

EFFECTS OF THE ABSENCE OF OLFACTORY INPUT IN THE REGULATION OF BRAIN ELECTRICAL ACTIVITY

A hypothesis concerning its participation in Neurodegenerative Diseases

The present report represents the first one of a forthcoming series in which we shall describe the several research lines, which are being undertaken at the Neuroscience Institute of the School of Medicine at the University of Morón (Argentina).

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The interactions between the olfactory system and the rest of the brain are being investigated.

Our research is aimed at contributing to know what happens to the brain when the olfactory system is damaged. It addresses issues related to the effects of:

- 1) The elimination of the olfactory receptors.
- 2) The interruption of the connections between the olfactory bulb and the rest of the brain.
- 3) Lesions of some structures involved in processing olfactory signals (lateral olfactory tract, anterior commissure, olfactory tubercles and piriform cortex).

These effects are being followed through the physiological states of Wakefulness and Sleep. The present report is referred to the first of those effects whereas the remaining ones will be addressed in the near future.

Why is the Olfactory System attracting the attention of an increasing number of researchers? What happens to the brain when the olfactory input is lacking? The answer to these questions resides in the rather surprising amount of peculiarities that it exhibits. As we shall see, some of them are considerably relevant in Medicine.

The sense of olfaction is primal for the life of humans and animals. Phylogenetically it is one of the oldest senses and most of the brain structures were modeled around it. Its function is to detect food, predators and mates whereas it gives warnings about food condition and the presence of dangerous chemicals.

There are some facts reflecting its crucial importance in human and animal life. These are:

- 1) The extraordinarily large amount of genetic information dedicated to it¹.
- 2) The numerous centrifugal fibers with different neurotransmitters arriving at the olfactory bulbs². These fibers arise from an unusual large number of brain regions. Therefore, it is one of the sensory systems more intensively submitted to control and modulation from the brain².
- 3) The diffusion, dispersion and interconnectedness of the central connections of the olfactory nerve³.

These facts suggest that probably there are still new functions to be discovered and not strictly related to the odor detection and discrimination.

The olfactory system exhibits rather peculiar features of exceptional relevance from a neurobiological point of view. Many precious data can be found in "*Handbook of Olfaction and*

¹ Zhang, X. and S. Firestein. (2002). The olfactory receptor gene superfamily of the mouse. *Nat. Neurosci.*, 5(2): 124-133.

² See references in: Kratskin, I.. (1995). Functional anatomy, central connections, and neurochemistry of the mammalian olfactory bulb. In: R. Doty (eds.). *Handbook of Olfaction and Gustation*. Marcel Dekker, Inc.. New York, pp. 103-126.

³ A brief overview can be found in: Affanni J.M. and Cervino C.O. (2005). Interactions between Wakefulness, Sleep and the Olfactory System. In "*The Nature of Sleep*". Edited by Parmeggiani P.L. and Velluti R. Imperial College Press. London.

Gustation" edited by Doty⁴. Among those features, the following stand out:

A) The olfactory neurons are the only ones to be in direct contact with the ambience. Variation of ambient parameters can deeply affect them.

B) The olfactory receptor neurons are continuously regenerated during the whole adult life (continued *neurogenesis*). This is an extraordinary fact of outmost importance in medicine. It means that olfactory neurons and their precursors are agents presumably withholding the secret of one the greatest hopes in medicine, namely the possibility to induce formation of new neurons. The persistence of continued neurogenesis and the genesis of new neuronal contacts (synaptogenesis) during the whole life span indicate that the olfactory system is a superb experimental model for studying the generation of new neurons. We suggest that intensive study of the implicated molecular mechanisms together with research on the conditions of the internal environment of the olfactory system might be extremely rewarding. Elucidation of those problems might provide new possibilities for medical developments since, unfortunately, neurogenesis in the adult brain is far too limited to repair damage in the central nervous system. Many interesting questions can be posed. As a case in point, consider how interesting it might be to know if there is any relationship between adult neurogenesis and the existing direct exposure to ambient air produced by the ebb and flow of the nasal respiratory rhythm. What happens with neuronal generation during chronic suppression of nasal breathing by submission to experimental tracheal breathing? And so on.

C) Olfactory bulb neurons and hippocampal neurons, closely connected with olfactory receptor neurons, also regenerate during the whole adult life.

D) As a consequence of the regeneration of olfactory receptor neurons, there is also generation of new synapses in the olfactory bulb (neosynaptogenesis).

⁴ Doty R. (eds.). (1995). *Handbook of Olfaction and Gustation*. Marcel Dekker Inc., New York, pp. 455-469.

E) The olfactory receptor neurons have the extraordinary ability to transport several materials from the nasal cavity to different brain regions. This represents another extraordinary characteristic. As a case in point, it has been suggested that Alzheimer Disease can be triggered by pathogen agents entering through the nose and spreading along olfactory neurons into several regions of the brain. In any way, the ability of olfactory neurons to transport varied materials (metals, toxins, viruses and proteins) is a well-established fact⁵. **Consequently, the brain appears to be highly vulnerable through this surprising route.**

F) The stimulation of the olfactory system is one of the most powerful agents capable of evoking memories.

G) The olfactory system, together with gustation, participates in the outstanding phenomenon of *flavor aversion learning*. For instance, if the ingestion of spoiled food is followed by nausea and vomiting, an extraordinary long lasting aversion to that food develops. In fact, after a single exposure to that association, the aversion may persist during a whole lifetime (in some cases for 60 years). This is a very strong form of associative memory that operates through food stimuli (taste and smell). Sometimes it plays a dramatic role in patients undergoing chemotherapy or radiation therapy for cancer. Nausea provoked by such treatments induces aversion to various kinds of foods. This occurs because an association between food stimuli and nausea is established.

H) There is interaction between the olfactory system and exogenous steroids. This was shown through the modification of testicular size, serum testosterone and spermatogenesis⁶.

⁵ Ferreyra-Moyano, H. and E. Barragan. (1994). *Environmental factors in the etiology of Alzheimer's dementia and other neurodegenerative diseases*. In: R. Isaacson and K. Jensen (Eds). *The vulnerable brain and environmental risks, Volume 3: Toxins in air and water*. Plenum Press, New York, pp. 43-63.

⁶ Anand Kumar TC, Sehgal A, David GF, Bajaj JS, Prasad MR. (1980). *Effects of intranasal administration of hormonal steroids on serum testosterone and spermatogenesis in rhesus monkey (Macaca mulatta)*. *Biol Reprod*;22(4): 935-940.

THE NON-SPECIFIC FUNCTIONS OF THE OLFACTORY SYSTEM.

In addition to the specific functions of odor detection and discrimination, unspecific functions are currently ascribed to the olfactory system. Herrick⁷ was the first to indicate that the central connections being "*characteristically diffuse, widely dispersed and interconnected*" were suggestive of unspecific functions.

We provided the first experimental evidence of non-specific functions of the olfactory bulb⁸. Our studies in the opossum showed that the olfactory bulbs are involved in the regulation of Sleep. The complete transverse section of the olfactory peduncles, an operation that separates the olfactory bulbs from the rest of the brain, produces striking changes in the Sleep patterns, particularly in paradoxical sleep⁹. In our original paper, we said that our results suggested that "*the olfactory bulb plays an important role in the regulation of the functional organization of the structures involved in the production and maintenance of sleep. This participation seems to be independent of impulses coming from the olfactory receptors since their destruction was unable to elicit the changes observed after the section of the olfactory peduncles*".

Cain¹⁰ in a very inspiring paper proposed that the olfactory bulb was involved in a forebrain arousal system mainly comprised of limbic system and hypothalamus. Later, Shepherd¹¹ in another lucid paper proposed that the olfactory system is not a system but several systems. According to this author, that plurality provides varied combinations of their

⁷ Herrick, J. (1933). *The functions of the olfactory parts of the cerebral cortex*. Proc. Nat. Ac. Sci., 19: 7-14.

⁸ Vaccarezza, O. and J. Affanni. (1966). *Influencia de los bulbos olfatorios sobre el sueño del marsupial (Didelphis azarae)*. Revta. Soc. argent. Biol. 42: 106-111.

⁹ See also Affanni and Cervino (2005).

¹⁰ Cain, D.P. (1974). *The role of the olfactory bulb in limbic mechanisms*. Psychol. Bull., 81(10): 654-671.

¹¹ Shepherd, G.M., M.C. Nowycky, C.A. Greer and K. Mori. (1981). *Multiple overlapping circuits within olfactory and basal forebrain systems*. In: G. Székey, E. Lábos and S. Damjanovich (eds.). *Neural Communication and Control*. Adv. Physiol. Sci., 30: 263-278.

connections, which collaborate in the control of different types of behavior.

Obviously, the non-specific functions can be studied in different ways. One of them consists in the chronic stimulation of the olfactory receptors followed by observation of non-specific effects. Far more attention was paid to the functional effects of stimulating the olfactory system than to its deprivation. However, when olfactory stimuli are delivered the rapid onset of adaptation phenomena represents a very serious hindrance. Contrasting with stimulation, olfactory deprivation offers the possibility of suppressing olfaction during long periods without that interference.

Therefore, we think that, regarding research on unspecific functions, a detailed study of the functional effects of chronic olfactory deprivation is urgently needed. It might provide new data regarding the nature and properties of those unspecific functions.

Our principal aim was to put our attention on the deprivation effects, not only during wakefulness but also during sleep and after the application of arousing stimuli within it.

The experiments described in this report can be classified within the broad range of OLFACTORY DEPRIVATION. This suppression of the olfactory input can be elicited by different methods, namely:

- 1) By unilateral occlusion of the nostrils.
- 2) By bilateral occlusion of the nostrils in non-obligatory nasal breathers.
- 3) By tracheal breathing after tracheotomy and insertion of a tracheal tube.
- 4) By elimination of the olfactory receptor neurons or destruction of some part of the Central Nervous System olfactory structures
- 5) By submission to odor-free or odor-truncated environments.

The experiments reported here were performed by elimination of the olfactory receptors or by the insertion of a tracheal tube, which abolishes nasal breathing.

Most of the authors who tried to find out the effects of these experimental procedures focused their attention on structural or biochemical changes¹². The structural ones can be clearly seen only after one or more months since the beginning of deprivation. Surprisingly, they can be induced at any age of the experimental animals. This contrasts sharply with what happens in other sensory deprivations, which have a short critical period. After it, they cannot be induced any more.

From the very beginning of our research project, we have considered that the order of appearance of the changes might have special significance. In fact, they might help to interpret the cascade of phenomena responsible for the post deprivation gross anatomical alterations. **This is why we decided to look for more precocious changes through the study of brain electrical activity. We thought that paying attention to that activity would provide the possibility of detecting early changes in brain physiology. We based our research strategy on our previous results, which pointed towards changes in sleep patterns. Consequently, we thought that the deprivation effects had to be studied along the three principal brain states (Wakefulness, Slow wave Sleep and Paradoxical Sleep). This is, obviously, one of the best ways to reveal the contribution of olfactory inputs to brain functioning and has led us to the discovery of interesting phenomena (see below).**

IN SEARCH OF AN ANIMAL MODEL FOR OUR STUDY

Our first decision was the choice of a suitable animal model. It is a well-known fact that very often the difference between success and failure of a given experiment resides in the choice of the animal species on which the experiments are going to be performed.

¹² See an excellent review by Maruniak, J. (1995). *Deprivation and the Olfactory System*. In: R. Doty (eds.) *Handbook of Olfaction and Gustation*. Marcel Dekker Inc., New York, pp. 455-469.

In our case, we selected the armadillo *Chaetophractus villosus* as experimental model. This animal exhibits several advantages for this kind of research, namely:

- 1) It is extremely resistant to varied types of surgical procedures.
- 2) It possesses highly developed olfactory structures (olfactory bulbs, olfactory tubercles, olfactory peduncles, lateral olfactory tract, piriform cortex)¹³.
- 3) The rhinal fissure occupies a high position in the lateral aspect of the brain hemispheres (see Fig. 1). This feature facilitates the making of lesions on the piriform cortex.
- 4) It is a very good sleeper under laboratory conditions.
- 5) We have some good knowledge of its sleep characteristics and of the electrical activity of the olfactory system¹⁴.

The brain of the armadillo is shown in Figure 1.

¹³ Benítez, I.; H. Aldana Marcos and J. Affanni. (1994). *The encephalon of Chaetophractus villosus. A general view of its most salient features*. *Comunicaciones Biológicas*, 12 (1): 57-73.

¹⁴ Affanni J. M., Morita E.K., García Samartino L. (1968). *Efecto de la sección de los pedúnculos olfatorios y de la comisura anterior sobre la actividad eléctrica del bulbo olfatorio del marsupial Didelphys azarae*. *Rev. Soc. Argent. Biol.*, 44: 183-188.

▪ Affanni, J.M., L. García Samartino, A.M. Scaravilli y J.S. Panizza. (1973). *Cambios en la actividad sinusoidal inducida después de la sección de los pedúnculos olfatorios en Chaetophractus villosus (Mammalia, Dasyopidae)*. *Physis*, 32: 101-105.

▪ Affanni, J.M. and L. García Samartino. (1984.) *Comparative study of electrophysiological phenomena in the olfactory bulb of some South American marsupials and edentates*. In: L. Bolis, R.D. Keynes and S.H.P. Maddrell (eds.) *Comparative Physiology of Sensory Systems*. Cambridge University Press, Cambridge, pp. 315-333.

▪ Affanni, J., E. Casanave, L. García Samartino and R. Ferrari. (1986). *Neocortical and olfactory bulb activity, in armadillos submitted to covering with soil*. *Arch. Int. Physiol. Biochim.*, 94: 271-279.

▪ Affanni, J.; L. García Samartino; E. Casanave and R. Dezi. (1987). *Absence of apnea in armadillos covered by soil*. *Resp. Physiol.*, 67: 239-243.

▪ Affanni, J.M., C.O. Cervino and H. Aldana Marcos. (2001) *Absence of penile erections during paradoxical sleep. Peculiar penile events during wakefulness and slow sleep in the armadillo*. *J. Sleep Res.*, 10: 219-228.

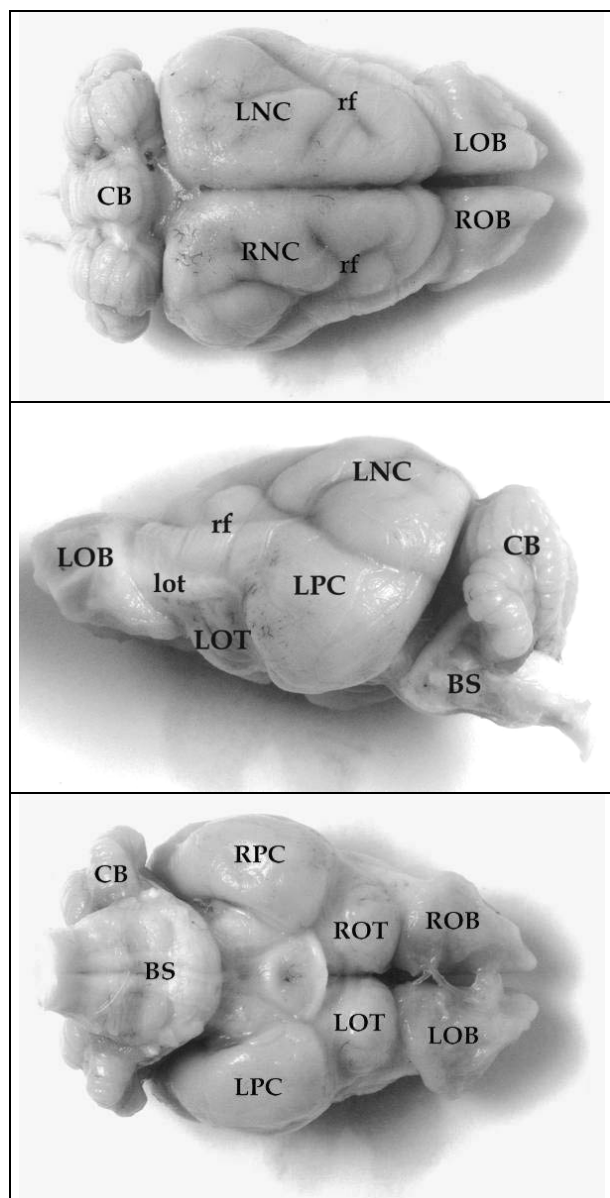


Figure 1. Dorsal, lateral and ventral view of the brain of the armadillo *Chaetophractus villosus*, adopted as experimental model.

BS, brainstem; CB, cerebellum; LNC, left neocortex; LOB, left olfactory bulb; LOT, left olfactory tubercle; LPC, left piriform cortex; lot, lateral olfactory tract; rf, rhinal fissure; RNC, right neocortex; ROB, right olfactory bulb; ROT, right olfactory tubercle; RPC, right piriform cortex.

THE EFFECTS OF PERIPHERAL OLFACTORY DEAFFERENTATION ON THE ELECTRICAL ACTIVITY OF THE BRAIN DURING WAKEFULNESS, SLOW WAVE SLEEP AND PARADOXICAL SLEEP

In the experiments herein summarized, *peripheral olfactory deafferentation* means the suppression of olfactory stimuli by elimination of the olfactory mucosa or by elimination of nasal breathing through tracheal breathing.

To date, our results have been published in "The Nature of Sleep" (Imperial College Press, London)¹⁵. Here follows only a brief summary.

Very soon (three hours approximately) after the elimination of the olfactory receptors or the initiation of tracheal breathing, dramatic changes in the electrical activity of the olfactory bulbs and other brain regions are observed. There are changes during both Wakefulness and Sleep. During Wakefulness, the induced olfactory waves and the rhino-central rhythm disappear¹⁶. These changes, in spite of being conspicuous, are not surprising in view of what is already known about the primal importance of olfactory afferences. **However, what is really surprising is that during Sleep high amplitude EEG-gamma waves [30-90 cycles per second (Hz)] invading almost two thirds of the brain surface can be seen. This very conspicuous activity is not only observed during Slow Wave Sleep but also throughout the whole phase of Paradoxical Sleep. It is also worth noting that when arousing stimuli are delivered the activity immediately disappears. Several figures illustrating these facts will be found in Affanni and Cervino (2005).**

During Sleep, the appearance of a powerful gamma rhythm in olfactory deafferented animals is extremely interesting. **This finding of our research group is, as far as we know, the first to show that without olfactory input the bioelectric patterns of sleep are deeply distorted.** There is another aspect of our studies, which appears interesting: until recently,

¹⁵ See Affanni and Cervino (2005).

¹⁶ See Affanni and Garcia Samartino, 1984.

olfactory input was considered to be strictly necessary for gamma activity in the piriform cortex¹⁷. From now on, it must be admitted that gamma activity can invade several brain regions, including the piriform lobes, without the participation of the olfactory signals. Of course, due allowance must be made to species differences and to the fact that we waited the initiation of sleep and that our recordings were prolonged through it. Gamma activity has been related to cognitive phenomena. The presence of this type of invasive activity during both phases of Sleep when the olfactory input is lacking suggests a particular significance. It is not inconceivable that such an invasion might be correlated with changes in the energy cost and biochemistry of the brain during sleep. We wonder whether the observed changes might also play some role in the generation of the anatomical changes and symptoms of nasal obstructive diseases or degenerative diseases.

Finally yet importantly, some participation of the above-mentioned phenomena in Sleep Apnea could not be excluded.

Several papers have been published indicating that nasal obstructions affect breathing in sleep. Disturbances in the quality of sleep and of daytime vigilance and behavior have been also reported. Lavie published a very instructive historical note¹⁸. In that paper there is a citation from William Hills' paper, which reads: "*The stupid looking lazy child who frequently suffers from headaches at school, breathes through his mouth instead of his nose, snores and is restless at night and wakes up with dry mouth in the morning, is well worthy of the solicitous attention of the school medical officer.*"

It cannot be denied that our experimental setup bears some resemblance with what happens in some pathological conditions. Such is the case of nasal obstructions and degenerative

¹⁷Vanderwolf C.H. (2000). *What is the significance of gamma wave activity in the piriform cortex?* *Brain Res*, 877: 125-133.

¹⁸ Lavie P. (1983). *Nasal obstructions, Sleep and Mental Function.* *Sleep* 6(3): 244-246.

diseases (Alzheimer, Parkinson and others) **in which there is loss of olfactory neurons.**

In view of our findings, perhaps a speculation might be posed. If similar results were confirmed in other animal species including humans, then it should be admitted that in those subjects the bioelectric patterns of sleep could be deeply distorted. **We wonder if the presence of early abnormal bioelectrical activity cannot initiate a cascade of morphological, biochemical and energetic changes.** Those changes might be responsible for some of the neurological deficits observed in those diseases

We do not know if these effects can be extrapolated to microsmatic animals like humans. Regarding this point, we think that these kinds of studies are badly needed in view of the fact that in humans there are pathological conditions -Alzheimer disease, Parkinson disease, etc-¹⁹ in which serious olfactory deficits and lesions of olfactory structures are seen. Is the quality of sleep affected? Of course; this might have far-reaching consequences revealing new features of brain development and brain health. Evidently, the lesson from comparative physiology of olfactory deprivation is that it is healthy for the researchers to display enough patience to wait for the onset of the different phases of sleep.

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¹⁹ See Ferreyra-Moyano and Barragán, 1994.

▪ Bylisma, F.W., P.J. Moberg, R.L. Doty and J. Brandt. (1997). *Odor identification in Huntington's disease patients and asymptomatic gene carriers.* *J. Neuropsychiatry clin. Neurosci.*, 9(4): 598-600.

▪ Meshulam RI, Moberg PJ, Mahr RN, Doty RL. (1998). *Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases.* *Arch Neurol.*;55(1): 84-90.

