2.5. Histo-anatomical verification

After the experiments, the armadillos were euthanized and were perfused via the aorta with saline solution followed by Bouin´s fluid. The sacrificed procedure was under anesthesia suggested in the AVMA Guidelines on Euthanasia for mammals reported by the American Veterinary Medical Association [51]. In order to verify the complete removal of the VNO, the noses were decalcified in buffered formic acid [52] for 25 days and washed in water for 12 hours. The tissues were dehydrated and embedded in paraffin. Serial sagittal and transverse sections were stained with either hematoxylin and eosin or Masson´s trichrome. The brains were removed and paraffin sections were stained with the Klüver-Barrera method in order to verify the placement of the electrodes and section of the olfactory peduncles.

2.6. Control Experiments

Control experiments, sham operations, consisted of six animals submitted to all the procedures required (anesthesia, antibiotics, dental acrylate, etc.) both before and after the surgical excision of the OVN except that excision. In three of these control animals, sham operations for the section of the olfactory peduncles were also made by the adequate procedures.

2.7. Analysis of the Electrical Activity

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; FNC, frontal neocortex; PC, pyriform 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 [42, 43, 50].

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

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

The animals were placed in individual cages located 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.

2.9. Experimental Schedule

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

The experiments were performed in three consecutive stages performed in the same animals. The electrical activity of the above mentioned structures (OB, FNC and PC) was studied during wakefulness (W), SWS and PS. The stages were as follows:

- *Stage 1*: in eleven experimental animals after the implantation of the electrodes, the brain electrical activities were studied during three days.
- *Stage 2*: when Stage 1 was finished, the same animals were submitted to the bilateral excision of the VNO. From the first day after the removal the electrical activity of the OB, FNC and PC was studied during seven days.
- *Stage 3*: in five experimental animals, when Stage 2 was finished, the animals were submitted to the section of the olfactory peduncles. From the first day after this surgical intervention the electrical activity of the previously mentioned structures was studied during seven days.

2.10. Statistical Analysis

t-Student test were performed to determine significance differences observed in frequency of induced gamma activity in W (VNO or VNX animals) and the gamma activity during PS (VNX animals). 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 the OB, dorsal surface of the FNC, and PC

3.1.1. During Wakefulness (W)

- *A) Before removal of the VNO (intact animals):* The classical Adrian's induced waves (induced gamma activity), Ottoson's waves and a peculiar alpha-rhythm were observed in the OB (**Fig. 3**). When the animals were in relaxed W the 8-12 Hz (rhino-central rhythm, RCR) dominated the tracings. Generally, the more this rhythm was observed the less Adrian's waves were seen. These bioelectrical activities of the OB are projected towards the FNC and the PC. The power spectra of the induced gamma activity showed maximal power at 24 Hz in the OB, dorsal surface of FNC and PC (**Fig. 3**).
- *B) After removal of the VNO (VNX animals):* The tracings were similar to those prior to VNO ablation (**Fig. 3**). Adrian's waves (induced gamma activity) were always present in the OB, FNC and PC. The same happened with Ottoson's waves and with the RCR. No theta rhythm was seen in the OB.

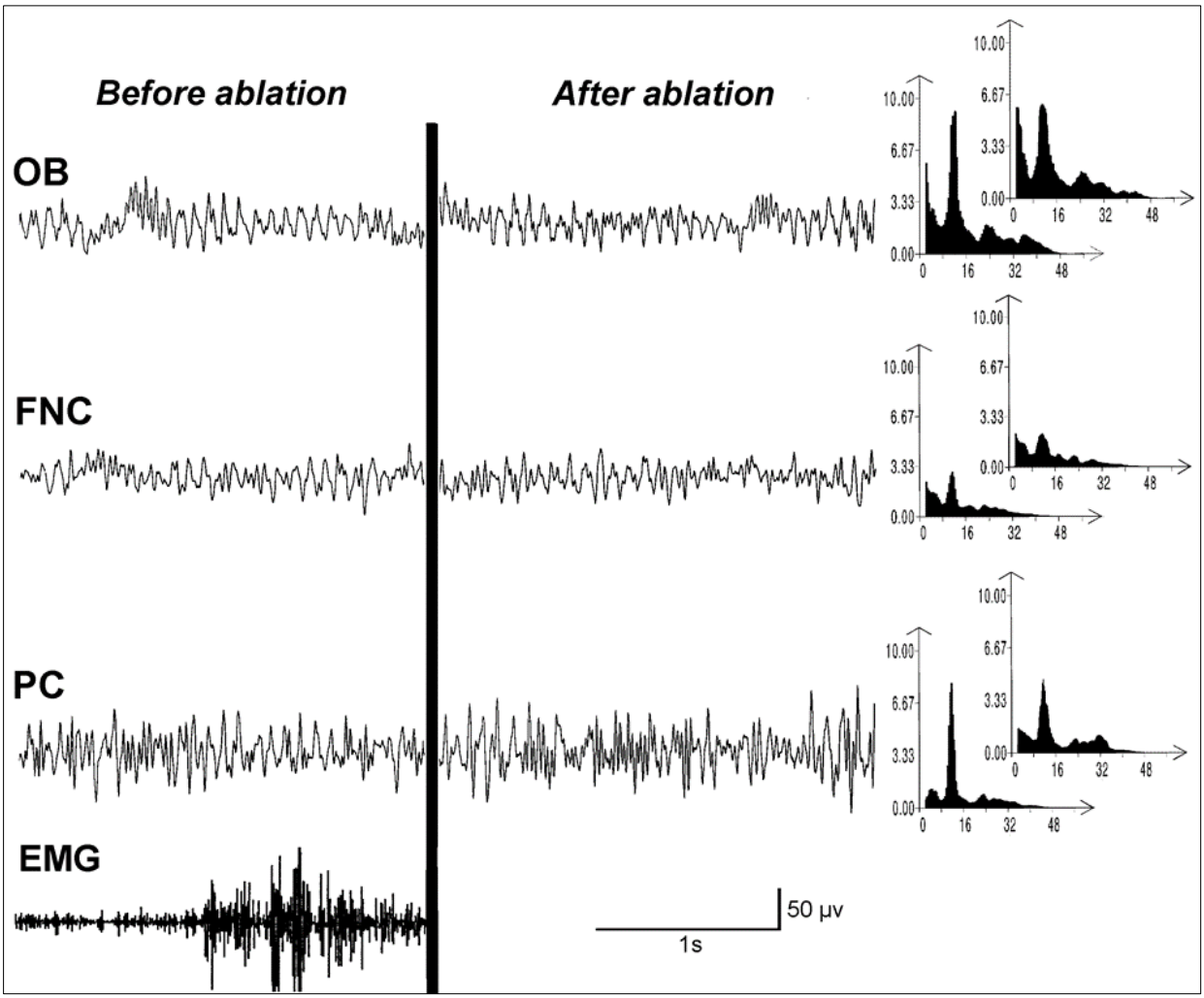

Figure 3 Electrical activity and power spectra before and after VNO ablation, during Wakefulness (N= 11). Recording of EMG and power spectra from EEG analysis (before ablation, bottom; after ablation, top) are included. OB, left olfactory bulb; FNC, left frontal neocortex; PC, left pyriform cortex. Vertical bars indicate the amplitude in microvolts; horizontal bars, 1second

3.1.2. During Slow Wave Sleep (SWS)

- *A) Before removal of the VNO (intact animals):* The Adrian's waves were reduced in amplitude or absent in the OB. The same happened with Ottoson's waves. The RCR was absent. In the three EEG tracings and the power spectra showed that there were abundant slow waves. Sleep spindles with maximal power at 14 Hz were seen in FNC (**Fig. 4**).
- *B) After removal of the VNO (VNX animals):* The same types of tracings than those recorded prior to VNO ablation (**Fig. 4**) were observed.

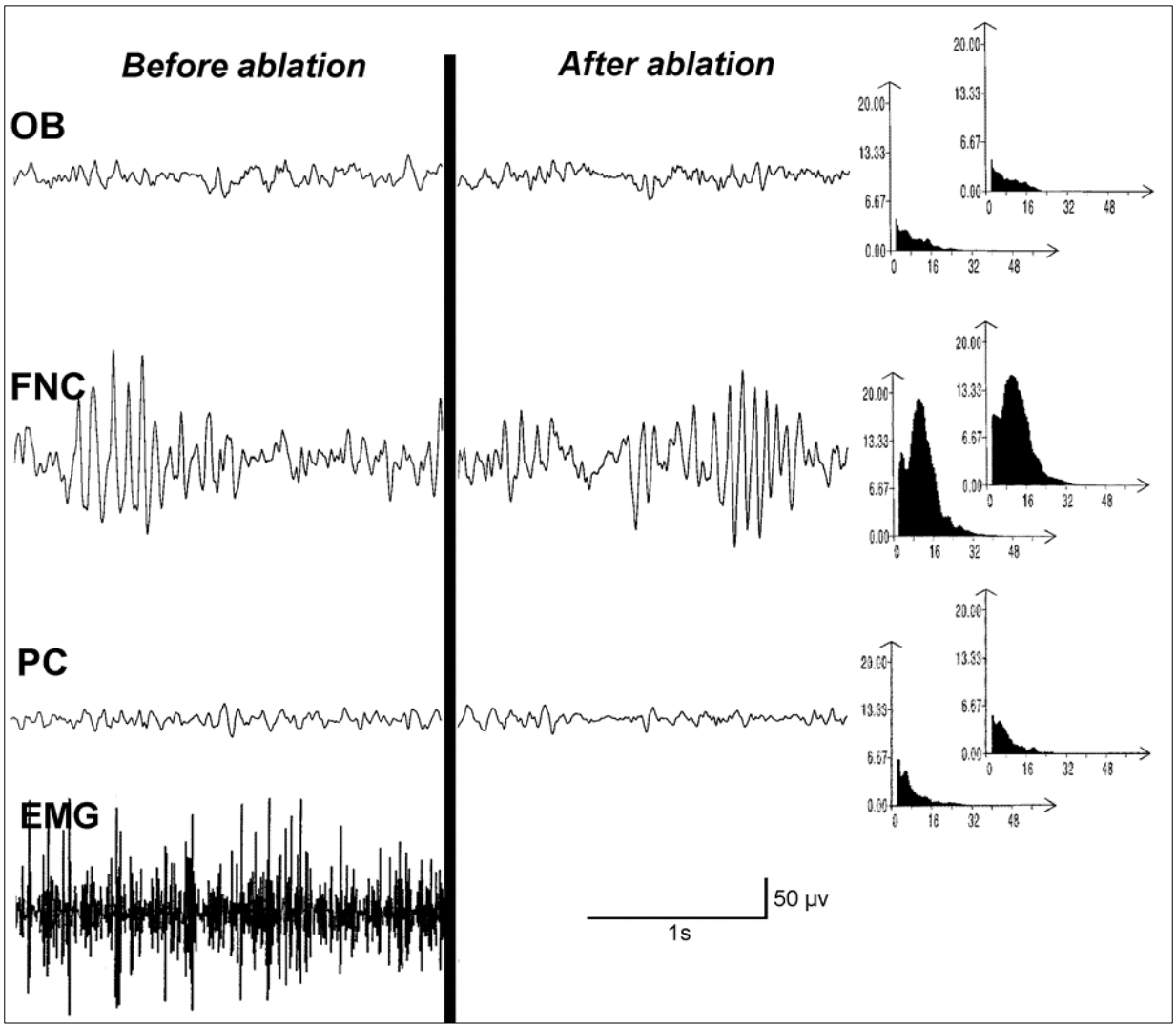

Figure 4 Electrical activity and power spectra before and after VNO ablation, during Slow Wave Sleep (N= 11). Note the big sleep spindle (14 Hz) in FNC. Recording of EMG and power spectra from EEG analysis (before ablation, bottom; after ablation, top) are included. OB, left olfactory bulb; FNC, left frontal neocortex; PC, left pyriform cortex. Vertical bars indicate the amplitude in microvolts; horizontal bars, 1second

3.1.3. During Paradoxical Sleep (PS)

- *A) Before removal of the VNO (intact animals):* The Adrian's waves were also greatly reduced or absent. The same happened with Ottoson's waves. The RCR was completely absent (**Fig. 5**).
- *B) After removal of the VNO (VNX animals):* A dramatic change in the electrical patterns of OB, FNC and PC was observed. Bursts of very conspicuous gamma activity were seen continuously throughout this phase of sleep. A clear modulation of their amplitude was also observed. The power spectra showed maximal power at ~32 Hz (**Fig. 5**). There was a significant difference ($P<0.01$, Paired *t*-test) in frequency between induced gamma activity in W (VNO or VNX animals) and the gamma activity of PS (VNX animals).

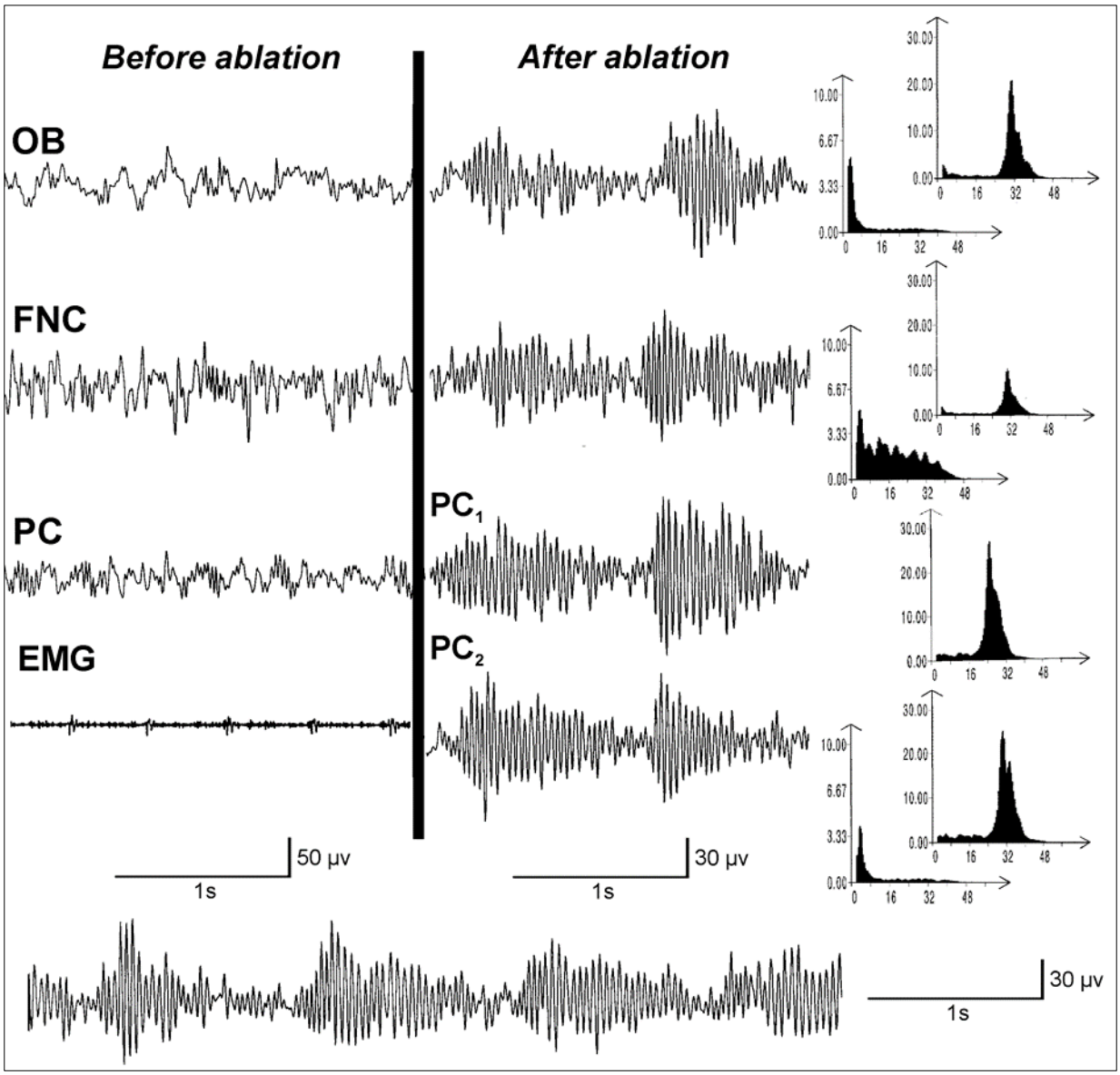

Figure 5 Electrical activity and power spectra before and after VNO ablation, during Paradoxical Sleep (N= 11). Recording of EMG and power spectra from EEG analysis (before ablation, bottom; after ablation, top) are included. Note the strong amplitude modulation in OB (lower recording). OB, left olfactory bulb; FNC, left frontal neocortex; PC, left pyriform cortex. Vertical bars indicate the amplitude in microvolts; horizontal bars, 1second

- *C) Effect of an arousing stimulus:* In VNX animals, when strong stimuli (auditory, tactile, etc.) were applied during PS, there was an immediate behavioral arousal with abrupt interruption of the gamma activity in OB, FNC and PC (**Fig. 6**).

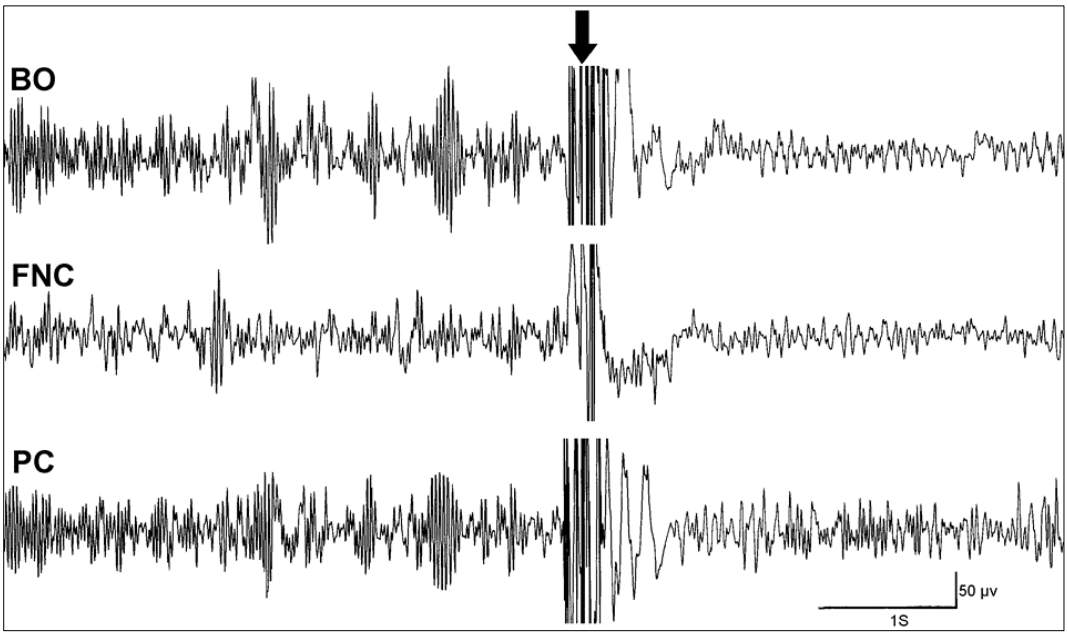

Figure 6 Arousal from Paradoxical Sleep in the VNX armadillo. OB, left olfactory bulb; FNC, left frontal neocortex; PC, left pyriform cortex. Arrow indicates application of an auditory stimulus. Vertical bars indicate the amplitude in microvolts; horizontal bars, 1second

- *D) Section of the olfactory peduncles:* During PS, when VNX animals were submitted to the section of the olfactory peduncles the high amplitude gamma activity disappeared from all the structures (**Fig.7**).

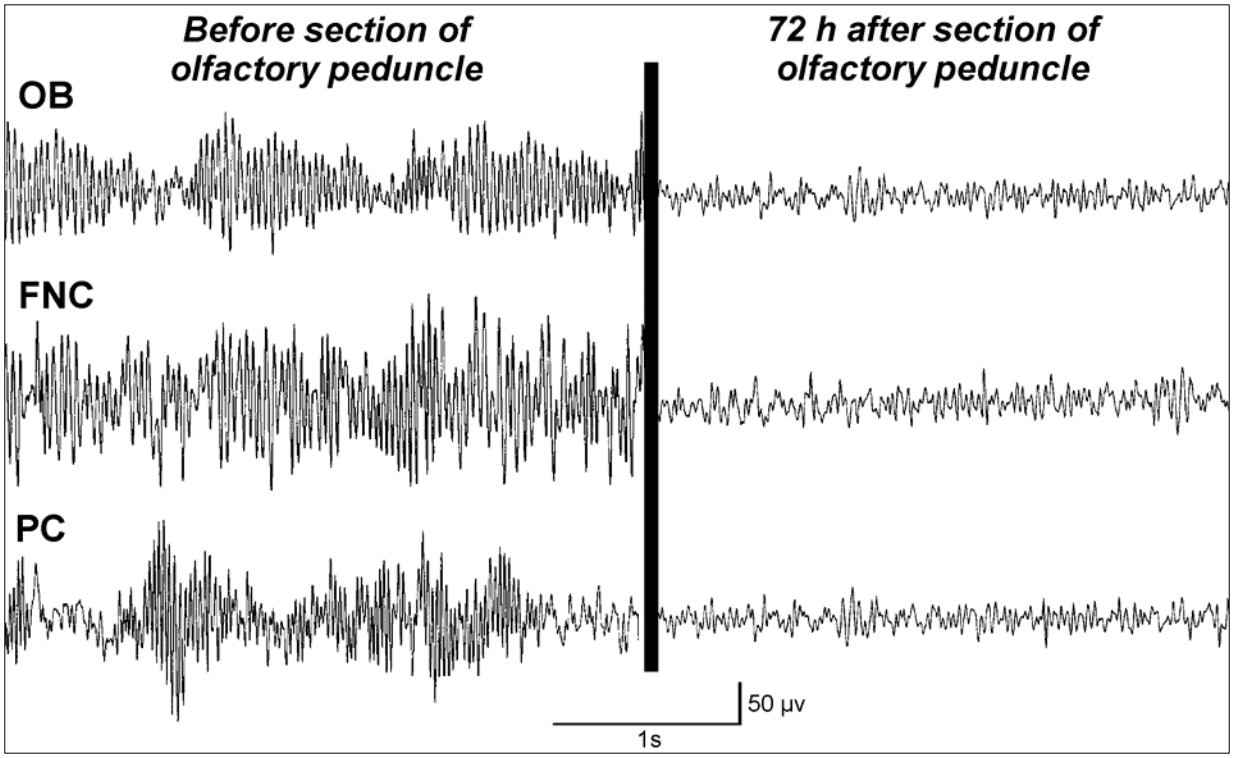

Figure 7 EEG from VNX animals subjected to olfactory peduncle section (N= 5), during PS. OB, left olfactory bulb; FNC, left frontal neocortex; PC, left pyriform cortex. Vertical bars indicate the amplitude in microvolts; horizontal bars, 1second

3.2. After the Sham Operations

In all the animals submitted to the sham operation and studied during W, SWS and PS, the electrophysiological patterns of all the structures studied showed no differences with those of intact animals.

4. Discussion

From our results, it is worth noting several points:

- The post mortem studies showed that the VNO were completely extirpated and that the olfactory mucosa was not damaged. The latter fact was corroborated by the presence of Adrian´s waves, Ottoson´s waves and the RCR. Moreover, the absence of gamma activity during SWS which is always seen when the olfactory mucosa is lacking [42, 43] adds evidence in this sense.
- During W and SWS, VNX no changes were observed in the electrical activity of the OB, PC or dorsal FNC. In fact, Adrian´s waves [53] Ottoson´s waves [54] and the RCR [41] of the operated animals did not show major general changes in comparison with control animals. The persistence of the RCR shows that the VNO are not necessary for its generation. This fact contributes to improve our knowledge concerning the fundamental characteristics of that peculiar electroencephalographic rhythm.
- In spite of the lack of effect of OVN removal during W and SWS, a most surprising phenomenon appears exclusively during PS. Removal this sensorial organ elicits dramatic changes in the brain electrical pattern. These changes are represented by the sudden appearance of a very conspicuous gamma activity (high amplitude, 32 Hz) as soon as PS begins. It remains present without interruptions during the whole this sleep stage, followed by their abrupt disappearance as soon as PS comes to an end. Only some amplitude oscillations of the gamma waves are noticed.
- The gamma activity was seen not only in the OB, but also in the PC and dorsal surface of the FNC. We observed them in the animals in which we put electrodes in some points of the paleocortex under the rhinal fissure (Fig. 1). Those areas represent almost two thirds of the total brain surface as was shown in our laboratory [55, 56]. The neocortical surface is reduced to a relatively small cap. In a sense it is somewhat surprising to verify that the removal of the OVN is capable of inducing such a notorious change throughout the paleopallial surface.
- In a way similar to what happens in olfactory deafferented animals [42, 43] the application of arousing stimuli immediately interrupts the peculiar gamma activity of PS. 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.
- The section of the olfactory peduncles provokes the disappearance of the gamma activity. This suggests that centrifugal influences from the brain are strictly necessary for the gamma invasion of extensive areas of the brain. From the OB they propagate to the PC, the rest of the paleocortex and the FNC.
- The presence of gamma activity in the dorsal surface of the FNC agrees with the neocortical projections of the olfactory cortex already described [2]. Apparently, armadillos have a powerful frontal representation of smell.

The disappearance of the gamma activity after the section of the olfactory peduncles provides an essential clue for beginning to understand the mechanisms of production of the gamma oscillations. This occurs because it abolishes not only the OB oscillations but also those of the PC, FNC and some paleocortical areas. The participation of the central centrifugal input to the OB is therefore mandatory for generating PS oscillatory dynamics. There are many brain structures from which centrifugal fibers arise: anterior olfactory nucleus, locus coeruleus, raphe nuclei, PC, lateral entorhinal cortex, and some amygdaloid nuclei [4, 57]. Our experiments do not permit to determine neither the precise source nor the transmitters of the centrifugal input to the OB. However, it is evident that it is active only during PS.

The OB is necessary for the propagation of gamma activity to the rest of the brain areas in which the oscillation were recorded. In fact it acts as a sort of distribution station for those areas.

It is worth noting that removal of the VNO produces striking changes in the bioelectric patterns of the MOS. This represents one of the most interesting findings of the present paper because it indicates an interaction between both systems which is manifested only during PS. The results indicate a new role of the VNO, which has not been communicated previously. Our experiments reveal that the AOS exerts, during PS, a powerful action on the MOS which determines, under normal conditions, the gamma activity “silence” of the latter system.

Why that kind of centrifugal influences are active only during PS? We think that the absence of VNO must play a major role. We suggest the following hypothesis: in control animals, during PS, the vomeronasal efferences either directly or

indirectly way block the action of PS. VNO removal would suppress the action permitting the arrival of centrifugal impulses at the OB. This might generate gamma activity in the OB and from there towards other brain areas.

There is another interesting result of our experiments. Anatomical studies of the central pathways of the olfactory and vomeronasal systems showed that these two systems converge on telencephalic neurons providing an anatomical substrate for functional interactions [5, 6, 7, 8]. Examples concerning to functional interaction are extremely scanty. It is already known that these systems exhibit a conjoint participation in the vocalization of male mice when exposed to freshly voided female urine [28]. As far as we know there are no reports of reciprocal or unilateral influences on the electrical activities of both systems. The VNO is a component of the AOS, whose removal induces striking electrical oscillations in the MOS. VNX deeply, modifies the electrophysiological expression of PS in the OB. Our results, therefore, show a new kind of functional relationship between both systems.

In view of these findings we think that it would be interesting to investigate if the inverse also occurs. That is, to inquire if peripheral deafferentation of the MOS modifies the electrical activity of the AOS.

Our experiments also suggest that under normal conditions the OVN impedes the PS changes in the OB bioelectrical oscillations. While being intact, it impedes the centrifugal influence on the OB. When absent (by the ablation) permits it. We wander if under normal conditions an intact OVN might suffer some kind of functional nullity imitating the experimental ablation. Future experiments recording the flow of impulses in the vomeronasal nerve would be capable of clarifying this point. In that case the VNO would act like a master switch that can assume the "on" or the "off" position regulating the absence or presence of gamma activity in the OB.

We think that perhaps some mammals would be able to maintain an inactive state during PS. In this sense there is an interesting report from Ganzha and Bogach [58]. They communicate the presence of gamma oscillations in the MOS of normal dogs. The oscillations were not related to the olfactory input since they persisted with tracheal breathing. The authors estimated that the oscillations were perhaps of central origin although they did no section the olfactory peduncle. Those findings are in sharp contrast with ours because we did not observe the oscillations in normal armadillo but in the vomeronasalectomized ones.

Last but not least, on reading the paper from Ganzha and Bogach we thought that perhaps the dog studied by them had some type of vomeronasal deficiency. In fact, we found a paper [59] in which they write: "*The V2R genes are expressed in the mammalian vomeronasal organ, and their products are involved in detecting pheromones. Here, we describe the evolution of the V2R gene family. We have found that the human, chimpanzee, macaque, cow and* ***dog*** *V2R gene families* ***have completely degenerated.***" (highlight is ours). We do not know if that degeneration bears some relationship with the presence of OB gamma waves in normal dogs. However, this could be a promising line of research.

There remains an intriguing point. We found the gamma activity abrupt appearance and disappearance coinciding with the beginning and end of each PS episode of VNX animals. This suggests that intact VNO exerts a suppressor influence on the generation of gamma activity in the OB. This influence interferes with the action of PS structures. This suppression must be originated by the VNO activation. Now, which is the stimulus for that activation? Experiments on other mammals indicate that the VNO are not continuously stimulated. The principal stimuli are represented by pheromones although it has been demonstrated that VNO are also responsive to odors [60, 61]. However, pheromones are not always available. In the case of our experiments, this availability appears highly improbable on account of two facts:

- The animals were deeply asleep immerged in PS with the characteristic lack of muscular tonus. This atonia invades even the penile muscles in which erections do not occur during this sleep phase [48].
- Each animal under observation was isolated in a special room within which the Faraday cage was installed (see 2. Material and methods).

Thus, in the absence of sampling activity for detecting pheromones and odors it seems difficult to admit that the VNO are capable of producing a continuous discharge of impulses during PS. Evidently, the difficulties for explaining the VNO activation by pheromones are particularly solid.

How is it that VNX generates a sequence of events that produces the permanent presence of gamma activity during the whole duration of all the PS?

In search of a more convincing explanatory argument we rescue an almost forgotten but most important observation from Adrian [36] with rabbits. In an epoch in which pheromones were not good known, this author compared the

electrical activity of the MOS and AOS. He showed that the effect of air borne smells was confined to the former. However, he observed that a slight mechanical pressure to the VNO elicited a powerful oscillatory activity in the accessory olfactory bulb. In view of that observation, we propose the following hypothesis: In armadillos exists a tonic discharge from the VNO receptors which inhibits the centrifugal nuclei during PS. Such a discharge is probably elicited by vascular changes in the organ that might exert mechanical pressure on the receptors.

There are facts that make our hypothesis plausible. Firstly, the development of mechanical pressure is facilitated by the fact that the organ is enclosed in a bony and cartilaginous capsule. In a previous investigation of our group [44] we studied the VNO of our animals (Fig. 2). It is a tubular organ enclosed in a cartilaginous canal communicating with the nasal cavity whereas caudally the tube ends blindly. The sensory epithelium is on the medial wall. Large vascular sinuses, nerves and glandular tissues are seen within it. Other animals also show a similar disposition. The VNO of the hamster also shows abundant cavernous, elastic tissues submitted to changes in blood pressure under autonomic nervous control [62].

Additionally and probably supporting our hypothesis, a paper from Homeyer et al. [63] made in Jouvet´s laboratory appears very significant. They reported an interesting phenomenon. In the chronic pontine cat, with the OB isolated from the brain stem, the Ottoson and Adrian´s induced waves were suppressed during PS. This observation could not be explained by centrifugal control since the OB were separated from the rest of the brain. The suppression of the waves was correlated with vasodilatation of the nasal mucosa. The authors considered that the control of the OB oscillations was presumably mediated through the vasomotor innervation from the medulla to the nasal mucosa. We propose that a similar phenomenon might occur in the VNO. The anatomical and histological conditions of the VNO are perfectly suited for the exertion of pressure. We think that probably during PS those changes submit the vomeronasal receptors of our animals to considerable excitatory pressure. Does PS stimulate the VNO through autonomic mechanisms generators of mechanical pressure? The question remains...

The data presented here lead us to an unforeseen and interesting result. According to them, it appears that the VNO plays an important role in determining the bioelectrical patterns of OB and other brain regions deployed during SP. The cortical surface area invaded by gamma activity (OB, PC and FNC) is relatively large. On the other hand, the great amplitude and percentage of time occupied by the activity during the PS, lead us to suspect that the appearance of the same can have important consequences on the brain energetics. Its absence, while the vomeronasal function remains intact, could also have important energetic consequences.

So far this curious new function of VNO had not been suspected. The extraordinarily interesting thing about this activity is that it appears only in the course of the PS. The findings of vomeronasal eliminations contrast markedly with those of olfactory deafferentation [42, 43]. The latter generates the appearance of the same type of activity but now for the entire duration of the SWS and the entire duration of the PS.

5. Conclusion

We hypothesized that in our animal model there could be a tonic discharge from VNO receptors that inhibits centrifugal nuclei during PS. If bilateral VNO excision causes the appearance of the gamma activity described above, it must be admitted that normal vomeronasal afferences somehow prevents its appearance. The new function of the VNO, then, would be to stop the appearance of this electroencephalographic activity when the brain enters the PS phase.

As described, peripheral olfactory deafferentation is able to determine the bulbopetal centrifugal activity triggering gamma activity, both during SWS and during PS. On the other hand, vomeronasal deafferentation only does so in the PS. Our experiments do not allow us to know the mechanism of such differential effects, but they clearly indicate a very interesting fact hitherto unknown.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare to have no conflict of interest.

Statement of ethical approval

Ethical approval was obtained from ethical committee of the University of Morón. The animals were treated following the code of ethics outlined by the Canadian Council on Animal Care [49] 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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