Brain Research Bulletin, Vol. 15, pp. 437–441, 1985. © Ankho International Inc. Printed in the U.S.A. 0361-9230/85 $3.00 + .00

Effects of Hypnogenic Vagal Stimulation on Thalamic Neuronal Activity in Cats

G. JUHÁSZ, L. DÉTÁRI AND T. KUKORELLI

Department of Comparative Physiology, University Eötvös Loránd, Budapest, Muzeum krt. 4/A, Hungary, 1088

Received 16 November 1984

JUHÁSZ, G., L. DÉTÁRI AND T. KUKORELLI. *Effects of hypnogenic vagal stimulation on thalamic neuronal activity in cats.* BRAIN RES BULL 15(5) 437–441, 1985.—Neuronal responses from the ventro-postero-medial (VPM) and reticular (NR) nuclei of cat thalamus to vagal stimulation was recorded during wakefulness (W), slow-wave-sleep (SWS), and paradoxical sleep (PS) using chronically implanted microelectrodes. Cellular firing was facilitated in NR and depressed in VPM when weak, hyponogenic stimuli were delivered to the vagal nerve during W and SWS. Higher intensity vagal stimulation increased firing frequency and duration of discharge in both nuclei. Vagally induced discharges of several VPM neurons were depressed by NR stimulation. We speculate that intrathalamic mechanisms play a role in the genesis of induced synchronization and sleep.

Sleep Vagal stimulation Unit activity Thalamus Cat

VISCERAL receptors and afferents modify the level of wakefulness. Both cortical synchronization and sleep can be induced by stimulation of carotid sinus [8] and vagal nerve [4,7]. In previous papers [12,13], we described a synchronizing and hypnogenic influence of intestinal origin which could be mediated by splanchnic and vagal afferents.

There are few data, however, which describe the recruitment of central neuronal mechanisms by synchronizing and hypnogenic afferent impulses originating from the visceral receptors. Previous studies have focused exclusively on the participation of the solitary tract and nodose ganglia in development of vago-aortic sleep [19].

However, sleep alters synaptic transmission of viscerosensory information at the thalamic level as well [14]. The hypnogenic intestinal, splanchnic and vagal stimulation increases the number of short clusters of neuronal firing, characterizing slow-wave-sleep (SWS) [15]. EEG synchronization accompanies the burst-pause cellular patterns generated by intrathalamic-mechanisms. The thalamic reticular nucleus (NR) may be involved in these events by controlling both spontaneous and sensory evoked activity in relay nuclei [9, 18, 20, 21, 23, 26, 27, 28]. In this study, the patterns of neuronal firing in NR and in the VPM of the thalamus induced by vagal stimulation were compared in all sleep-wakefulness stages in cats. In addition, the effects of NR stimulation on VPM responses to vagal excitation were also examined.

METHOD

Surgery

Eight adult male cats (aged 2–4 years, weighing 3–5 kg) were implanted under Nembutal anaesthesia (50 mg/kg) with cortical, ocular and muscular macroelectrodes for sleep recording as described earlier [12]. Mechanical microdrives were fitted onto the scull above the right VPM and anterior part of NR (coordinates by Jasper's atlas: VPM Fr: 8.5 L: 5–2, V: −1, −2, NR Fr: 13, L: 2–5, V: 0–2). Each microdrive contained an array of four microelectrodes made of teflon coated stainless steel wire of 75 μ diameter. A bipolar platinum electrode was fixed on the cervical portion of the left vagal nerve. The chronic vagal electrode was made from a teflon body with a slot for the vagal nerve. Two platinum wires were fixed in holes of the slot base and they were wrapped around the nerve. The electrode leads run through the holes of the electrode body and they were soldered onto the head plugs. A teflon plate covered the slot which insulated the electrodes from the surrounding tissue. At least one week of recovery was allowed after surgery before recordings began.

Recording and Stimulation

Both recording and stimulation were performed by means of a multistranded cable containing leads attached to the head plugs. EEG, EOG and EMG were recorded on a BECKMAN (R 611) polygraph. Unit activity was amplified by an asymmetric amplifier, monitored on a Tektronix oscilloscope and stored by a PHILIPS (Analog-7) tape recorder. Square-wave pulses of a NIHON-KOHDEN stimulator were used for stimulation of the vagal nerve. Two different intensities of stimulation were applied in each experiment: weak, hypnogenic stimuli which induced cortical synchronization and sleep by exciting vagal afferents of the largest diameter [6,11]; strong, activating stimuli, which evoked a short desynchronization in the cortical EEG without overt signs of behavioral arousal when they were delivered in SWS. The reticular nuclei were stimulated by electric pulses of 0.05 msec duration and the stimulus voltage was 1.5 times higher than the threshold of appearance of evoked responses in VPM.

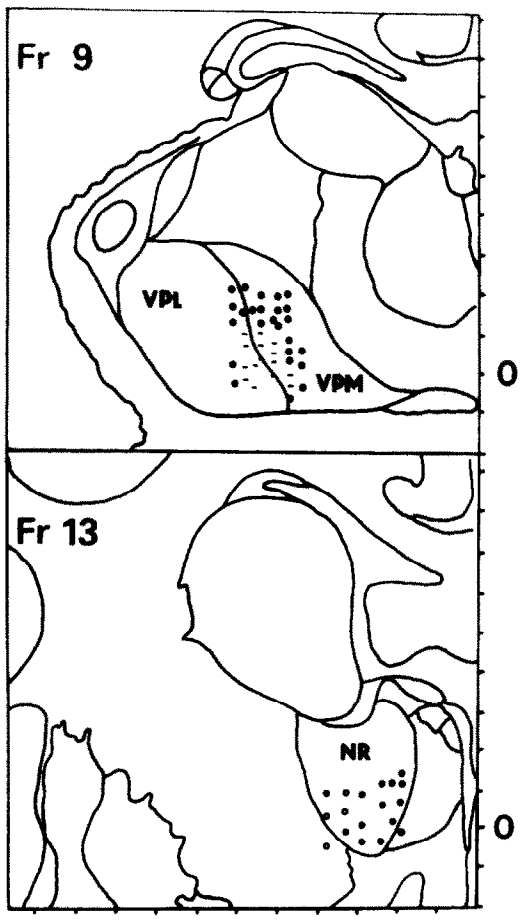

FIG. 1. Localization of recording points in VPL, VPM and NR. Electrode positions in which NR stimulation induced inhibition in responses of VPL-VPM neurones are marked by "—". The antero-posterior dispersion of electrode tracks was less than 0.8 mm.

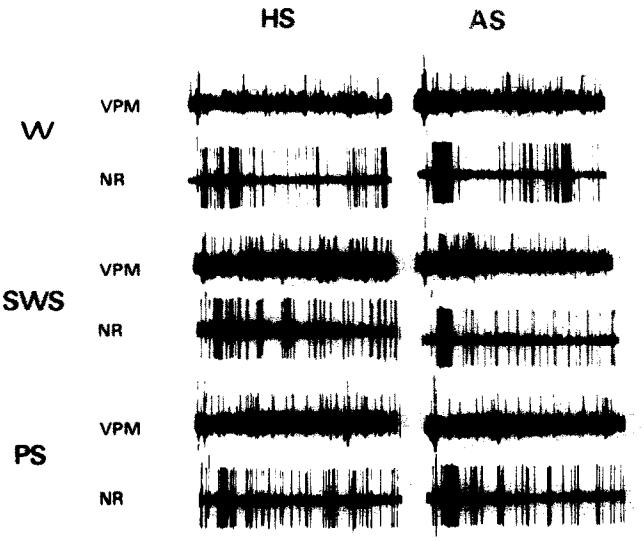

FIG. 2. Neuronal responses of VPM and NR during W, SWS and PS. 10 responses are summarized in each curve. AS indicates the responses to activating vagal stimulation; HS shows responses to hypnogenic stimulation. Total length of the curve is 500-msec. Each sweep was triggered by the stimulus. The long inhibitory component in W can be seen here was not typical.

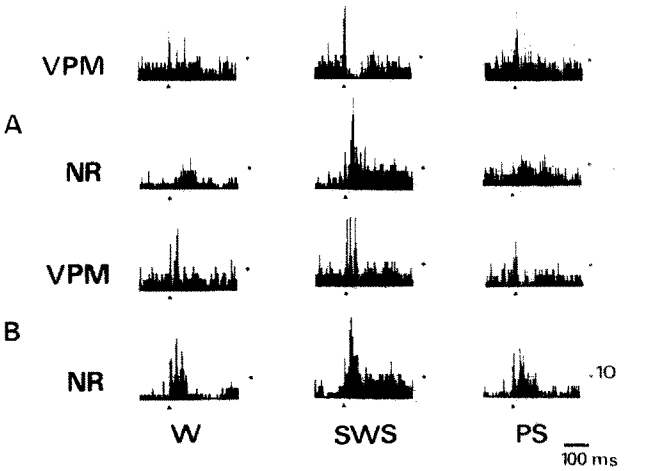

FIG. 3. PST histograms of HS (A) and AS (B), recorded during W, SWS and PS. Each histogram is composed of 30 responses. Stimulus is marked by △.

Experimental Procedure

Recordings were performed in daily sessions from 9 a.m. to 13 p.m. with the cats in a soundproof cage. Microelectrodes were lowered into VPM and NR until stable neuronal activity was encountered. Cellular discharges were then recorded continuously during several sleep-wakefulness cycles. Relaxed wakefulness (W), slow-wave sleep (SWS) and paradoxical sleep (PS) were distinguished by usual polygraphic criteria. Appropriate parameters of hyponogenic stimuli were determined in W and those of activating ones were determined in SWS. During W and during 2–3 consecutive episodes of SWS and PS, single pulses of hypnogenic stimuli were delivered randomly to the vagal nerve, with the shortest interstimulus interval being 5 sec. In the course of the following sleep episodes, activating stimuli were applied in a similar fashion. On termination of the recording the microelectrodes were advanced by 200 μ and the recording procedure was repeated. Not more than two points of VPM and NR were explored in one day.

When the effects of NR stimulation on the evoked neuronal activity of VPM were studied, the conditioning weak vagal stimuli were followed by NR test ones. The intervals between them were 2, 5, 10, 20 and 50 msec. Only those VPM points were explored in which evoked responses were elicited by both NR and vagal stimulation.

Final electrode positions were marked by electric lesions (200 mA, 10 sec). The brain was perfused by 10% formalin solution, and a disk containing the exposed structures was dissected from the brain by stereotaxic knife. After paraffin embedding, 10 μ slices were made and stained by cresyl-violet for light microscopic verification of electrode tracks. The sites of the individual recording spots were identified taking into account the contraction of brain tissue during the histological procedures.

Data Processing

The neuronal discharges recorded on magnetic tape were

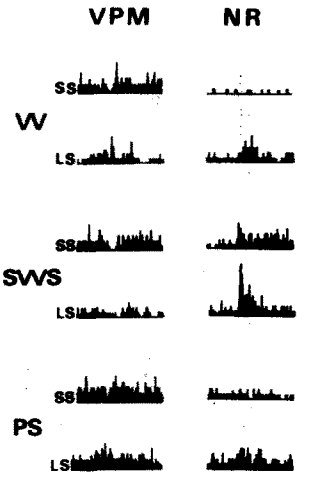

FIG. 4. Responses of neurons generating small (SS) and large (LS) spikes to hypnogenic vagal stimulation. PST-s summarize data of 30 responses recorded from VPM and NR during W, SWS and PS. PST-s were made from −100 to +300 msec; stimulus is marked by △.

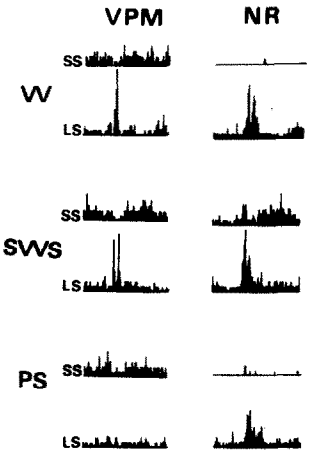

FIG. 5. Responses of neurons discharging with small (SS) and large (LS) spikes to activating vagal stimulation. PST-s summarize data of 30 responses recorded from VPM and NR during W, SWS and PS. PST-s were made from −100 to +300 msec; stimulus is marked by △.

analysed by a TPA-i type computer. Noise was filtered by a level discriminator and the amplitudes of spikes were measured by a four-channel interface. Following A/D conversion at the time of spike generation, the amplitude and the input channel marker data of each discharges were stored in the computer memory. The stimulus triggers were also measured by a parallel input register. First, amplitude histograms were composed from one minute long periods of spontaneous activity recorded at the beginning and at the end of the recording session. Only those samples were analysed in which the amplitude histograms remained unchanged indicating that no neurons was lost during a long recording period of 2–4 hours.

When the neuronal amplitude histograms showed two separate distributions indicating two populations of cells, one of larger amplitude than the other, the activity of the large and small cells were separated and the data processing was repeated on each spike train.

Neuronal responses were studied by means of peristimulus-time-histograms (PST) obtained from both total and separated multineuronal activity. Each PST included 30–50 individual responses and they were displayed on an X-Y plotter. On the basis of a χ^2 test, a response component of a PST was considered "inhibitory" when at least 5 consecutive data points were significantly lower than the average value of prestimulus activity and it was considered "excitatory" when at least 5 data were above this level. It should be noted that the word "inhibitory" will not be used in its strict sense. Certainly, the reduced level of firing does not prove a direct inhibition of examined cells. However, to simplify the description of neuronal responses, inhibition will be used in the sense of transient, decrease of firing. The lowest points of inhibitory components and the highest points of excitatory components were measured. Peak latencies of excitatory components in NR were compared to those of the inhibitory components in VPM responses by using a t-test. Evaluating the influence of stimulation in NR the effect of NR stimulation on VPM responses to vagal stimulation was termed "inhibitory" when: the excitatory component of VPM response was reduced or the inhibitory component of VPM response was enhanced or a new inhibitory response component was developed. The occurrance or reduction of PST components was considered by application of χ^2 test.

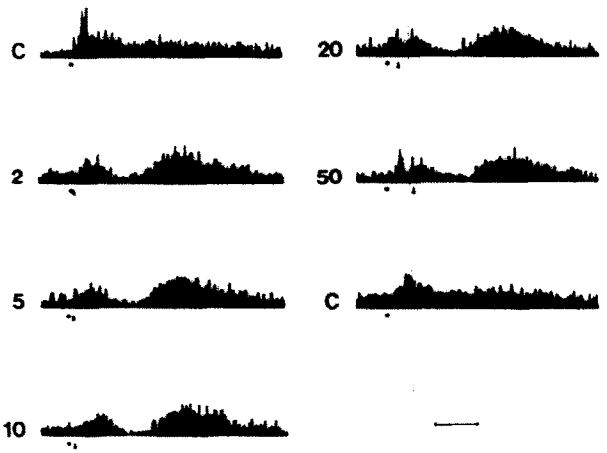

FIG. 6. Inhibitory effect of NR stimulation in vagal responses of VPM neurons. C shows vagal responses before and after application of NR stimulation. NR stimulus (△) were delayed 2, 5, 10, 20, 50 msec to vagal one (●).

RESULTS

Neuronal Responses to Vagal Stimulation

Neuronal responses of VPM and NR were recorded simultaneously from 64 point pairs (Fig. 1). In all of the recorded points, vagal stimulation elicited marked responses (Fig. 2). However, the parameters of induced neuronal activity changed with stimulus intensity, sleep stages and recording sites.

Hypnogenic stimulation resulted in clear-cut alteration of neuronal firing in VPM only during W and SWS. The VPM responses started with a short facilitation of firing which was

frequently followed by a depression of cellular discharges. The inhibitory component was much longer in SWS than in W. The excitatory component was enhanced and the inhibitory component was reduced in response to activating stimulation. In addition to this effect, activating stimulation elicited responses were composed of two components in PS (Fig. 3). Reticular nuclei neurons responded to both hypnogenic and activating stimulation by increasing their discharge in all stages of the sleep-wakefulness cycle. The latency of firing increase in NR did not differ statistically from that of the inhibitory component of the VPM response. In both of the recorded structures the responses showed this pattern, no exceptions were found.

In five recording location pairs of VPM and NR, the simultaneously recorded neuronal activity proved to be separable into spike groups of small and large amplitudes during both wakefulness and sleep. In VPM, hypnogenic stimulation induced inhibitory components in small amplitude responses without preceding excitatory component in all stages of the sleep-wakefulness cycle. The averaged latency of the peak of the inhibitory component was 38.7 msec. This effect of hypnogenic stimulation was observed during wakefulness and sleep, however, it was most obvious in SWS (Fig. 4). At the same time, a short excitatory component in W and an inhibitory component in SWS were induced by hypnogenic stimulation in the large amplitude activity, but no response was observed in PS (Fig. 4). In W, the averaged peak latency of excitatory components was 55.6 msec during SWS. Compared to the effects of hypnogenic stimulation, the small amplitude responses were not changed (average latency of inhibition 34.8 msec) but excitatory components of large amplitude responses were increased by activating stimulation during W, SWS and PS (Fig. 5). Vagal stimulation did not influence small amplitude discharges in NR. Large amplitude activity was enhanced by both hypnogenic and activating stimulation (Figs. 4 and 5). The average peak latency of excitatory component in NR was 34.9 msec which was not significantly different from the latency of inhibitory component in small amplitude responses of VPM.

Influence of NR Stimulation on Induced VPM Activity

In W, the neuronal response of VPM to weak conditioning vagal stimulation was modified by a test stimulus of NR. This effect depended on the position of recording electrodes in VPM and the delay of NR stimulation. Sixty-four point pairs of the two nuclei were explored in four cats. The influence of NR stimulation was found to be depressive to the VPM responses in half of the examined points (51.5%). The positions of these locations are shown in Fig. 1. Cells located near the border of VPM and VPL and close to the zero plane, exhibited an inhibitory component from NR stimulation. Nevertheless, responses of these cells to vagal stimulation were poorly excitatory (Fig. 6). When the VPM responses had inhibitory and excitatory componets, NR stimulation reduced excitatory component and increased inhibition. All these effects proved to be most marked when the delay of NR stimulation was less than 20 msec (Fig. 6). The inhibitory effect of NR stimulation was not observed in the neuronal responses of the other half of the sixty-four VPM neurons.

DISCUSSION

Both hypnogenic and activating stimulation of the vagal nerve modified firing patterns of neurons in thalamic nuclei. The responses of VPM neurons were always composed of excitatory and inhibitory components which were extremely well developed in SWS. However, an increase of firing was observed in NR in association with an inhibitory pattern detected in the VPM. Consequently, vagal stimulation could activate neurons in NR which might be able to inhibit the VPM cells, especially when hypnogenic stimulation was applied. Activation of such a mechanism has been suggested by others. For example stimulation of certain NR neuronal populations produces inhibition of neuronal discharge in thalamic relay nuclei [9, 17, 18, 26, 27]. A similar interaction of vagal and NR stimulation was also observed in our experiments, supporting the concept that inhibitory components of VPM responses to vagal stimulation were due to the marked inhibition produced directly by NR stimulation. However, it could not be excluded that local inhibitory processes also contribute to the development of inhibitory response component because the burst-pause discharge pattern of thalamic relay neurons have been observed without input from other cells [16].

In SWS, when the inhibitory influence of NR on VPM was found to be the most intensive, the excitatory component of neural responses was also increased in the VPM. This response is consonant with our previous findings in which an increase of the thalamic transmission of viscerosensory evoked potentials was observed in SWS [14]. It is widely known that the facilitation of primary component of evoked potentials is usually accompanied with increased excitatory processes of neurons. Vagal impulses can reach the thalamus both on the lemniscal and extralemniscal pathways. The lemniscal path is shorter and is directed to VPM while the extralemniscal route is longer and projects diffusely in the nonspecific thalamic nuclei [6]. It can be supposed that, similarly to the somatosensory input, NR also receive vagal input [22]. The neuronal pathways on which the inhibitory effect of NR neurons reach the VPM is not yet clear. The large latency of NR induced inhibition of VPM responses suggests the existance of a long, multineuronal pathway between them.

Separate analysis of neuronal activity generating large or small spikes showed that both hypnogenic and activating stimulation induce inhibitory responses in small-spike cell responses. The same inhibitory effect, however, was observed in the activity of cells discharging with large spikes only in the case of hypnogenic stimulation. When stimulus intensity was strong, the long and intensive excitatory component overlapped the inhibition. Amplitude differences of spikes recorded simultaneously on extracellular microelectrodes depends on the size of cell bodies rather than on distances of the neurons from recording electrodes [10, 20, 24, 25]. The anatomic structure of the thalamus suggests that the neurons, firing with small spikes might be interneurons. It is possible that relay cells were discharging with large spikes. The implication of this suggestion is that interneurones of relay nuclei are always inhibited by NR activation induced by vagal stimulation without respect to stimulus intensity. The interneuronal inhibition, however, could spread to relay cells only when hypnogenic stimulation is not too strong. We conclude that hypnogenic vagal stimulation is capable of driving inhibitory mechanisms of the NR which control VPM neurons. This inhibitory effect could contribute to the development of alternating excitation and inhibition of neuronal firing in relay nuclei. Such a neuronal pattern is characteristic of EEG synchronization.

REFERENCES

1. Baust, W. and H. Heinemann. The role of baroreceptors and of blood pressure in the regulation of sleep and wakefulness. *Exp Brain Res* **3**: 12–24, 1967.
2. Ben-Ari, Y., R. Dingledine, J. Kanazawa and J. S. Kelly. Inhibitory effects of acetylcholine on neurons in the feline nucleus reticularis thalami. *J Physiol (Lond)* **261**: 647–671, 1976.
3. Bonvallet, M., P. Dell and G. Hiebel. Tonus sympathic et activité electrique corticale. *Electroencephalogr Clin Neurophysiol* **6**: 119–144, 1954.
4. Chase, M. H., Y. Nakamura, C. D. Clemente and B. Sterman. Afferent vagal stimulation: Neurographic correlates of induced EEG synchronization and desynchronization. *Exp Neurol* **5**: 236–249, 1967.
5. Creutzfeld, O. Electrical activity from the neuron to the EEG and EMG. *Handbook of Electroencephalography and Clinical Neurophysiology*, vol 2, edited by A. Remond. Amsterdam: Elsevier, 1974.
6. Dell, P. and R. Olson. Projections thalamiques, corticales et cerebelleuses des afferences viscerales vagales. *C R Soc Biol* **145**: 1084–1088, 1951.
7. Dell, P. and Y. Padel. Endormissement rapid provoqué par la stimulation sélective d'afferences vagales chez le chat. *Rev Neurol* **111**: 381, 1964.
8. Dingledine, R. and J. S. Kelly. Brainstem stimulation and the acetylcholine-evoked inhibition of neurones in the feline nucleus reticularis thalami. *J Physiol (Lond)* **271**: 135–154, 1977.
9. Frigyesi, T. L. Intracellular recordings from neurons in dorsolateral thalamic reticular nucleus during capsular, basal ganglia and midline thalamic stimulation. *Brain Res* **48**: 157–172, 1972.
10. Grover, F. and J. Buchwald. Correlation of cell size with amplitude and background fast activity in specific brain nuclei. *J Neurophysiol* **33**: 160–171, 1970.
11. Kleitman, N. *Sleep and Wakefulness*, 2nd edition. Chicago: University Press, 1963.
12. Kukorelli, T. and G. Juhász. Electroencephalographic synchronization induced by stimulation of small intestine and splanchnic nerve in cats. *Electroencephalogr Clin Neurophysiol* **44**: 491–500, 1976.
13. Kukorelli, T. and G. Juhász. Sleep induced by intestinal stimulation in cats. *Physiol Behav* **19**: 355–358, 1977.
14. Kukorelli, T. and G. Juhász. Tonic and phasic modification of viscerosensory evoked potentials during sleep in cats. *Acta Physiol Hung* **64**: 237–246, 1983.
15. Kukorelli, T., G. Juhász and L. Détári. Modification of cortical and thalamic unit activity by visceral stimulation during sleep in cats. In: *Adv Physiol Sci, vol 1, Regulatory Functions of the CNS. Motion and Organization Principles*, edited by J. Szentágothai, M. Palkovits and J. Hámori. New York: Pergamon Press, 1981, pp. 309–313.
16. Llinás, R. and H. Jahnsen. Electrophysiology of mammalian thalamic neurones in vitro. *Nature* **297**: 406–408, 1982.
17. Massion, J. and L. Rispal-Padel. Differential control of motor cortex and sensory areas on ventrolateral nucleus of the thalamus. In: *Corticothalamic Projections and Sensorimotor Activities*, edited by T. L. Frigyesi, E. Rinvik and M. D. Yahr. New York: Raven Press, 1972, pp. 357–374.
18. Nanobashvili, Z. J. and S. P. Narikashvili. Unit activity of the reticular and relay nuclei of the cat thalamus. *Neurophysiology* **13**: 19–25, 1981.
19. Puizillout, J. J., G. Gaudin-Chazal, A. Sayadi and D. Vigier. Serotonergic mechanisms and sleep. *J Physiol (Paris)* **77**: 415–424, 1981.
20. Rinaldi, P., G. Juhász and M. Verzeano. Analysis of circulation of neuronal activity in the waking cortex. *Brain Res Bull* **1**: 429–435, 1976.
21. Schlag, J. D. and M. Waszak. Characteristics of unit responses in nucleus reticularis thalami. *Brain Res* **21**: 286–288, 1970.
22. Shosaku, A., Y. Kayama and I. Sumitomo. Somatotopic organization in the rat thalamic reticular nucleus. *Brain Res* **311**: 57–63, 1984.
23. Skinner, J. E. and C. D. Yingling. Regulation of slow potential shifts in nucleus reticularis thalami by the mesencephalic reticular formation and the frontal granular cortex. *Electroencephlogr Clin Neurophysiol* **40**: 288–296, 1976.
24. Tyler, T. J., R. A. Roemer and R. F. Thompson. Relation between gross and unit evoked activity in precurciate cortex of cat. *Physiol Behav* **6**: 375–479, 1971.
25. Verzeano, M. and J. H. Mahnke. Serotonin and thalamic synchronization. *Physiol Behav* **9**: 649–653, 1972.
26. Waszak, M. Effect of polarizing currents on potentials evoked in neurons of the ventral lead of nucleus reticularis thalami by intralaminar stimulation. *Exp Neurol* **40**: 82–89, 1973.
27. Waszak, M. Effect of barbiturate anaesthesia on discharge pattern in nucleus reticularis thalami. *Pharmacol Biochem Behav* **2**: 339–345, 1974.
28. Yingling, C. D. and J. E. Skinner. Regulation of unit activity in nucleus reticularis thalami by the mesencephalic reticular formation and the frontal granular cortex. *Electroencephalogr Clin Neurophysiol* **39**: 635–642, 1975.
29. Zepelin, H. and A. Rechtschaffen. Mammalian sleep longevity and energy metabolism. *Brain Behav Evol* **10**: 422–470, 1974.