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Sleep as an instinct: the last experimental research of Giuseppe Moruzzi

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ABSTRACT

This short paper summarises the last experimental research period (1970-1975) of Professor Giuseppe Moruzzi on the topic of the sleep as an instinctive behaviour. The main goal was "the study of the levels of reticular activation which are required for the onset of different type of instinctive behaviours". By the earliest research line the sleep-waking cycle in acute and chronic thalamic pigeons and the effects of the reticular formation stimulation were studied. The electrical stimulation of the pontine reticular formation produced a range of effects according to the animal's level of wake or sleep: the spontaneous pecking towards the grain, for example, was interrupted and the pigeon shifted toward other behavioural activities, but started again to peck at the end of stimulation.
Moreover, repeated trains of stimulations at higher voltage were able to induce sleep. These results suggested the existence of ascending fibres from the caudal brainstem able to block the instinctive activities which were distinct from the fibres responsible of the awakening phenomena. In the very last experiments the level of general arousal (general drive) and its relationship with the motivated behaviour (specific drive) were studied by Professor Moruzzi. The experiments were aimed to establish a setting of strong motivation for selected "specific drive" such as bow-cooing behaviour – induced by testosterone administration – and pecking behaviour – induced by apomorphine administration –, in order to test the effect of "general drive" by modulating the intensity of the background illumination, being this latter considered a natural activator of general arousal. The results obtained supported the hypothesis that any kind of "specific drive" requires a given level of general activation "general drive" for its overt manifestation which, in turn, is the result of the balance between activating and de-activating reticular structures.

Key words
Reticular formation • Thalamic pigeon • Testosterone and apomorphine administration

Introduction

In 1969 "Archives Italiennes de Biologie" published a review article entitled "Sleep and instinctive behaviour" written by Giuseppe Moruzzi. Where he described how the studies on physiology of sleep developed. He starts from Berger's milestone experiments based on observational approaches up to the extremely refined studies based on electrophysiological techniques. And these latter caused the behavioural aspects to fad in the background. In fact, at that time the general opinion considered sleep "purely a problem of neurophysiology, with the additional information coming from neuropharmacology and neurochemistry" and this "has led us to forget that sleep is also an ethological problem. With a few exceptions … the relation of sleep to other manifestations of instinctive behaviour has been completely disregarded".
However Moruzzi believed that "time is now ripe for an attempt to bridge the gap between ethology and physiology in the field of hypnic studies".
In the end, after an exhaustive analysis of all the pharmacological, clinical and other sleep characteristics so far studied, he concluded his paper suggesting the

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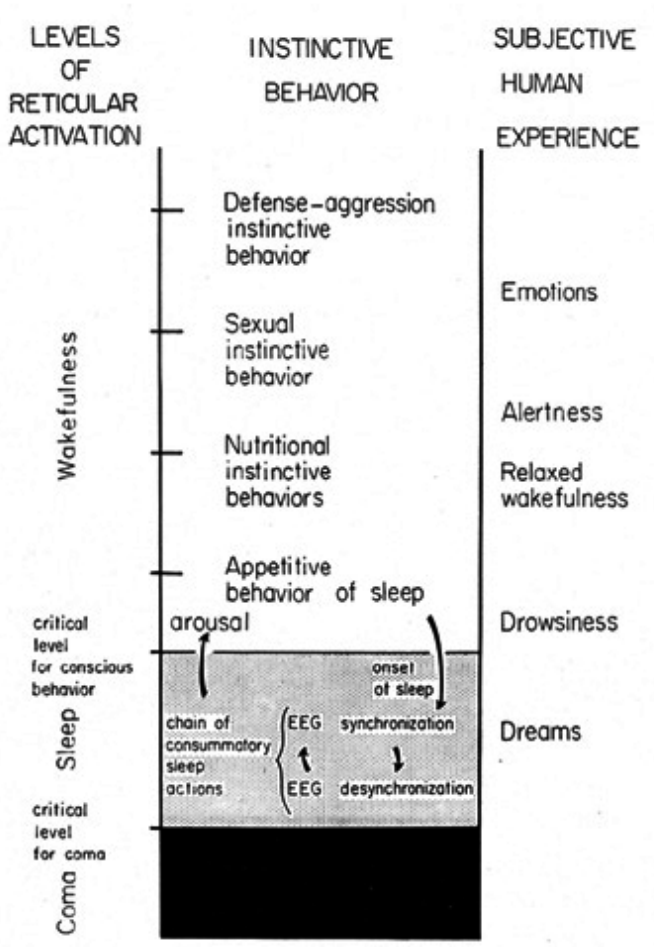

Fig. 1. - The scheme postulates the relationship between level of reticular activation, instinctive behaviour and subjective human experience.
When the reticular activation is above the critical level required for conscious behaviour, i.e. during the waking state, all kinds of instinctive behaviour are possible provided there is an adequate excitation of the specific motivational system and a proper environmental situation. Reticular activation has no steering function, but there might be optimal ranges of activation for each type of instinctive behaviours, as suggested in the scheme. Viceversa it is very likely that the level of activation raises during the appetitive phase and declines after the consummatory action is over.
The instinctive behaviour of sleep starts for levels of reticular activation just above the threshold for consciousness, as an appetitive phase which is typical for each animal. Any further deactivation occurring during, and probably facilitated by, the appetitive phase leads to the chain of sleep consummatory actions, characterized by synchronized or desynchronized EEG. Below a further critical level of reticular deactivation, sleep instinctive behaviour is impossible (coma) (in Moruzzi 1969).

model shown in Fig. 1. Where reticular activation levels are related to the instinctive behaviour. Moruzzi asserted that "Sleep is an active state, one however that requires a level of reticular activation too low to be compatible with conscious behaviour"; and at the same time he hypothesized "sleep may be regarded as a chain of consumatory acts, represented by an alternation of synchronized and desynchronized episodes. It is preceded by an appetitive phase, whose subjective correlates is drowsiness". "This type of instinctive behaviour requires a low but critical level of reticular activation: a level too low to permit consciousness, one however still adequate to maintain stereotyped patterns of motoneuronal and interneural discharge, and to permit the occurrence the hallucination, the dreams".
Moruzzi drew the review to a close observing that: "Sleep regulation may be regarded as an aspect of a wider problem: the study of the levels of reticular activation which are required for the onset of different type of instinctive behaviours".

A new research line
Studies on the sleep-waking cycle in acute and chronic thalamic pigeons and the effects of the reticular formation stimulation

The opportunity to start this original research line on sleep-waking cycle and on the role of the ascending reticular formation by means of neuroethological methods happened together with my admission in the research group of Professor F. Magni[1] and his assistant Dr. M. Brunelli[2]: it was the 1st November 1970 and I had just taken my biology degree.

Moruzzi read all the ethological literature with great attention (Stellar, 1960; Hinde, 1966; Valenstein, 1969), and requested articles from his colleagues around the world.

He was particularly interested in the syndrome of Rolando-Flourens described in birds after the ablation of cerebral hemispheres (in Cate, 1936). Schrader (1889) reported that these animals recovered some of the waking activities following several days of lethargy.

Moruzzi thought that the experimental model of the thalamic pigeon was the more appropriate and easier to test his hypothesis (than mammals) and our research began at once.

A great number of pigeons were recruited to develop the surgical ablation of cerebral hemispheres. I was completely lacking in both neuroscience and neurosurgical techniques, anyway I could start on this scientific adventure thanks to the experience and the expertise of my research partners, Magni and Brunelli.

The development of the surgical technique of inserting a thin and narrow spatula to protect the thalamus; while the overhanging telencephalon was aspired with a vacuum pump proved to be very successful (Fig. 2a). To check if the pigeon was fit for the experiment, once the effect of the anaesthetic had vanished, the animal was thrown into the air so that became stretch his wings: a good landing was evidence of a successful surgery (Fig. 3a). A few hours after surgery, the acute thalamic pigeons awoke for short periods and wandered around aimlessly night and day (Fig. 3b).

The recording of the pigeons' behaviour required accurate organisation. Shifts were arranged, even in the deepest darkness, and infrared glasses and a small lamp that did not disturb the animals were used. The number and duration of the waking-ups were meticulously noted down for each animal. As the pigeons' situation became chronic, their wake phase was enriched by their instinctive repertory. Besides walking they began preening, pecking and then bow-cooing with the typical male premating dance. In the end they also started nest-calling, which is the characteristic crouching hatching position. At that point, chronic electrodes in the pontine reticular region as well as electrodes for ECG registration in free-moving were implanted.

The first results of this new line of research were obtained in May 1971 and were presented to the Accademia Nazionale dei Lincei by Moruzzi himself (Brunelli et al., 1971). In the first six months of experiments, 58 thalamic pigeons were studied, with many ethological observations and videos of their behaviour. Data on the pontine reticular formation stimulation in the acute and chronic animals were also processed. Finally, the histological controls of the telencephalon ablations (Fig. 2b), and of pontine stimulation sites were carried out by Mrs. Bruna Margheritti[3], our excellent histology technician.

The stimulation of the pontine reticular formation, with different stimulation parameters, produced various effects according to the level of wake or sleep; in this way the spontaneous pecking towards the grain came be interrupted and the pigeons generally shifted to other motor research activities by rotating their heads and/or walking around. They started the pecking activity again at the end of the stimulation. Moreover, a repeated train of stimulation with higher voltage induced sleep with their typical feathers attitude, closure of the eyes, and a slowing of the heartbeat (Fig. 4).

Moruzzi presented these results at the International Congress of Physiological Sciences in Munich, Bavaria, (Brunelli et al., 1971). Our results aroused surprise and satisfaction in the scientific world for the renewal of his experimental activities. Afterwards the data were also presented at the Bruges Symposium. The main purpose of the Bruges Symposium was to stimulate a debate between the specialists of the various disciplines of sleep research (neurophysiologists, neurochemists, neuropharmachologists, and clinicians). On this occasion Moruzzi showed a short film recorded during the first experiments concerning the effects of reticular stimulation on pigeon's behaviour[4].

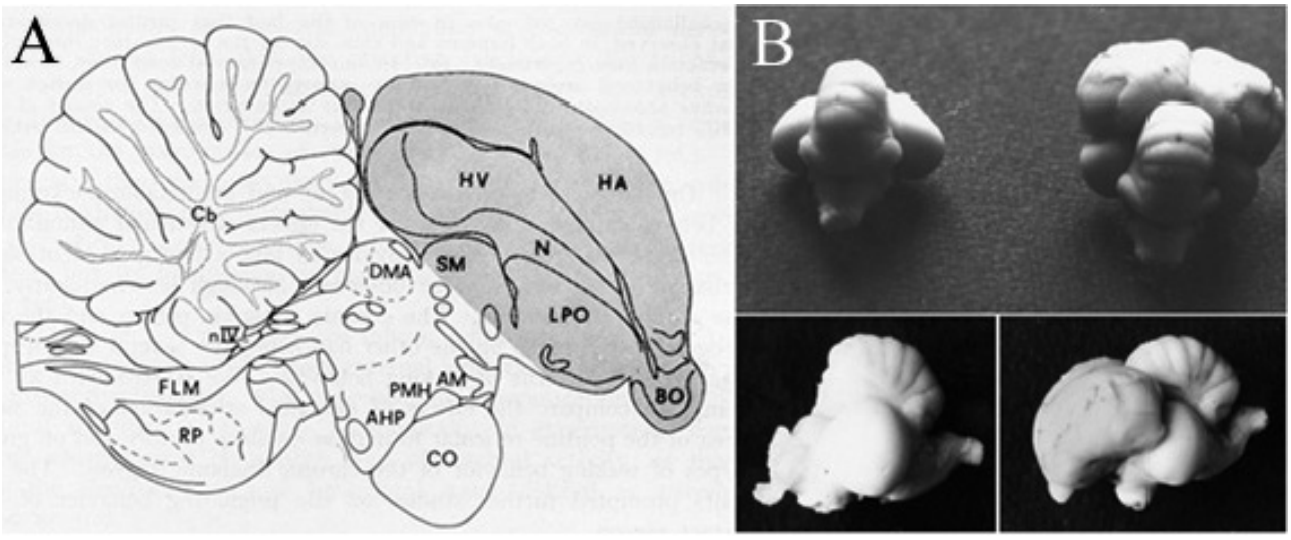

Fig. 2. - Diagrammatic drawing of midsagital section through the pigeon's brain, showing the extension of the ablation of the telencephalon hemispheres and histological control of brain.
A: BO = *bulbus olfactorius*; HA = *hyperstriatumaccessorium*; HV = *hyperstriatum ventrale*; LPO = *lobus paraolfactorius*; N = *neostriatum*; SM = *n. septalis medialis* (in Brunelli et al., 1972).
B: Posterior (top) and lateral (bottom) view of formalin fixed brain of intact pigeon (right) and after bilateral hemisphere ablation (left).

Our results suggested the existence of neurons or fibers ascending from the caudal brainstem separate, both for anatomical origin and function from the fibers responsible of the awakening phenomena. They were co-stimulated by the pontine electrode, resulting in the reversible blocking or shift of spontaneous pecking. The effect of the stimulation could not be revealed when the animal was asleep, because this state lacked the background of instinctive activities on which the blocking action could be exerted. During sleep only the effect of awakening could be disclosed and, it required a slightly more intense stimulation. Therefore in the awake pigeon was possible to selectively stimulate a reticular system other than the activator system: a system able to block the instinctive activities that we were specifically studying (Brunelli et al., 1972).

The last experiments
The level of general arousal (general drive) and its relationship with the motivated behaviour (specific drive)

The results were encouraging, mostly because the behavioural repertory was ample, recurring and long lasting, therefore the experiments were continued.

The experiments aimed to establish a background of strong motivation for selected "specific drives" by means of pharmacological induction (Brunelli et al., 1974) in order to test the effect of reticular system stimulation. Courtship and pre-mating behaviours such as bow-cooing were induced with testosterone administration. They were reversibly blocked by pontine reticular stimulation with marked decreases in heart rate. Moreover, the threshold level to induce this effect was always lower than the threshold to activate the arousal response.

Finally, in the last experiments the interest was focussed on the pecking as "specific driver".

Techniques of pecking recording were improved. The ingenious and imaginative technician staff (Mr. L. Nicotra[5] and his assistant Mr. M. Morelli[6]) provided us with innovative methodological tools (Morelli et al., 1975). The simple microphone system for recording the pecking of the pigeon was replaced by a selective apparatus based on the different capacitative properties of the areas of the bowl where the black spots placed. The background of strong pecking motivation was induced pharmacologically with apomorphine administration and the "general drive" modulated by the intensity of ambient illumination; the latter being considered a natural activator of general arousal, mediated by direct and

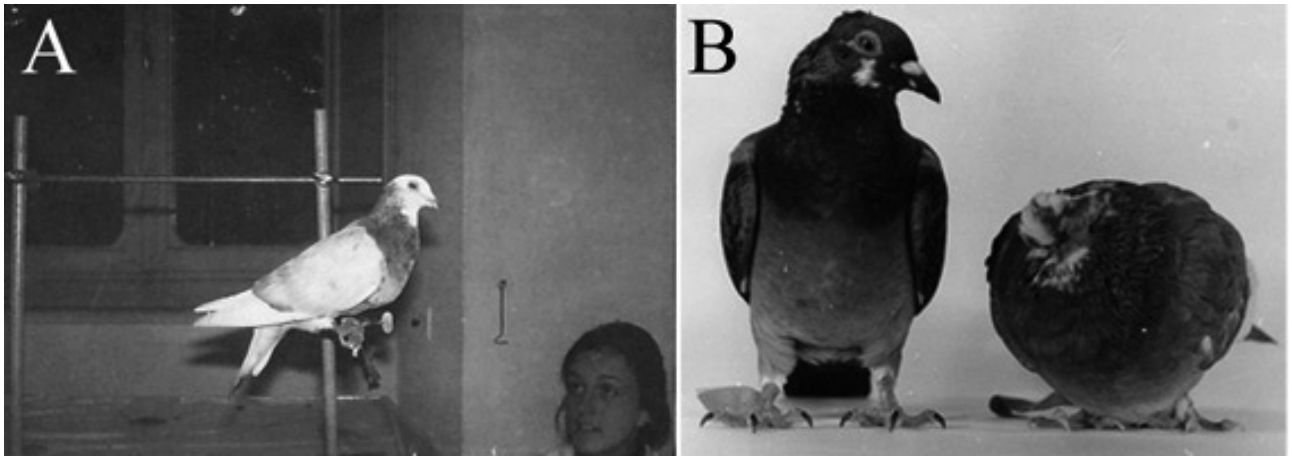

Fig. 3. - Picture of an experimental session.
A: The author tests the sense of balance in the acute thalamic pigeon.
B: Picture of acute thalamic pigeons during wake (left) and during sleep (right).

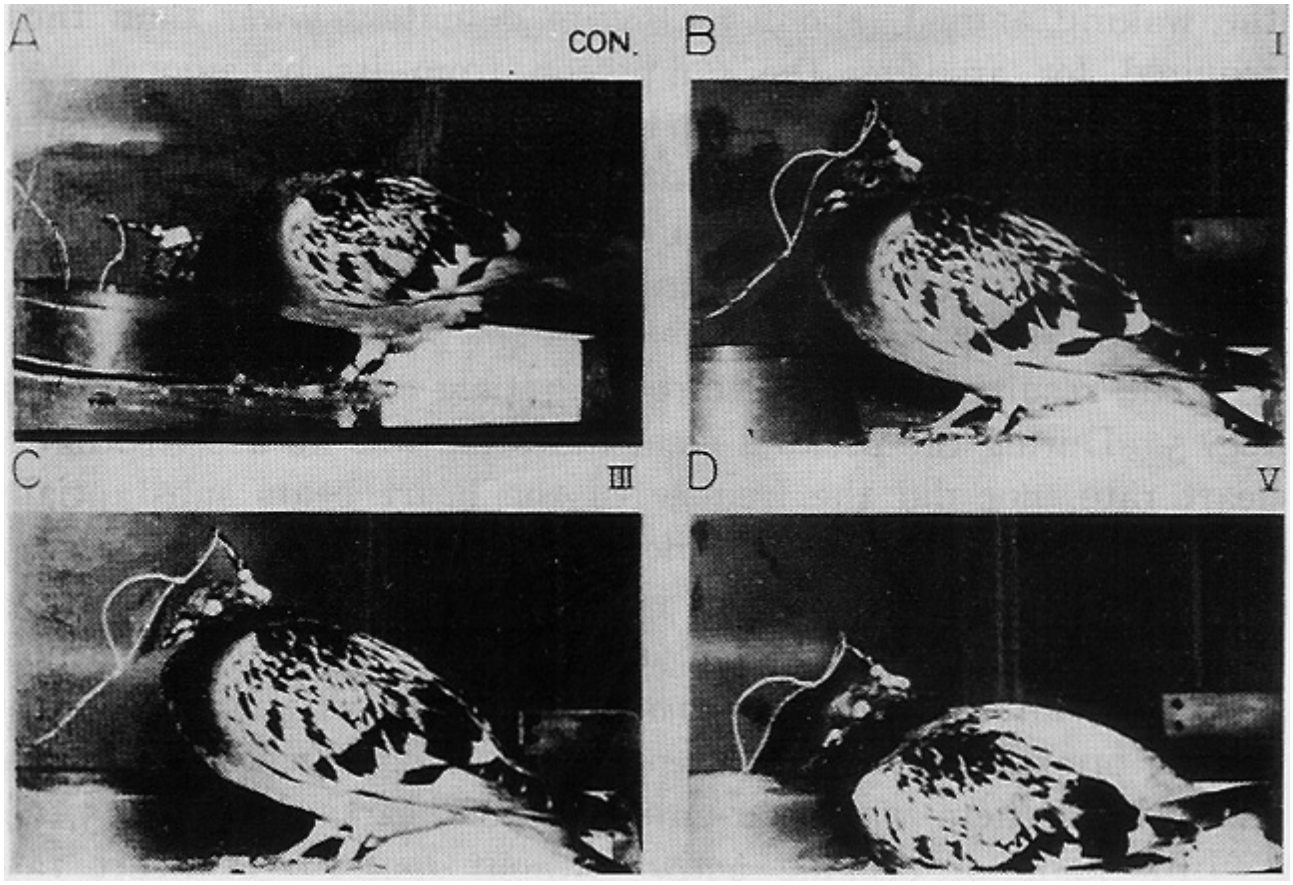

Fig. 4. - Sleep induced by repetitive stimulation of the reticular formation in the chronic thalamic pigeon.
Stimulation of *n. reticularis pontis oralis* with groups of trains of 60 shocks (300/sec, 0,1 msec pulse duration, 1,5 V) at a repetition rate of 1/sec for 15 seconds, followed by 10 seconds of rest.
A = control before stimulation; B, C and D = after the first, third and fifth group of trains. Notice arrest of pecking in B, with gradual onset of sleep behaviour in C, D (in Brunelli et al., 1972).

indirect projections from the retina to the activating reticular system (Rossi and Zanchetti, 1957).
Apomorphine pecking, absent in acute pigeons, simultaneously reappeared in the chronic preparation with the recovery of spontaneous pecking. Reticular pontine stimulation produced reversible blockade of both spontaneous and apomorphine-induced pecking with the same stimulation parameters. The effect of the drug only appeared in the presence of a specific stimulus, namely the visual contrast of a black spot as trigger of pecking activity. A decrease in the contrast of the spot produced a reduction in the number of pecks delivered to the stimulus and an increase in those missing the target, while the total number of pecks was unaffected. Conversely, a lowering of the background luminance was followed by a decrease in the total number of pecks, which was mostly due to a reduction of those aimed at the target, as "consequence of a reduction of general arousal to levels at which the specific feeding motivation cannot achieve its full behavioural expression" (Brunelli et al., 1975).

Conclusion

Taken all together these results supported the hypothesis that: "the level of general arousal (general drive) is a critical factor for appearance of any specifically motivated behaviour. In other words, any kind of motivated activity, which appears as a result of specific factors, requires for its overt manifestation a given level of general activation (general drive), which, in turn is the result of the balance between activating and de-activating reticular structures" (Moruzzi, 1973, 1974).
These were the last results published by Moruzzi.

Author's personal notes

Forty years have gone by, since we started this adventure, when professor Moruzzi allowed me to enter the University world, at first as a contract "ad horas" for student physiology practical, then with a fellowship, and in 1974 as his assistant professor. As a matter of fact I was professor Moruzzi's last assistant. I cannot forget my first interview with prof. G. Moruzzi during which I requested to attend his esteemed institute. During the interview Moruzzi listened to me, whilst sitting in his well-known green armchair in his study. He stared at me with his penetrating eyes which seemed to read my inner thoughts. In the end he said with his typical rhythmic intonation: "Bene, consulterò i miei collaboratori responsabili dei vari gruppi di ricerca, le farò sapere; mi lasci il suo recapito" (Well, I'll consult the head of the research groups in the Institute, I'll let you know. Leave me your telephone number). After a few days, Moruzzi gave me a positive answer and permission to join the group of the Professor Magni. I am convinced that having passed that initial interview means for me much more than having passed all the most important examinations in my scientific career.

Notes

[1] Prof. Franco Magni, dead in 2003.

[2] Prof. Marcello Brunelli, retired in 2010.

[3] Mrs. Bruna Margheritti, istologycal technician, retired in 1995.

[4] The 16 mm film was realised with contribution of the technical photographer Mr. Alberto Bertini (retired in 1996). Being today ruined, has been digitized, unfortunately the audio track is lost; (the CD is available).

[5] Mr. Leopoldo Nicotra, chief in electronic technician, retired in 1994.

[6] Mr. Marcello Morelli, electronic technician, retired in 2005.

Acknowledgments

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References

Brunelli M., Magni F., Moruzzi G., Musumeci D. Effetti di stimolazioni elettriche localizzate del ponte su attività istintive del piccione talamico e del piccione integro. *R.C. Accad. Lincei, Cl. Sci. fis. mat. nat., Ser. VIII*, **50**: 603-606, 1971.

Brunelli M., Magni F., Moruzzi G., Musumeci D. Sleep-waking cycle in the acute thalamic pigeons. *Proc XXV Intern. Congr. Physiol. Sci.*, **9**: 87, 1971.

Brunelli M., Magni F., Moruzzi G., Musumeci D. Brain stem influences on waking and sleep behaviors in the pigeon. *Arch. Ital. Biol*., **110**: 285-321, 1972.

Brunelli M., Magni F., Moruzzi G., Musumeci D. Effects of pontine stimulations on sleep and waking behaviors in the pigeon. In: Petre-Quadens O. and Schlag J.D. (Eds.) *Basic Sleep Mechanisms*. New York, Academy Press: 33-49, 1974.

Brunelli M., Magni F., Moruzzi G., Musumeci D. Apomorphine pecking in the pigeon. *Arch. Ital. Biol.*, **113**, 303-325, 1975.

Cate J. ten. Physiologie des Zentralnervensystems del Vögel (Rolando-Flourens). *Ergebn. Biol.*, **13***:* 93-173, 1936.

Hinde R.A. *Animal behavior*. New York, McGraw-Hill Book Co, 1966.

Morelli M., Musumeci D., Nicotra L. Apparatus for quantitative evaluation of visually guided pecking in the pigeon. *Pharm. Biochem. & Behav*., **3**: 919-922, 1975.

Moruzzi G. Sleep and instinctive behaviour. *Arch. Ital. Biol*., **107**: 175-216, 1969.

Moruzzi G. Les systémes deactivateurs ascendants du tronc cerebral. *Arch. Ital. Biol.*, **111**: 546-552, 1973.

Moruzzi G. *Neural mechanisms of the sleep-waking cycle*. In: Petre-Quadens O. and Schlag J.D. (Eds.) *Basic Sleep Mechanisms*. New York, Academy Press: 13-31, 1974.

Rossi G.F. and Zanchetti A. The brain stem reticolar formation. *Arch. Ital. Biol*., **95**: 199-435, 1957.

Schrader M.E. Zur Physiologie des Vogelgehirns. *Plugers Arch.*, **44**: 175-238, 1889.

Stellar E.D. Drive and motivation. In: Field J., Magoun H.W., Hall V.E. (Eds.) *Handbook of Physiology. Section I: neurophysiology*. Washington D.C., Amer. Physiol. Soc.: 1501-1527, 1960.

Valenstein E.S. Biology and drive. *Neurosc. Res. Symp. Sum*., **3**: 1-108, 1969.

Von Holst E. and von Saint Paul U. Electrically controlled behaviour. In: McGaugh J.L., Wemberger N.M., Whalen R.E. (Eds.) *Psycology*. San Francisco and London, Freeman W.H. a. Co: 56-65, 1966.