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# CONTRIBUTION OF REM SLEEP TO FOS AND FRA EXPRESSION IN THE VESTIBULAR NUCLEI OF RAT LEADING TO VESTIBULAR ADAPTATION DURING THE STS-90 NEUROLAB MISSION

O. POMPEIANO

*Dipartimento di Fisiologia e Biochimica, Università di Pisa, Via San Zeno 31, 56127 Pisa*

## PREFACE

At the end of 1993, I was asked to contribute to the development of a NASA research project to be held in April 1998 during the space lab Mission called Neurolab (STS-90). No attempt was made in the past to investigate the molecular changes which occurred in the whole brain during the different space flight conditions. I proposed, therefore, to study in rats the changes in the expression of immediate early genes (IEGs) which affected different brain structures and could contribute: *a*) to the adaptive changes of impaired functions which occurred when the animals were exposed to microgravity after launch, and *b*) to readaptation to the terrestrial environment which occurred at the reentry after landing.

The project entitled "Gene expression in the rat brain during space flight" (Pompeiano et al., 2003) was examined by the Committee of Cellular and Molecular Biology of the National Institute of Health, Bethesda, MD and classified as the sixth among the 180 applications submitted for analysis to that Institution. It was then included among the 25 research projects which could contribute to the space lab Mission. A four-years period (1995-1998) was required for the preparation of this research project, while the period between 1998 and 2004 was necessary to qualitatively and quantitatively evaluate the results obtained in different brain structures of 72 rats sacrificed at different time points of the space flight.

The development of our study was made possible thanks to the finantial support of the Italian Space Agency (ASI) and the results so far obtained were published in several international Journals (Pompeiano et al., 2001a, b, 2002, 2003, 2004; Pompeiano M. et al., 2002; Balaban et al., 2002; d'Ascanio et al., 2002, 2003).

The present study represents the revisitation of one of our previous studies, in which we first examined the changes in gene expression which occurred in the vestibular nuclei (VN) during the space flight (Pompeiano et al., 2002). The extension of this study was required following the results of a recent investigation in which we examined in detail the changes in gene expression which affected specific brain structures involved in different phases of the sleep-waking cycle, as shown in rats sacrificed at different time points of the space flight (Centini et al., 2006). The identification of these animal states could allow us to differentiate the effects of

labyrinthine (gravity) signals acting on the VN during the space flight from the effects of extralabyrinthine signals, which may impinge on the same structures either during wakefulness, possibly associated with stress, or during appropriate phases of slow wave sleep (SWS) or paradoxical sleep (PS), possibly associated with rapid eye movements (REM) bursts.

## INTRODUCTION

One of the main aims of our study was first to examine the changes in the expression of IEGs which occurred in the VN of rats during different conditions of the space flight. In particular, we examined the expression of the IEGs c-fos and FRA (fos-related antigens) in different vestibular structures during and after the space flight. It is known that c-fos and FRA code for transcription factors (Fos and FRA) regulating the expression of target genes (Ceccarelli et al., 1989; Morgan and Curran, 1991; Herdegen and Leah, 1998). It is known that fos-protein levels peak within 2-4 h after exposure to a given stimulus and return to baseline values eithin 6-8 h. FRA levels, however, peak somewhat later and persist in the brain for days ("acute FRAs", eg, FRA-1, FRA-2, Fos B, Δ Fos B) or weeks ("chronic FRA's", eg. modified forms of Δ Fos B) after the initial stimulus (Sharp et al., 1991; Nestler et al., 1999). It appears, therefore, that Fos lies in the pathway mediating short-lived changes in gene expression that reflect short-term compensation for labyrinthine-induced postural and motor deficits. On the other hand, FRA proteins mediate long-term molecular changes leading to long-lasting adaptation of these modified functions. Thus a preliminary aspect of our study was to investigate whether exposure of the animal to *microgravity*, such as it occurs after launch, would decrease the expression of IEGs such as the c-Fos and the FRAs in the VN, while just the opposite result would occur during exposure of the same animal to *hypergravity* at the re-entry. These molecular changes could then contribute to the plastic changes responsible for the adaptive processes, occurring in the new experimental conditions.

*Responses of VN neurons to natural stimulation of macular labyrinthine receptors.* Experimental anatomical studies have first studied the distribution of labyrinthine afferents originating from macular (utricular and saccular) receptors on different components of the vestibular complex (cf. Brodal et al., 1962; Buttner-Ennever, 2000).

These findings were then followed by the results of electrophysiological experiments performed particularly in decerebrate cats, and showing that neurons located in different parts of the vestibular complex responded to natural stimulation of macular labyrinth receptors. These neurons included either vestibulospinal (VS) neurons originating from the lateral (LVN) and spinal (descending) vestibular nucleus (SpVN) (cf. Boyle and Pompeiano, 1980, 1981a, b; Wilson and Peterson, 1981), which contribute to the vestibulospinal reflex (VSR) (cf. Lund and Pompeiano, 1968; Pompeiano, 1975 for ref.), or neurons originating from the medial vestibular

nucleus (MVN), which contribute particularly to the vestibulo-ocular reflex (VOR) (cf. Ito, 1974 for ref.)

In addition to these labyrinthine signals which may trigger the discharge of VN neurons even in the absence of forebrain structures such as after decerebration, i.e. under conditions of postural activity which resemble that occurring during waking (or SWS), evidence was presented indicating that extralabyrinthine signals may trigger the activity of vestibulo-ocular neurons particularly during episodes of PS (Bizzi et al., 1964a, b), which are characterized by the occurrence of bursts of REM (Pompeiano and Morrison, 1965, 1966c cf. Pompeiano, 1974 for ref.).

*Responses of VN neurons to REM sleep.*

In our previous study, in which we investigated the changes in Fos and FRA expression which occurred in the VN of rats during different space-flight conditions (Pompeiano et al., 2001, 2002), no direct evidence was presented to relate these changes in gene expression to a specific animal state. Further experiments were then required to differentiate the changes in gene expression which affected different brain structures, and could be related to different phases of the sleep-waking cycle, as they occurred at specific time points of the space flight. In a recent study (Centini et al., 2006) we tried to identify whether episodes of waking possibly associated with stress, or episodes of sleep ranging from slow wave sleep (SWS) to paradoxical sleep (PS), occurred during appropriate time periods of the space flight.

While SWS is characterized by a pattern of EEG synchronization which is usually associated with a tonic activity of the extensor musculature, the PS episode is characterized by the occurrence of tonic and phasic behavioural changes (cf. Steriade and Hobson, 1976 for ref.). The tonic manifestations are represented by a pattern of EEG desynchronization, which is usually associated with a suppression of the postural activity lasting throughout the PS episode, as monitored by EMG recordings (cf. Jouvet, 1962, 1967; Pompeiano, 1966, 1967). As to the phasic manifestations, they are characterized by the occurrence of rhythmic pontine waves (cf. Callaway et al., 1987; Escudero and Vidal, 1996), which are associated with geniculo-occipital waves, thus being referred to in the literature as ponto-geniculo-occipital (PGO) waves (cf. Jouvet, 1962; Pompeiano, 1970). As reported in the following section, these pontine waves may actually lead to the occurrence either of isolated ocular movements or of bursts of REM.

Experiments of unit recording performed in unrestrained, unanesthetized cats (Bizzi et al., 1964 a, b) have previously shown that the discharge of VN neurons, which was quite regular during quiet waking and SWS, was greatly modified during PS, due to the occurrence of bursts of high frequency unit discharge, invariably associated with bursts of REM. This effect occurred particularly at the level of the medial (MVN) and spinal vestibular nucleus (SpVN), but not at the level of the superior (SuVN) and the lateral vestibular nucleus (LVN). Moreover, none of the units recorded from the VN during REM sleep showed changes in spontaneous discharge during the eye movements occurring in the awake animal.

Since the second order vestibular neurons particularly originating from the MVN, project to the extraocular motoneurons and control their activity (Lorente de Nó, 1933; Szentagothai, 1950), we postulated that these VN neurons were involved in the development of the bursts of REM tipical of PS. The crucial proof that the discharge of the VN neurons was related to the REM was shown by the fact that in unrestrained, unanesthetized cats a bilateral lesion of the VN (Pompeiano and Morrison, 1965, 1966c) did not prevent the occurrence of PS episodes, which were still characterized by the occurrence of low-voltage fast-activity in the EEG and the postural atonia typical of this phase of sleep. However, the bursts of REM were severely impaired, while sporadic and isolated ocular movements could still be observed in the electrooculogram (EOG). This effect persisted throughout the survival period of the animals, i.e. up to 36 days after the lesion. Histological controls indicated that an almost complete and persistent abolition of the bursts of REM occurred only when the lesion affected the MVN and SpVN of both sides throughout their rostrocaudal extent, but not when it involved the SuVN and the LVN. This abolition of REM during PS episodes was neither due to interruption of fibers of the vestibular nerves, nor to lesion of cerebellar efferent fibers which cross the VN before reaching the brain stem (Walberg et al., 1962 a, b). In fact, a regular occurrence of REM could still be observed after bilateral chronic lesion of the vestibular nerves and/or complete ablation of the cerebellum (cf. also Jouvet, 1962, 1967). These findings led us to conclude that the MVN and the SpVN respond not only to labyrinthine signals during waking, but also to extralabyrinthine signals which contribute to the occurrence of bursts of REM during PS. A detailed description of these findings has been reported in previous review articles (Pompeiano, 1967, 1970, 1974).

*Pontine origin of the rhythmic discharges of the VN neurons during REM sleep.*

After the demonstration that pontine waves are associated with the occurrence of geniculo-occipital waves possibly related either to isolated ocular movements or to bursts of REM, evidence was provided in our laboratory indicating that the extralabyrinthine signals, which drive the discharge of VN neurons, also originate from the pontine structures underlying the PGO waves.

This finding was grounded on the results of electrophysiological experiments, in which we recorded the integrated activity of the lateral geniculate nucleus (LGN), which occurs during PS before and after bilateral electrolytic lesion of the MVN and the SpVN (Morrison and Pompeiano, 1966; Pompeiano and Morrison, 1966 a, b). The integrated tonic activity of the LGN, which is small during SWS, increases during wakefulness and even more during PS (cf. also Benoit, 1964).

Two kinds of geniculate activity were differentiated with this technique during PS. The first was represented by short-lasting rhythmic enhancements which preceded by 10-90 sec the onset of PS and the disappearance of the EMG activity in the dorsal cervical muscles. These waves, referred to as *type I LGN waves*, were usually related to single ocular jerks or isolated twitches of the limb musculature. As to the second type of geniculate activity, it was characterized by phasic increases, 1.5 and

2.0 times larger in amplitude and 2 to 6 times longer in duration than those described above. This type of waves, designated *as type II LGN waves*, was strictly related in time to the occurrence of large bursts of REM. The differentiation of these two types of LGN activity was not made by previous authors (Mouret et al., 1963; Brooks and Bizzi, 1963; Bizzi and Brooks 1963; Jeannerod, 1965; cf. Pompeiano 1974 for ref.), because of the limitation of the technique they used.

The main result of our experiments was that a bilateral lesion of both the MVN and the SpVN abolished in cats not only the bursts of REMs, but also the related type II LGN waves (cf. Pompeiano 1974 for ref.). However, the rhythmic type I geniculate activity, which preceded by several sec the occurrence of the other signs of PS, persisted throughout the episode of PS. Monophasic geniculate activity closely related to pontine waves (Callaway et al., 1987; Escudero and Vidal, 1996), was also observed in the awake animal prior to the PS episode (Gottesmann, 1966a, b). Thus after the VN lesion, a rhythmic type I LGN activity was also observed at irregular intervals during quiet wakefulness and/or SWS.

In summary, *extralabyrinthine* signals originating from pontine structures, may affect the activity of the MVN and SpVN neurons during PS. These signals are responsible not only for the occurrence of bursts of REM, but also for the discharge of lateral geniculate neurons related to these bursts of REM. A bilateral lesion of the VN, while abolishing the bursts of REM and the related type II LGN waves, did not prevent the occurrence of the rhythmic type I geniculate activity and the isolated ocular jerks which occur during PS.

When we first investigated the changes in gene expression which occur in the VN during the space flight (Pompeiano et al., 2001a, 2002a), we did not know precisely whether and at which time point of the space flight did a PS episode occur. This information was obtained in a more recent study in which the analysis of the Fos and FRA expression which occurred in several brain structures during appropriate time points of the Neurolab Mission allowed us to identify the time period in which a PS episode, possibly associated with bursts of REM occurred (Centini et al., 2006).

With the present study we decided to re-examine the changes in Fos and FRA expression which occurred in the VN during the space flight, and relate the results obtained with those occurring during well identified episodes of the sleep-waking cycle (Centini et al., 2006). We could then verify whether the changes in Fos and FRA expression observed in the VN during the space flight were induced either by the gravity changes occurring during wakefulness and/or SWS, or by extralabyrinthine signals of pontine origin possibly related to the occurrence of PS or REM sleep.

## MATERIAL AND METHODS

Seventy-two adult male albino Fisher 344 rats were used in our project. They were divided in three groups of 24 rats: *a*) The flight (FLT) group, *b*) the Asynchronous

Ground Control (AGC) group, and *c*) the vivarium (VIV) group. The AGC and VIV rats represent ground-based controls (no flight, no FLT).

FLT, AGC and VIV animals were sacrificed at the following time points of the Neurolab Mission: *a*) FD2 = flight day 2 (N = 4 rats), i.e. 12-24 h after launch, when the peak G force changed over a period of 9 min from 1 to 3 G before reaching a condition of microgravity, corresponding to about 0 G; *b*) FD14, anticipated by 2 days (N = 9 rats), i.e. 12 days after launch, when adaptation to approximately 0 G had occurred; c) R + 1 = re-entry day 1 (N = 5 rats), i.e. 12-24 h after landing, when the peak G force increased in about 16 min from about 0 to 1.5-1.6 G, followed by 12.5 additional min before reaching 1 G at landing; d) R + 13 (N = 6 rats), actually delayed by 1 day, i.e. 14 days after landing, when readaptation to 1 G had occurred.

We have retained the nomenclature for the time points originally assigned by NASA, because these terms were used in all official mission documents, as well as in all the publications made in our previous research studies (Pompeiano O. et al., 2001a, b, 2002, 2003, 2004; Pompeiano M. et al., 2002; Balaban et al., 2002; d'Ascanio et al., 2002, 2003). Animal care was provided by a Veterinian crew member in flight and by specialized personnel on ground. All animal procedures complied with the National Institute of Health Guide for the Care and Use of Laboratory animals.

Temperature (23 ± 1°C) and lighting conditions (12 h: 12-h, light/dark (LD) cycle, light intensity = 30 lux) were the same for all animals with the exception of the 18 animals (VIV, AGC and FLT) killed 2 weeks after landing (R + 13 subjects, see above), which were exposed to a constant dim light (light intensity <30 lux). The animals were killed during the first half of the dark circadian period (the waking phase). In order to attenuate spontaneous fluctuations in the animal state which could modify the patterns of neuronal responses to different flight conditions, 50% of the FLT, AGC and VIV rats were submitted prior to killing to a 60 min exposure to bright light (about 300 lux) (light pulse, LP treated rats), while the others we not (no LP, NLP rats).

Both flight and ground control rats were implanted with a biotelemetry unit in order to monitor functional parameters such as body temperature, motor activity, feeding and drinking. Of special interest was the finding that the motor activity of FLT subjects virtually ceased for 30 min after landing, i.e. at R + 1, despite the animals being at the peak of their waking or active phase (Dr. C.A. Fuller, personal communication). This episode could be attributed to acceleration stress related to landing (arrest reaction, Brodal 1981, or freezing reaction, Sanford et al., 2001), possibly followed by a rebound episode of REM sleep (cf. Centini et al., 2006). Once activity was finally resumed after landing, it remained at or lower level than on the previous day (Dr C.A. Fuller, personal communication) All the animals were euthanized by decapitation, the brainstem being dissected and submitted to appropriate fixation.

Adjacent 40 μm brainstem sections from all animals were alternatively reacted with Fos and FRA antibodies, according to standard immunocytochemical protocols (Pompeiano et al., 2002a). In particular, a commercial polyclonal antibody was used

against Fos (1: 10.000; Oncogene Research Products, Cambridge, MA, USA), while the antibody used to detect FRA (1: 2000) was a generous gift of Dr M.J. Iadarola (NIPR, NIH, Bethesda, MD, USA). This antibody was directed against the M peptide of c-fos and cross-reacted with Fos, FRA-1, FRA-2 and Fos B. Fos protein reaches peak values within 2-4 h after the original stimulus, and returns to baseline values within 6-8 h, while FRA proteins were also induced soon after stimulation, but persisted somewhat longer than Fos protein, i.e. for days or weeks (Nestler et al., 1999).

All space flight animals were matched with ground control animals, kept at the same temperature and with the same housing and lighting conditions. The reason for choosing the AGC group as the control group was that AGC rats were housed in cages of the same size as those used for the FLT rats (4 x 4.25 x 10"), this size being smaller than that of the cages used for the VIV rats (18.5 x 12.25 x 8.5"). Similar results were obtained in both LP and NLP rats.

## RESULTS

*Effects of different space flight conditions on Fos expression observed in the medial (MVe) and spinal (SpVN) vestibular nuclei.*

As reported in the Introduction, electrophysiological experiments performed in decerebrate cats have previously shown that neurons located in different parts of the vestibular complex respond to natural stimulation of macular labyrinthine receptors (Boyle and Pompeiano, 1980, 1981 a, b; Wilson and Peterson, 1981). We expected, therefore, that gravity (G)–induced changes in the number of Fos-positive cells occurred in the VN complex during appropriate time points of the space flight. In particular, a moderate nearly significant increase in Fos-expression occurred at FD2, i.e. 12-24 h after take–off with respect to control subjects. However, a more prominent and significant increase in Fos-positive cells was seen at R + 1, i.e. 12-24 h after landing. Fig. 1 shows the localization of Fos-positive cells observed in the VN of three rats sacrificed at R + 1 and allows to compare the results obtained in a FLT rat with those obtained in corresponding rats of the vivarium (VIV) and the asynchronous ground control group (AGC). The large number of Fos positive cells observed in the FLT rat exposed to the increase in gravity force at the reentry contrasts with the few labeled cells observed in the control groups during exposure to the terrestrial environment. Fig. 2 illustrates the schematic drawings of coronal sections of the medulla to show the distribution and thus the amount of Fos-positive cells observed in the VN of four representative rats of the FLT group sacrificed at different time points of the space flight.

The moderate increase in Fos expression observed at FD2 was due either to the short-lasting exposure of the animal to linear acceleration, which varied at launch from 1.0 to 3 G and/or to the fact that the effects of this gravity change were partially attenuated by the subsequent microgravity the animals were exposed to for most

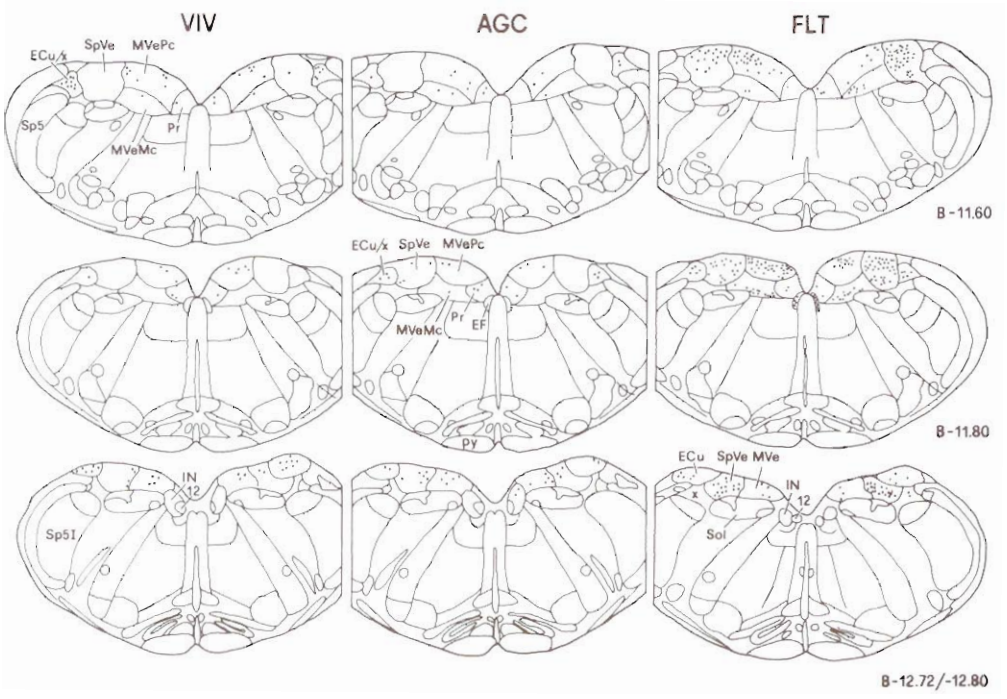


Fig. 1. - *Schematic drawings from camera lucida of frontal sections of the medulla, showing the localization of Fos-positive cells in the vestibular nuclear complex and neighbouring structures of both sides, in three rats of the vivarium control group (VIV), the simulated control group (AGC) and the flight group (FLT) sacrificed at R + 1, i.e. 1 day after return to 1G. In this as well in the following figures the schemes are shown in the rostrocaudal direction, and indicated by numbers which correspond to the stereotaxic planes of bregma (B), as reported in the atlas of Paxinos and Watson (1998).*

In the VIV (R17) as well as in the AGC rat (R17) only a few labeled cells were found bilaterally in the dorsal (MVePc) and to a lesser extent in the ventral part of the medial vestibular nucleus (MVeMc), as well as in the caudal part of the SpVe, the MVe and the Pr. A discrete number of labeled cells were found in the somatosensory structures ECu/X. In the FLT rat (R17) there was a prominent and bilateral increase in number of labeled cells in the rostral part of the medial vestibular nuclei, particularly at dorsal level (MVePc) and in the ventralmost strip of the MVeMc, as well as throughout the caudal extent of the MVe and the SpVe, including the group F. A large number of labeled cells occurred also in EF, but neither in the Pr nor in the nuclei Ecu/X.

of the 12-24 h period after launch. On the other hand, the prominent increase in Fos-positive cells at the reentry (R + 1) could to attributed to the increase in gravity force from about 0 G to 1.5-1.6 G, followed by the sustained exposure of the rat to the terrestrial environment (1 G) at the reentry. In contrast to these findings, the number of Fos positive cells observed in the VN was neither increased with respect to controls at FD 14, i.e. after 12 days of stable exposure to microgravity, nor with respect to controls at R + 13, i.e. 14 days after return to the terrestrial environment. Thus Fos expression increases or decreases in the VN of rats for at least several h of the 24 h

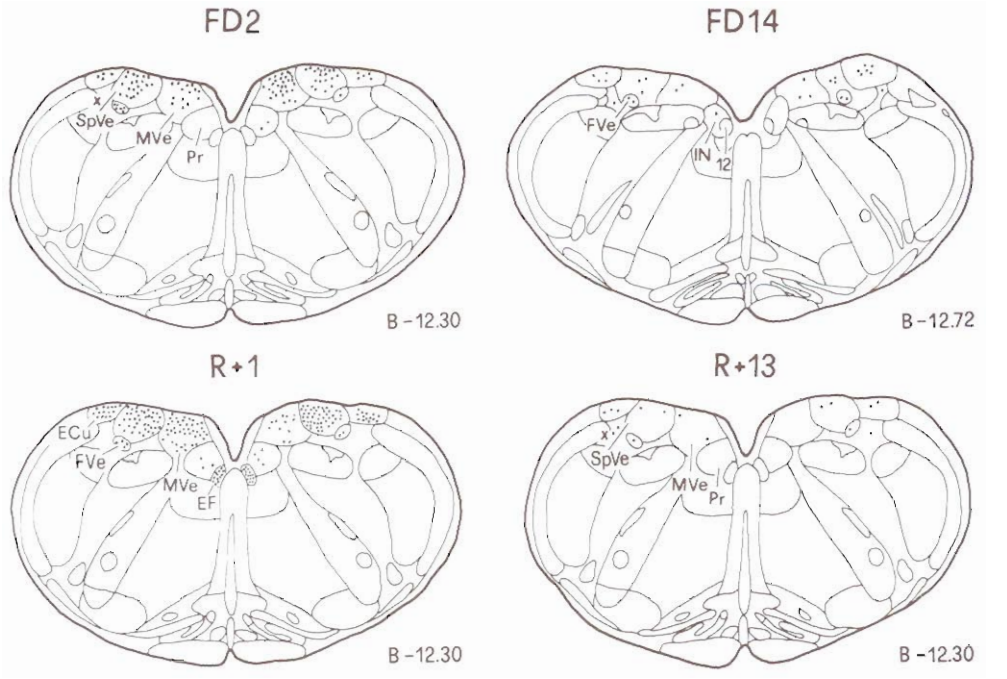


Fig. 2. - *Schematic drawings of coronal sections of the medulla, showing the localization of Fos-positive cells in the VN of both sides in four representative rats of the FLT group (NLP) sacrificed at FD2 (R3), FD14 (R5), R + 1 (R17) and R + 13 (R19), respectively.*

Each animal was identified by a number following the letter R (rat). The numbers at the bottom of each scheme correspond to the stereotaxic plane relative to the bregma (B), as reported in the atlas of Paxinos and Watson (1998). Each dot represents one Fos-immunoreactive cell. At FD2 (R3) a good number of labeled cells occurred in the medial vestibular nucleus (MVe) and spinal vestibular nucleus (SpVe) of both sides at caudal level, while scattered cells where found in the group F (FVe), the prepositus hypoglossi (Pr) and the intercalated nucleus (IN), as well as in the external cuneate/group X nuclei (ECu/X). At FD14 (R5) the number of labeled cells was reduced in all the structures indicated above. At R + 1 (R17) there was a prominent and bilateral increase in number of labeled cells in the caudal part of the MVe and SpVe (including the FVe) and to some extent also in the nuclei ECu/X, but not in the Pr. However, a large number of labeled cells occurred in epifascicular (EF) nuclei. At R +13 (R19) the number of labeled cells was greatly attenuated in all the structures indicated above, reaching the level obtained in the AGC control.
(From Pompeiano O. et al., 2001 a, Fig. 1 and 2002, Fig. 5).

period after transient exposure to increases or decreases of the G-force, respectively. At FD2 and R + 1 the effects of gravity affected the MVe and SpVe in both LP and NLP subjects, while smaller effects were observed in both the parvicellular (MVePc) and the magnocellular (MVeMc) part of this nucleus. Fos-positive cells were also observed in the SuVe at FD2 and more prominently at R + 1. However, as reported in our previous study (Pompeiano et al., 2002), *no Fos expression* was observed in the LVN at any time point of the space flight.

*Effects of different space flight conditions on FRA expression observed in the medial (MVe) and spinal (SpVe) vestibular nuclei.*

Observations made in our experiments have shown that FRA-positive cells occurred in the MVN, the SpVN and the SuVN. In particular, FRA-positive cells were observed in FLT rats sacrificed either at FD2 or at R + 1, while only a few labeled cells were observed at FD14 and R + 13. There were, however, great differences in the pattern of FRA expression observed at the reentry, i.e. in rats sacrificed at R + 1, with respect to the described pattern of Fos expression reported in the previous section. Since FRA expression can be detected for longer periods of time than Fos expression after its induction (i.e. for 1 up to several days, rather than for 6-8 hours, as reported by Nestler et al., 1999), we expected that the labeled VN structures observed 12-24 h after launch or the reentry were due to the G force occurring during these periods of the space flight. If however, during the space flight VN structures showed only Fos but not FRA labeling, the conclusion would be that these selected areas of the VN complex were not specifically activated by labyrinthine signals driven by the gravity force, but rather by extralabyrinthine signals converging on these vestibular structures at a given period of the space flight.

In particular, at the reentry FRA-positive cells were found throughout the whole extent of the SpVe including the group F (FVe), while only few FRA-positive cells were found to be scattered within rostral parts of the MVe (including both the MVe Pc and MVeMc); moreover, no labeled cells were observed in the caudal part of this structure.

Fig. 3 allows to compare the pattern of FRA expression (A) and Fos expression (B) in two representative rats of the FLT group (R15 and R17) sacrificed at R + 1. The FRA positive cells were particularly concentrated throughout the whole extent of the SpVe, while only few scattered FRA positive cells were observed in rostral portions of the MVN, observed at the selected coronal sections of the medulla, the caudal part of this structure being free of labeled neurons. The limited expression of FRA positive cells observed in the rostral parts of the MVN contrasts with the large number and density of Fos-labeled neurons particularly located in the dorsal aspect of the MVN (MVePc), and to a lesser extent also in the ventral aspect of this structure (MVeMc).

As was the case with Fos, *no FRA expression* was observed in the LVN at any time point of the space flight. This negative result affected both the rostroventral and the dorsocaudal part of this structure, and involved all the small- and large-size LVN neurons (cf. Pompeiano, 1991). This finding which occurred also at the reentry (R + 1) suggests that the absence of Fos-positive cells observed in this vestibular structure during the space flight was a genuine phenomenon, that could not be attributed to the time interval which elapsed between landing and killing the animals. Thus neither the labyrinthine-induced effects which can be visualized by the FRA labeling, nor the extralabyrinthine effects which were visualized by comparing the patterns of Fos and FRA labeling could reveal gene expression in the LVN during the space flight.

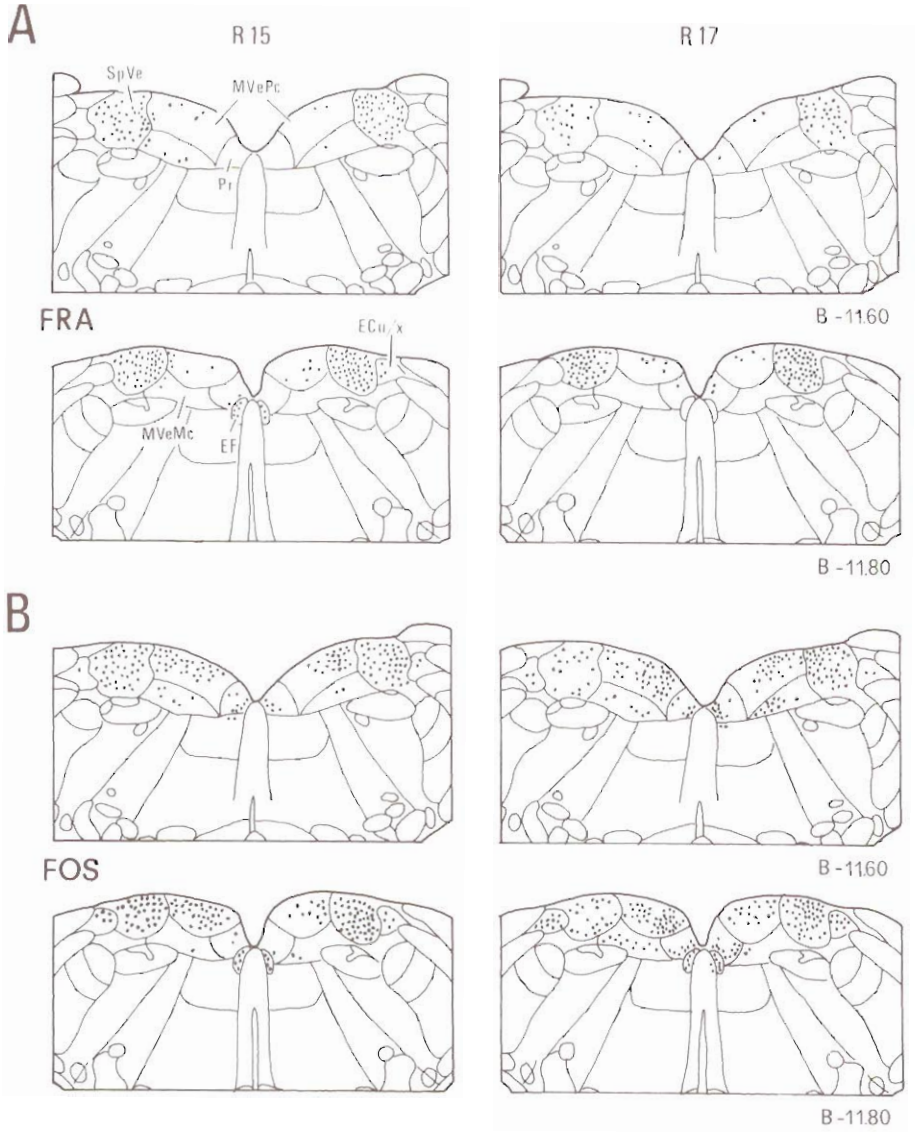


*Fig. 3. - FRA and Fos staining in vestibular structures. The panels show schematic drawings of coronal sections of the medulla indicating the localization of FRA-positive cells (A) and Fos-positive cells (B) in the VN of two FLT rats (NLP), killed at R + 1 (R15 and R17).*
The illustrated sections passed through the caudal part of the vestibular complex In both animals, FRA-positive cells (A) were very abundant in the caudal SpVe. Only few labeled cells were observed in the caudal part of the MVe, where they were observed particularly at dorsal rather than ventral level. Few labeled cells were seen in the Pr and EF. No staining was found in surrounding structures. In the same experiments and at the same levels, large numbers of Fos-positive cells (B) were observed not only in the caudal SpVe but also in the MVePc and to a lesser extent in the MVeMc Labeled cells were found occasionally in the Pr, but were more numerous in EF as well as in external cuneate nucleus (ECu/X). (From Pompeiano O. et al., 2002, Fig 8)

## DISCUSSION

The analysis of the gene expression which involves the VN during the space flight has been used in the present study to identify the nature of the mechanisms which contribute to activation of various components of the vestibular complex, as they occur at different time points of the Neurolab mission. As reported in the Introduction, two are the modalities by which the VN neurons can be activated in ground-based experiments. The first is represented by macular labyrinthine signals, particularly originating from gravity receptors. The second is represented by changes in the activity of VN neurons which may occur in relation to the animal state. Observations made in intact cats had actually shown that the discharge of VN neurons particularly located in the MVN, where vestibulo-ocular neurons are located (Ito, 1984), increased not only during head movements in awake animals, but also in the absence of head movements during the REM typical of PS episodes (Bizzi et al., 1964, a, b). It appeared also that a bilateral electrolytic lesion limited to the MVN (but extended also to the SpVN) reduced or suppressed the bursts of REM typical of PS (Pompeiano and Morrison, 1965, 1966c; cf. Pompeiano, 1967, 1970, 1974 for ref.). Evidence was also presented indicating that extralabyrinthine signals probably originating from pontine reticular structures or pontine waves (Bizzi and Brooks, 1963; Brooks, 1967 a, b), which drive the geniculo-occipital system (Sakai, 1980) thus giving rise to the so-called PGO waves (cf. Jouvet, 1962, 1967), also produced rhythmic discharges of the vestibulo-ocular system.

It appeared, therefore, that pontine waves, while contributing to the occurrence of bursts of REM, also acted on the corresponding sensory system by sending an efferent copy to the lateral geniculate nucleus (LGN), which represents the first relay station of the visual system (Pompeiano and Morrison, 1966 a, b; Morrison and Pompeiano, 1966; cf. Pompeiano 1967, 1970, 1974). Lesion experiments performed in unrestrained cats have also shown that a complete bilateral lesion of the MVN, while reducing or suppressing the bursts of REM during PS, did not prevent the occurrence of the typical pontine waves which persisted throughout the PS episode and were associated with the occurrence of isolated ocular jerks (Morrison and Pompeiano, 1966; Pompeiano and Morrison, 1966 a, b; cf. Pompeiano, 1970, 1974, for ref).

The discussion will be subdivided into five sections dealing with different aspects of the problem under investigation:

A. Gene expression in the SpVN and MVN during the space flight and their dependence on labyrinthine and extralabyrinthine signals.
B. Changes in the sleep-waking activity during the space flight.
C. Neurochemical systems involved in the occurrence of pontine and vestibular influences on the oculomotor system during REM sleep.
D. Pontine and vestibular influences on the LGN related to REM sleep during the ontogenesis.
E. Lack of gene expression in the LVN during the space flight.

A. *Gene expression in the SpVN and MVN during the space flight and their dependence on labyrinthine and extralabyrinthine signals.*

Experiments of molecular biology had previously shown that the IEG c-fos examined in the CNS under ground-based conditions, generally show low basal levels of expression in several brain structures. Its mRNA level increases in the cytoplasm after several min of appropriate cell stimulation, peak after 30-60 min, and then declines to baseline levels. As reported in the Introduction, the corresponding Fos protein, which is synthesized and subsequently transported to the cellular nucleus, peaks within 2-4 h and returns to baseline levels within 6-8 h (Ceccarelli et al., 1989; Morgan and Curran, 1991; Herdegen and Leah, 1998). In contrast to Fos, proteins called FRA (Fos B, FRA-1, FRA-2, Δ Fos B) can also be induced following appropriate neuronal stimulation, but remain detectable in the nucleus for longer periods, ranging from 12 - 24 hrs to several days after the initial stimulus (Sharp et al., 1991; Nestler et al., 1999).

Because of the brevity of normal PS episodes, which last in the rat for about 2 min, and the short-lasting persistence of the Fos protein in the labeled neurons after its induction, we decided to use as markers of neuronal activation in the VN of rats sacrificed at different time points of the space flight not only the Fos protein, but also the FRA proteins, which once induced even after short episodes of PS, are likely to persist in the brain tissue for longer period of time. We have previously shown that during the space flight an IEG expression occurred in the VN when the gravity force increased during launch, but particularly after landing (cf. Pompeiano et al., 2001a, 2002). In the present study, a clear-cut difference in the patterns of Fos and FRA labeling was observed at the reentry, i.e. 12/24 hrs after landing.

While *FRA* expressing cells which occurred soon after landing, i.e. during the early part of the period related to the reentry, were particularly located throughout the whole extent of the SpVN (Fig 3A), *Fos* expressing cells, which presumably occurred during the last part of the 12-24 hrs preceding killing were located not only in the SpVN, but also in the MVN, as well as in the MVePc and to a lesser extent also in the MVeMc (Fig 3B). We postulate that the increase in gene expression observed in the SpVN can be attributed to *labyrinthine* signals driven either during the dynamic changes in the gravity force occurring at the reentry (Fos) or during the static changes due to persistence of the animal at the terrestrial G (FRA). On the other hand, the Fos expression observed in the MVN and its subdivisions can be attributed to *extralabyrinthine* signals, possibly related to the behavioural state occurring at the reentry during the few hrs immediately prior to sacrifice.

In *conclusion*, it appears that the increase in Fos expression which occurs in the MVN and its rostral subdivisions within 6-8 hrs after landing is temporarily separated from the increase in Fos expression which occurs in the SpVN and outlasts the 12/24 hrs period after landing. These findings suggests that exogen labyrinthine signals originating from macular receptors are potentiated by signals of endogenous source. As reported in the following section this source is likely to originate from the pontine structures, which are responsible for the occurrence of the so-called pontogeniculo-occipital (PGO) waves typical of REM sleep.

B. *Changes in the sleep-waking activity during the space flight.*

Electrophysiological correlates of sleep and waking have been studied not only in ground-based experiments (Steriade and Hobson, 1976; Hobson and Steriade, 1986), but also under different space-flight conditions (cf. Hobson et al., 1998, for ref.). In particular, attempts were made to investigate whether exposure of astronauts to different space-flight conditions could influence the amount of SWS and PS or REM sleep. Evidence presented in previous studies indicate that astronauts undergo a consistent increase of REM sleep during the first sleep epoch in space (Quadens and Green, 1984). Further experiments, performed during Skylab missions, have shown that a marked increase in REM sleep and a decrease in REM latency consistently occurred after return to 1 G (Frost et al., 1976, 1977). Similar results were also obtained during the Neurolab Mission, where crew members showed marked increase in REM sleep after return to earth, while post-flight latency of this phase of sleep significantly decreased (Dijk et al., 2001, 2003). Evidence was also presented indicating that this post-flight REM increase was not due to changes of the circadian rhythm, but rather reflects a homeostatic response of the loss of REM sleep, which occurred during the space flight. A more speculative explanation is that this massive increase in REM sleep represents a response which may contribute to readaptation of brain functions after return to 1 G.

More recently, changes in the expression of IEGs were studied in rats and cats to identify the cellular and molecular changes which occurred in ground-based experiments during different phases of the sleep-waking cycle (Pompeiano M. et al., 1994; cf. Cirelli and Tononi, 1998, 2000a, b; Cirelli et al., 1993). It was previously shown that in intact animals the discharge of noradrenergic LC neurons, which increases in spontaneously awake rats (cf. Aston-Jones and Bloom, 1981; Barnes and Pompeiano, 1991; Foote et al., 1983, Hobson et al., 1975; Hobson and Steriade, 1986), was associated with an increase in Fos expression which occurred not only in this structure (and the lateral parabrachial nucleus) (Pompeiano M. et al., 1994; Cirelli et al., 1995), but also in different areas of the neocortex and other forebrain structures, while a decrease in Fos expression occurred in the same structures during SWS.

Observations reported in a recent study (Centini et al., 2006) have shown that flight rats sacrificed at FD2 and to some extent also at FD 14 showed a large number of Fos-positive cells in several areas of the forebrain including many cortical and subcortical regions, which are indicative of a state of spontaneous or forced (for a few hours) *waking*. However, rats sacrificed at the reentry (R + 1) showed very low levels of Fos expression in most of the forebrain regions indicated above, which are indicative of a state of *sleep*, particularly of SWS. In this instance, however, a large number of labeled cells was observed in the central nucleus of the amygdala (CeA) of FLT rats with respect to the control. This finding should be integrated with the results of FRA expression indicating that at this time point of the space flight there was a condition of *stress* followed by a rebound of REM sleep (see below). Unfortunately, due to the brevity of the PS episodes and the short-lasting persistence

of the Fos protein in the labeled neurons, it was not easy to identify in ground-based experiments the effects of PS episodes on gene expression in rats. However, attempts were made in the literature to investigate whether episodes of PS could be induced by appropriate activation of well identified neurochemical systems.

C. *Neurochemical systems involved in the occurrence of pontine and vestibular influences on the oculomotor system during REM sleep.*

Experiments performed in decerebrate cats have previously shown that i.v. injection of an anticholinestherase produced catapletic episodes characterized by a complete suppression of postural activity (Hoshino and Pompeiano, 1976; Pompeiano and Hoshino, 1976a,b Pompeiano, 1980), similar to that occurring during PS. Evidence was also presented indicating that these cataplectic episodes depend on the steady discharge of pontine reticular neurons, which are cholinergic and/or cholinoceptive and act on the bulbospinal inhibitory system, thus suppressing posture (see Tononi and Pompeiano, 1995 for ref.). An interesting result of these experiments was that VN neurons, histologically identified as being located in the MVN, showed rhythmic discharges which preceded the occurrence of the cholinergically-induced bursts of REM (Thoden et al., 1972; Mergner et al., 1978). This finding closely resembled that observed during the bursts of REM which occur in intact animals during PS (Bizzi et al., 1964 a, b). Evidence was also presented indicating that the MVN neurons activated during the cholinergically induced bursts of REM were mono-(and/polysynaptically) activated by stimulation of the ipsilateral labyrinth and antidromically activated by stimulation of the ascending medial longitudinal fasciculus projecting to the contralateral abducens motoneurons (cf. Pompeiano, 1980; Mergner and Pompeiano, 1978). It appears, therefore, that these neurons belonged to the vestibulo-ocular system. Experimental anatomical studies have also shown that dorsal pontine reticular structures, located in the paramedian pontine reticular formation, project not only to the ipsilateral abducens nucleus (Pompeiano, 1980), but also to the ipsilateral MVN (Pompeiano, Mergner and Corvaja, 1978). Moreover, the dorsal part of the paramedian medullary tegmentum sends afferents to the contralateral abducens nucleus as well as to the contralateral MVN (Pompeiano et al., 1978).

It is likely, therefore, that a close link exists between the pontine structures responsible for the pontine waves and these paramedian pontine and medullary reticular structures, whose activity contributes to the rhythmic discharge of VN neurons responsible for the horizontal eye movements during the bursts of REM.

After these studies had been performed, observations made in cats have shown that microinjection of the cholinergic agonist carbachol into dorsal pontine structures, such as the peri-LC$\alpha$, the laterodorsal tegmental (LDT) and the pedunculopontine nuclei (PPN) (Jones, 1991; Shiromani and McGinty, 1986; Vanni-Mercier et al., 1989; Sakai, 1988; Sakai et al., 1981, 2001), where cholinergic and non-cholinergic neurons are located (cf. Boissard et al., 2002), produced an increase in PS episodes characterized by low-voltage, fast-cortical activity, hippocampal theta activity, postural atonia, PGO waves and bursts of REM (Sakai et al., 1981, 2001). Similar results

were also obtained in rats (Boissard et al., 2002, 2003), where pressure injection of carbachol in the sublaterodorsal (SLD) nucleus, the rat homologous of the cat peri-LCα and the PPN (Swanson, 1992), induced a PS-like state (Gnadt and Pegram, 1986; Kubin, 2002; cf. Boissard et al., 2002, 2003).

It is of interest that a rebound of PS (or REM sleep) can be elicited not only by a prolonged period of sleep deprivation (SD) (Rechtshaffen et al., 1999), but also in the absence of sleep loss by a waking period following *stressors*. One of the conditions which may lead to the occurrence of a PS episode at the reentry after the space flight is represented by the acceleration stress (cf. Centini et al., 2006 for ref.), which follows the period of some sleep loss after exposure to microgravity. Behavioral observations performed in our experiments have shown that an inactivation period characterized by immobilization occurred at the reentry during the first half an hour after landing (see Material and Methods). Of some interest is also the demonstration that short-lasting periods (0.5-2 hrs) of *immobilization stress* lead to the occurrence of PGO waves typical of REM episodes (Rampin et al., 1991; Marinesco et al., 1999; Duarte Palma et al., 2000). Observations reported in our previous study have clearly shown that an increase in FRA expression occurred in the locus coeruleus (LC) and the lateral parabrachial nucleus (Pompeiano M. et al., 2002) during the *acceleration stress* at the reentry, and that this finding was followed by an increase in Fos and FRA expression which affected structures involved in the tonic ascending and descending manifestations of PS (Centini et al., 2006).

In addition to the tonic manifestations, there are also phasic events which occur during the PS episodes, characterized by PGO waves and the related bursts of REM (Callaway et al., 1987). Neurophysiological studies had previously shown that the central nucleus of the amygdala (CeA) contributes to the occurrence of PGO waves, which appear either during waking or during episodes of PS or REM sleep (Sanford et al., 1995, 2001; Calvo et al., 1996; Morrison et al., 1999). Evidence was presented in our flight experiments (Centini et al., 2006) indicating the existence of a functional link between CeA and PGO waves (Calvo, 1984, 1987, 1996; Deboer et al., 1998, 1999). In particular, it appears that in rats the dorsolateral part of the pontine tegmentum, i.e. the lateral parabrachial nucleus (Silvestri et al., 1998) and the nucleus of the tractus solitarius (NTS), which receives projections from the vestibular nuclei (Balaban, 1998; Balaban and Beryozkin, 1994; cf. Yates and Miller, 1998 for ref.), exert a facilitatory influence on the amygdala which is involved in the generation of PGO waves and REM bursts (Datta et al., 1998). Observations made in our flight experiments have also shown that all these brainstem and forebrain structures underwent an increase in Fos and FRA expression at the reentry (cf. Centini et al., 2006).

The demonstration that Fos and FRA expression occurs in appropriate regions of the vestibular complex, particularly in the MVN and SpVN at the reentry, indicates the occurrence of specific forms of vestibular plasticity. It is known that Fos and FRA proteins form heterodimers that regulate the transcription of a number of target genes (Morgan and Curran, 1991; Herdegen and Leah, 1998). Because the target

genes control cellular functions, Fos and FRA induction observed particularly at the reentry represent steps in a process through which relatively short-lived signals at the cellular membrane can be transduced into longer-acting biochemical and structural changes, as indicated by the short-lasting changes in Fos protein expression, followed by the long-lasting modifications of FRA protein expression.

The time course of these inductions may parallel the occurrence of transcriptional changes which determine the plastic phenomena crucially involved in the adaptation to 0G following exposure to microgravity, as well as in the readaptation to normal G after return to the terrestrial environment (Hobson et al., 1998). In interpreting the results obtained in the VN at the reentry, we have seen that the increase in both Fos and FRA expression which affected at the reentry the SpVN can be attributed to *labyrinthine* signals originating from macular receptors. On the other hand, the increase in Fos (but not in FRA) expression which affected particularly the rostral part of the MVN could be attributed to *extralabyrinthine* signals, which drive their neurons during the bursts of REM. Thus, the increase in gene expression which occurs during REM sleep affects parts of the vestibular complex to some extent different from those influenced by the gravity force at the reentry. Due to the different time of persistence of Fos and FRA proteins in the brain tissue for hours and days respectively (Nestler et al., 1999), it appears that both short-lasting and long-lasting changes in plasticity affect different components of the VN complex during the space flight. In conclusion, it appears that after landing the effects of labyrinthine macular signals are augmented by extralabyrinthine signals possibly emanating from pontine (PGO) waves, which trigger the discharge of MVN neurons. This activity may thus contribute to development of the plastic changes which lead to readaptation of this vestibular nucleus to normal gravity. These findings contrast with the limited amount of gene expression observed in the MVN and the SpVN following either exposure to microgravity or in the absence of REM sleep.

It is worth to mention that the MVN neurons project not only to the extraocular motoneurons, but also to Purkinje cells of the cerebellar flocculus, where they terminate as mossy fibers (MF) (cf. Ito, 1984). In our space flight experiments the increase in Fos and FRA expression which affected the MVN at the reentry was also temporally associated with an increase in Fos and FRA expression which affected the dorsomedial cell column of the inferior olive, a structure which send climbing fibers (CF) to the flocculus (d'Ascanio et al., 2003). It is well known that both these MF and CF intervene in the plastic changes which are at the basis of the VOR adaptation (cf. Ito, 1984 for ref). We postulate, therefore, that the response of the MVN to REM sleep which occurred at the reentry contributes not only to readaptation to 1 G, but is also likely to be implicated in the acquisition of the most appropriate learning process which leads to VOR adaptation, during sustained visuo-vestibular stimulation.

D. *Pontine and vestibular influences on the LGN related to REM sleep during the ontogenesis.*

The pontine waves which occur during the PGO activity typical of REM sleep may serve as an endogenous source of activation of neuronal systems involved in mechanisms of sensorimotor integration (Pompeiano, 1970). Evidence has been presented indicating that the corresponding discharge contributes to structural maturation and differentiation of sensory and motor areas during the ontogenesis (Roffwarg et al., 1966; Pompeiano, 1970; Jouvet, 1978;). Classical experiments had previously shown that kittens submitted during the critical period to monocular visual deprivation (MD) undergo not only a profound functional reorganization of visual cortical areas (Hubel and Wiesel, 1970; Wiesel and Hubel, 1963 b; cf. Sherman and Spear, 1982 for ref.), but also show a notable cell hypotrophy in the visually deprived layers of the LGN (Wiesel and Hubel, 1963 a). Since in addition to the retinal input, the LGN also receives extraretinal signals related to the occurrence of PGO waves (cf. McCarley et al., 1978; Nelson et al., 1983; Sakai and Jouvet, 1980; Steriade and McCarley, 1990), we postulated that the rhythmic discharges of pontine and vestibular structures acting on the LGN and the visual system during REM sleep could exert a protective influence against the severe deficits in the visual function following MD (cf. Pompeiano, 1970). We have clearly seen that in kittens *total* sleep deprivation (SD) greatly enhanced the structural abnormalities produced by MD in the visual-deprived geniculate layers (Pompeiano and Corvaja, 1983, 1986, Pompeiano et al., 1995). These findings were extended by Roffwarg and his group (Shaffery et al., 1993; Marks et al., 1995), who found that the susceptibility of LGN neurons to MD (Wiesel and Hubel, 1963) could also be elicited by *selective* deprivation of REM sleep.

The demonstration that PGO waves can be traced from the region of the brachium conjunctivum to the LGN (cf. Steriade and McCarley, 1990 for ref.) and that peribrachial neurons discharge phasically 10-25 msec prior to the LGN PGO waves (McCarley et al., 1978; Nelson et al., 1983; Sakai and Jouvet, 1980), cannot allow to attribute the source of this ascending pathway solely to cholinergic PPT and LDT neurons projecting to the LGN (Steriade and McCarley, 1990). Excitatory pontine neurons may actually act on the LGN not only directly, but also through the MVN (Pompeiano and Morrison, 1966a, b; Morrison and Pompeiano 1966; cf. Pompeiano, 1970, 1974 for ref.), whose afferents to the LGN are likely to be glutamatergic in nature. Coincident activation within the LGN of cholinergic and glutamatergic afferents would lead to strengthening of coactivated synapses, thus counteracting the response of the LGN to the asymmetric input produced by MD.

In conclusion, it appears that the rhythmic discharge of pontine structures which impinge on the LGN during REM sleep represent the *endogenous* source of activation leading to periodic read out of the synaptic connections between primary optic fibers and LGN neurons. *Extraretinal* pontine and vestibular signals may thus collaborate with the *retinal* signals to facilitate neural maturation of the LGN.

The demonstration that the noradrenergic locus coeruleus (LC)-neurons, which cease firing during REM sleep (cf. Hobson et al., 1975; Steriade and McCarley,

1990), but actually increase their activity during waking and stress (Abercrombie and Jacobs, 1987), such as that occurring during SD (Rechtstaffen et al., 1999), represent an additional factor which may contribute to plasticity in the visual system during the sleep-waking cycle (Kasamatsu et al., 1979; Bear and Singer, 1986; Kasamatsu, 1991; cf. Pompeiano et al., 1995). We postulate that specific noradrenergic and cholinergic systems acting during REM sleep at the reentry may also intervene in the postnatal development of the visual (LGN) system during the space flight.

E. *Lack of gene expression in the LVN during the space flight.*

In contrast to the increase in Fos and FRA expression which affected at the reentry particularly the SpVN, due to labyrinthine signals, and also the MVN due to extralabyrinthine signals of pontine origin following REM sleep, no increase in gene expression occurred in the LVN at the reentry (cf. also Pompeiano et al., 2002). This finding is at first surprising since LVN neurons (as well as other parts of the vestibular complex), receive vestibular afferents from otolith receptors, as shown both in neuroanatomical (Brodal et al., 1962; Buttner-Ennever, 2000) and neurophysiological studies (Boyle and Pompeiano, 1980, 1981a, b; Wilson and Peterson, 1981). On the other hand, no changes in their rhythmic discharge affected the LVN neurons during the bursts of REM which occurred either in intact cats during PS (Bizzi et al., 1964a, b), or in decerebrate animals during the cholinergically induced cataplectic episodes (Mergner and Pompeiano, 1978; cf. Pompeiano, 1980).

The demonstration that gravity-inertial signals may modify the discharge of LVN neurons from which descending vestibulospinal (VS) neurons originate without affecting Fos and FRA expression at the reentry following the space flight is surprising, given the prominent changes in postural activity which occur in astronauts during exposure to microgravity and after return to the terrestrial environment. These changes were attributed to changes in the facilitatory influence that the LVN neurons exert on both the $\alpha$- and $\gamma$-extensor motoneurons (Lackner and DiZio, 2000), by utilizing the lateral VS system (cf. Pompeiano, 1972, 1975).

Since Fos (and FRA expression) can be induced by activation of excitatory (glutamatergic) but not of inhibitory (GABAergic) pathways (Gillespie et al 1999), we postulate that the lack of Fos (and FRA) expression revealed in the LVN neurons of rats at the reentry is due to reflex activation of Purkinje cells of the paramedian zone B of the cerebellar anterior vermis; it is known that these cells project directly to the LVN neurons (cf. Corvaja and Pompeiano, 1979; Voogd et al., 1991) on which they exert an inhibitory influence (cf. Pompeiano, 1972; Ito, 1984). This hypothesis is supported by the fact that during the Neurolab Mission an increase in Fos and FRA expression affected at the reentry precerebellar structures, such as the lateral reticular nucleus and the inferior olive (d'Ascanio et al., 2003), which send MF and CF respectively to the cerebellar cortex, including the spinal areas of the cerebellar anterior vermis. By the combined influence that these afferents exert on the cerebellar cortex, one may expect an activation of the P-cells particularly located in these cortico-cerebellar areas, prevents the occurrence of Fos and FRA expression in the LVN at the reentry.

An alternative possibility which may account for the postural changes which occur during the space flight is represented by the fact that the LC neurons, from which the descending ceruleospinal system originates, receive afferent projections from the VN either directly (Cederbaum et al., 1978; Fung et al., 1987a) or through medullary reticular structures (Pompeiano et al., 1990; van Bockstaele et al., 1993). Electrophysiological experiments performed in decerebrate cats have in fact shown that the noradrenergic coeruleospinal neurons responded to static and dynamic changes in head position, leading to selective stimulation of macular gravity receptors (Pompeiano and Hoshino, 1976b; Pompeiano et al., 1990). Evidence was also presented indicating that the descending coeruleospinal system exerts a facilitatory influence on posture; this influence was due not only to a direct excitatory influence on α-extensor (and flexor) motoneurons (Barnes et al., 1989, White et al., 1980, 1983, 1991), but also to an inhibitory influence that this system exerts on Renshaw cells (Fung et al., 1987 b, 1988), a finding which would release both tonic α- and static γ-extensor motoneurons from recurrent inhibition (cf. Pompeiano, 1984). The demonstration that in our Neurolab experiments the gravity force acts on the LC, thus being able to produce postural changes during the space flight, is documented by the fact that the expression of IEGs (Fos and FRA) which is extremely low in the LC following exposure to microgravity, greatly increased during launch and more prominently at the reentry (Pompeiano M. et al., 2002).

It is of interest that in ground-based experiments, the resting discharge of the LC neurons, which contributes to the postural activity during waking, decreased or disappeared during the episodes of postural atonia typical of PS (Aston-Jones and Bloom, 1981; Foote et al., 1983; Hobson and Steriade, 1986; Nitz and Siegel, 1997 for ref.). Similar result occurred also in decerebrate cats during the cholinergically induced cataplectic episodes (Pompeiano and Hoshino, 1976a, b cf. Pompeiano, 1980). In these cases, the reduced discharge of the LC neurons and the related loss of muscle tone was also associated with a suppression of their responses to natural stimulation of macular labyrinth receptors, a finding which could be attributed to GABAergic inhibition of the LC neurons (Nitz and Siegel, 1997). In conclusion, it appears that the inhibitory process which affects the LC neurons during the cataplectic episode is responsible for the suppression not only of the resting discharge of the LC neurons, but also of their response to labyrinth stimulation.

We have seen that during the Neurolab Mission the PS episode observed at the reentry occurred as a rebound event following an *acceleration stress* (cf. Centini et al., 2006). In this case, a prominent increase in FRA expression affected several forebrain structures which produce corticotropin releasing factor (CRF) and send their afferents to the noradrenergic LC neurons. CRF in a neuropeptide that promotes the synthesis and release of adrenocorticotropin hormone (ACTH) in the rat brain and pituitary during stress. The same neuropeptide in also implicated as a neurotransmitter that increases the discharge rate of the LC neurons during stress (Curtis et al., 1994; 1997; Valentino et al., 2001), being able to increase the FRA expression in LC at the reentry (Pompeiano M. et al., 2002). Thus the decrease in neuronal discharge

which occurs in the LC during PS after landing is likely to be counteracted by excitatory influences, due either to macular labyrinthine signals, or to activation following acceleration stress of neuronal systems, which promote the synthesis and release of CRF, the neuropeptide that activates the noradrenergic LC neurons during stress.

## SUMMARY

1. Electrophysiological studies performed in ground-based experiments have shown that VN neurons respond to *labyrinthine* signals following stimulation of macular gravity receptors. Additional evidence indicates that VN neurons may also respond to *extralabyrinthine* signals of pontine origin, which occur during the PGO waves typical of REM sleep (Bizzi et al., 1964a, b; cf. also Pompeiano, 1967, 1970, 1974 for ref.).
2. In a previous study (Pompeiano et al., 2002) changes in Fos and FRA expression were used to identify the short-term (Fos) and the long-term (FRA) molecular changes which affect the VN neurons at different time points of the space flight. In particular, while Fos protein persists in the brain tissue only for a few hours (6-8 hrs) after its induction, FRA proteins, which can also be induced in the same experimental conditions, persist in the brain tissue for longer periods of time (i.e. from 12/24 hrs to days).
3. In order to relate the changes in gene expression which occurred in the VN during the space flight either to gravity changes or to REM sleep, we investigated in a recent study (Centini et al, 2006) the changes in Fos and FRA expression which occurred in different phases of the sleep-waking cycle, thus being indicative of the animal state. We could then compare the results obtained during the space lab Mission with those previously observed either in ground-based experiments during the physiological state of waking and slow-wave (SWS) or during neurochemically induced episodes of PS, as obtained after microinjection of appropriate agents in dorsal pontine structures of rats.
4. Our findings indicated that a waking state possibly associated with episodes of SWS, occurred at FD2 and FD14, i.e. at launch and after exposure of the animal to microgravity. It appeared also that at the reentry (R + 1) rather than at launch (FD2), an increase in Fos and FRA expression affected the noradrenergic LC neurons, as well as several related structures. These findings probably resulted from the acceleration stress, or *immobilization stress* as shown by the appearance of a starle reaction (or arrest reaction) which occurred after landing.
   This condition of stress was followed after landing by an increase in Fos and FRA expression which affected ventromedial medullary reticular structures, whose descending projections are involved in the suppression of postural activity during PS. Moreover, their ascending projections were likely to increase the FRA expression in the neocortex as well as in several regions of the limbic system, such as the dentate gyrus and the hippocampus, which lead to EEG desynchronization

and the theta activity during PS. FRA expression affected also at the reentry pontine and diencephalic structures, such as the lateral parabrachial nucleus and the central nucleus of the amygdala, which are known to contribute to the occurrence of pontine waves and the related bursts of REM.

5. Observations made on the various components of the vestibular complex indicated that no Fos and FRA expression occurred in the LVN at the four different mission time points. However, an increase in Fos and FRA expression occurred particularly in the medial (MVN) and spinal vestibular nuclei (SpVN) at FD2 and at R + 1, i.e. 1 day after launch and 12-24 hours after landing, respectively. The pattern of FRA expression observed in the VN during the space flight was generally similar to that of Fos, except at the reentry, when *FRA positive cells* were observed throughout the whole SpVN, but not the MVN, which showed only a few labeled cells in its rostral part. In contrast to this finding, a prominent *Fos expression* was found not only in the SpVN, but also throughout the entire MVN. In this case the Fos labeling affected not only the caudal but also the rostral part of this structure, including the dorsal (MVePc) rather than the ventral aspect (MVeMc). Grounded on their different time of persistence, both Fos and FRA expression which occurred in the SpVe could be attributed to the increase in gravity force experienced during take-off and landing, while the Fos pattern which affected particularly the MVN soon after the reentry could additionally be attributed to the rebound episode of PS following the forced period of waking which occurred after landing and after the prolonged (12 days) exposure to microgravity.
6. The results of the present experiments provide the first molecular evidence that pontine activity sources producing rhythmic discharges of vestibulo-ocular neurons during REM sleep may substitute for labyrinthine signals after prolonged (12 days) exposure to microgravity, thus contributing to activity-related plastic changes in the VN leading to readaptation of the vestibular system to 1 G.

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Abbreviations

ACTH adrenocorticotropin hormone
AGC asynchronous ground control
CeA central nucleus of the amygdala
CF climbing fibers
CNS central nervous system
CRF corticotrophin releasing factor
EEG electroencephalogram
EMG electromyogram
EOG electrooculogram
FLT flight

FRA Fos related antigens
FVe group F of the VN
G gravity
IEGs immediate early genes
LC locus coeruleus
LD light/dark cycle
LDT laterodorsal tegmental nucleus
LGN lateral geniculate nucleus
LP light-pulse rats
LVN lateral vestibular nucleus
MD monocular visual deprivation
MF mossy fibers
mRNA RNA messenger
MVe or MVN medial vestibular nucleus
NLP no light-pulse rats
NTS nucleus of the tractus solitarius
PGO ponto-geniculo-occipital
PPN pedunculopontine nucleus
PS paradoxical sleep
REM rapid eye movements
SD sleep deprivation
SLD sublaterodorsal nucleus
SpVe or SpVN spinal vestibular nucleus
SuVN superior vestibular nucleus
SWS slow wave sleep
VIV vivarium
VN vestibular nuclei
VOR vestibulo-ocular reflex
VS vestibulospinal
VSR vestibulospinal reflex

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