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RESPONSES OF VESTIBULAR NEURONS TO STIMULATION OF CORTICAL SENSORIMOTOR AREAS IN THE CAT

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INTRODUCTION

The execution of voluntary limb movements involves a precise interaction between anticipatory postural adjustments and the primary movement (2, 3, 15). The anticipatory postural adjustments which provide the means of minimizing the equilibrium disturbances (1), are reported to be preprogrammed together with the focal movement (16, 17) and, as far as the involved structures are concerned, a prominent role has been attributed to the vestibular system (7, 9, 13, 14, 16, 19). In particular, the lateral vestibular nucleus (LVN) neurons with axons projecting to all segments of the spinal cord, not only exert a static and dynamic control of posture (4, 12, 19) but also receive direct bilateral influences from the sensorimotor cortex (22).

The present experiments have been performed to further extend the analysis of the relation between cortical motor areas and the LVN neurons, by studying the effects on single LVN neurons produced by stimulation of given motor cortex sites yielding specific movements and associated postural adjustments. The experiments have been carried out in cats, where a fine somatotopically organized motor system exists (18). The types of movement and postural adjustment were first analyzed in the implanted awake cat and, in a second experimental session, the LVN neural activity was recorded in the same anesthetized cat without ablating the cerebellum. Evidence of a strict relationship between neck and shoulder areas of the motor cortex and vestibulospinal neurons which respond with characteristic nervous activity is presented.

A brief communication of the preliminary data has been published (5).

METHODS

The experiments were carried out on 16 adult cats weighing 2.8-3.2 kg. A group of 5 animals were used for semichronic experiments and the remaining 11 for acute experiments.

Animal preparation. — Acute and semichronic experiments were performed in cats anesthetized with ketamine hydrochloride (5 mg/kg, preanesthesia) and Nembutal (20 mg/kg sodium pentobarbital) during the surgical preparation and with Nembutal (30 mg/kg) or chloralose (50 mg/kg) during the recording session (additional doses of 5 mg Nembutal or 25 mg/kg of chloralose were given later). Artificial respiration and at times pneumotorax were performed, the animals being tracheotomized and immobilized with d-tubocurarine.

Rectal temperature was monitored and maintained at 36°-38°C and expiratory CO_2 was monitored.

The cortical stimulating electrodes were implanted according to a technique used in previous works (18, 20). In particular, 10-16 nickel chrome electrodes (sharpened and insulated except at the tip 250 μm, 50-80 KΩ) were implanted, by the aid of a micromanipulator, through tiny trephine holes (0.5-1 mm) drilled bilaterally in the skull, into the pericruciate area (area 4, area 6 and area 3). The electrodes were placed perpendicular to the cortical surface (Fig. 1 A) and penetrated through the dura mater at a depth of 1.5 mm and

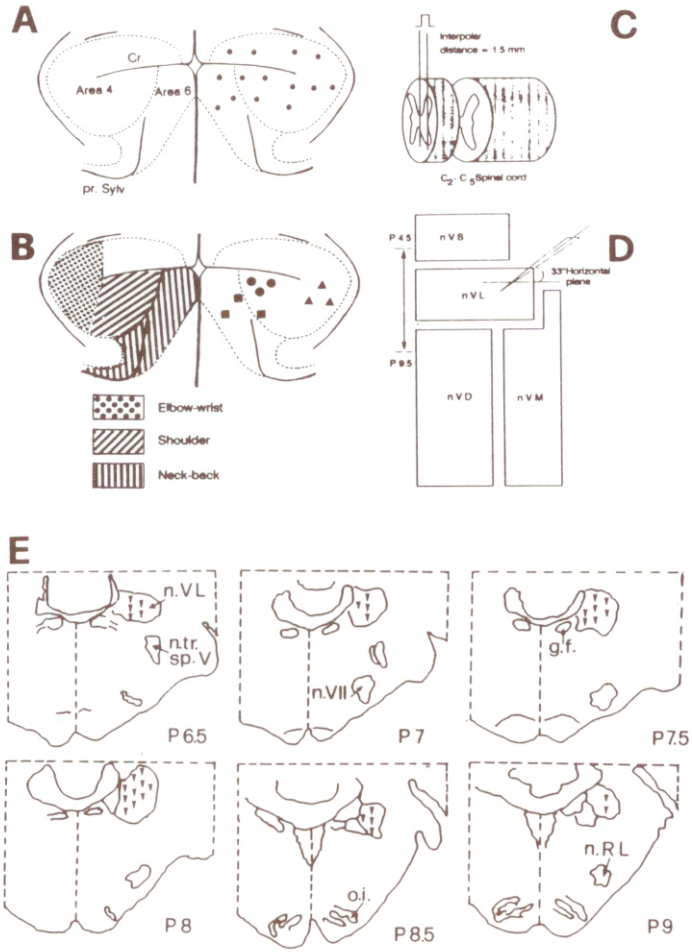

Fig. 1 – *Experimental arrangement and vestibular explored regions.*

Schematic diagram summarizing in A the distribution of cortical electrodes, in B the position of the electrodes and related cortical control areas of different parts of musculature. C and D illustrate respectively the spinal cord stimulation and the vestibular complex registration procedure.

Diagram E shows, in a series of transverse sections extending from P 6.5 to P 9, the anatomical localization of the recorded LVN neurons identified by electrolytic lesion and dye marks.

Abbreviations: nVL, nVM, nVS, nVD, nucleus vestibularis lateralis, medialis, superior, inferior; n.tr.sp.V, nucleus tractus spinalis n. trigeminus; n.VII, nucleus n. facialis; g.f., genu n. facialis; O.I., n. olivaris inferior; n.RL, nucleus reticularis lateralis.

2 mm apart; they were then sealed to the bone by dental cement and soldered to an electrical plug connector (20). An indifferent silver wire electrode was placed in the frontal sinus cemented to the bone and used at times as anode during monopolar stimulation.

A laminectomy was performed to expose spinal segments C1-C5 (Fig. 1 C) while two stainless steel electrodes (diameter 150 μm) sharpened, aligned sagittally with an interpolar distance of 1,5 mm and insulated except for 500 μm at the tip, were inserted at a depth of 4-5 mm from surface into the ventromedial funiculus, ipsilaterally to the recording site. A posterior craniotomy was performed to expose the caudal brain stem and the cerebellum which were protected in a pool of warm mineral oil.

In the semichronic experiments, a preliminary operation was conducted under sterile conditions in order to implant the cortical electrodes. The animals were left to recover from anesthesia and tested during the following days to verify the movements and postural adjustments induced by intracortical stimulation (bipolar and monopolar stimulations) and to evaluate the threshold parameters for each movement.

After 10 days from the initial operation, the semichronic animals were reanesthetized for the recording session. Posterior craniotomy for recording and laminectomy for the antidromic stimulation were performed as above described.

Recording electrodes characteristics. — In order to record the activity of vestibular neurons (Fig. 1 D), glass microelectrodes with diameter of 2-8 μm and resistance of 1500-2500 KΩ, filled with saturated Na citrate solution, were introduced according to stereotaxic parameters into the vestibular nuclear complex, at an angle of 33° from the horizontal plane, to avoid tentorium bone. Parallel tracks were repeated mediolaterally between L 1-L 6, rostrocaudally between P 4.5-P 10 and dorsoventrally between H-1- H-7.

Stimulation parameters. — In the semichronic experiments, the movements and postural adjustments evoked by cortical stimulation were analyzed by videocamera recording; the threshold parameters for each movement were marked and both threshold values and movement were checked during several testing sessions in the awake animal. Motor responses evoked by a given electrode and its threshold value remained constant during successive trials and different testing sessions.

In all animals cathodal stimulation of the cerebral cortex (1/sec trains with the following characteristics: duration 10 msec, frequency 300 Hz, pulse width 0.5 msec, 20-200 μA, delivered by a constant current stimulator) was performed by connecting the implanted electrodes in pairs, being each electrode alternatively cathode and anode; at times, the silver indifferent electrode was used as anode and monopolar stimulation was performed.

The antidromic bipolar stimulation of the vestibulospinal tract was obtained by applying single shocks (0.2 msec, 1 Hz). The neurons were identified as being antidromically driven when they responded at fixed latencies and followed high-frequency stimulation (200 Hz) and, when neurons fired spontaneously, by collision of the antidromic response with the orthodromic spike.

Hystological control. — In order to check the track location, the tip of the glass microelectrode was cut in its last track at the depth H O. Two additional reference tracks were performed with stainless steel electrodes and an anodal current (30 μA, 20 sec) was passed for tip visualization (symmetrical tracks at P 5, L 3 and depth H O). The location of the cortical stimulating electrodes was checked by applying a steady anodal current (30 μA for 20 sec) through each electrode.

At the end of each experiment, the animal was sacrificed with a lethal dose of anesthetic, the brain was removed, firstly photographed frontally after marking the stimulation sites on the surface of the pericruciate area with India ink, fixed in formalin and then cut in serial sections, which were stained by means of the Nissl method. The exact position of the cortical electrodes was ascertained from the sagittal hystological sections and by referring to the Hassler and Mush-Clement maps (8).

The position of the recording sites was constructed on the basis of the glass microelectrode track and of the location of the reference electrolytic lesions.

Data analysis. — All recordings were stored on magnetic tape and digitalyzed for subsequent latency and frequency analysis.

RESULTS

The cortical stimulating electrodes were placed in the pericruciate area according to the cartography established by Nieoullon and Rispal-Padel (18). Forelimb (wrist-elbow and shoulder areas), neck, back and hindlimb regions were implanted bilaterally. The exact position of the stimulating electrodes into the corresponding motor fields (Fig. 1 B) was evaluated in the semichronic experiments: neck and distal forelimb muscle contractions (wrist-elbow) were achieved in the awake animal with threshold (T) values of 40-60 μA, whereas back, shoulder and hindlimb movements required 80-100 μA. These T values remained constant both during the successive trials and the different testing sessions. Furthermore, T values and electrode location for each motor response did not vary significantly in different animals. During the recording session, the effect of cortical stimulation was tested by using the standardized T values and multiples of T.

1. *Analysis of vestibular neuronal activity.* — The activity of 113 units was recorded. A total of 90 units were located in the vestibular complex, while the remaining 23 units in the pontine reticular formation; the latter units will not be considered in the present study. The location of 32 units was identified by electrolytic lesion and dye marks, while that of the remaining units was reconstructed on the basis of the corresponding tracks. The 32 units, shown in Fig. 1 E, were histologically identified as LVN (Deiters) neurons, other 29 units were also found after reconstruction to be located in the Deiters' nucleus (mainly in the rostral portion), 8 units in medial vestibular nucleus neurons, while the remaining were located in the descending vestibular nucleus. The identification of the vestibular units as projecting to the spinal cord was obtained by stimulating the vestibulospinal tract at C2-C3 segmental levels. In particular, 25 of the 90 vestibular complex units (28%) were identified as vestibulospinal neurons. The conduction velocity of the corresponding axons, calculated on the basis of the antidromic activation latency of vestibular (mainly Deiters) neurons (between 0.5 and 1.1 msec), ranged from 50 to 70 m/sec.

At rest 71 vestibular units fired spontaneously with a frequency of 5-80 imp/sec and the average calculated in 10 units was 19.3 imp/sec ± 8.4, SD; the remaining 19 units were silent and were evoked by the stimulation of the cortical areas.

2. *Pericruciate area stimulation* . — As shown in Fig. 1 B, the major sites which, at T stimulation values, modulated the vestibular neuronal activity corresponded to the neck-back (squares), shoulder (dots) and elbow-wrist (triangles) cortical areas, both ipsi- and contralateral. The other areas (hindlimb, forelimb digit, face) required higher intensity values of stimulation of 4-5 shock trains; since the resulting responses were not constant, they were excluded from the analysis.

The activity of the vestibular units could be clearly affected by the stimulation

of neck-back, shoulder and elbow-wrist cortical areas. The units were subdivided in two main categories: 1) «modulated-response units», which were either activated or inhibited by cortical stimulation and showed either a variable latency (>10 msec) or a fixed latency (<10 msec); 2) «evoked-response units», which were driven with a fixed latency. The effects of stimulation of the above reported cortical areas on the discharge of 90 vestibular complex neurons have been reported in Fig. 2.

3. *Modulated-response units.* — A total of 34 vestibular units (37.8% of the total population) were modulated by the stimulation. The most efficacious cortical areas, in a decreasing order, were the elbow-wrist, the shoulder and the neck areas which respectively affected 41.6%, 37.5% and 31.8% of the 34 vestibular units (Fig. 2). Among these units, 13 (14.4% of the total 90 units) were identified as spinal projecting neurons. It can be noted from Fig. 2 that the vestibular units presented a better responsiveness to elbow-wrist areas, but were only in a small percentage (8.3%) of cases identified as vestibulospinal neurons. Almost all (6 out of 7) units responsive to neck area stimulation were identified antidromically. The modulation presented latencies ranging between 10 and 100 msec, consisted predominantly of an increase of frequency rate up to 60% of control value and lasted 100-500 msec. Inhibition could produce a large firing depression (about

	Modulated	Identified	Evoked	Identified	No Response
Elbow-Wrist	41.6 %	8.3 %	13.9 %	11.1 %	44.5 %
(tested units: 36)	(15)	(3)	(5)	(4)	(16)
Shoulder	37.5 %	12.5 %	18.5 %	15.6 %	43.8 %
(tested units: 32)	(12)	(4)	(6)	(5)	(14)
Neck	31.8 %	27.3 %	36.3 %	13.6 %	32 %
(tested units: 22)	(7)	(6)	(8)	(3)	(7)
Total	37.8 %	14.4 %	21.1 %	13.3 %	41.1 %
	(34)	(13)	(19)	(12)	(37)

Total 90 tested vestibular units

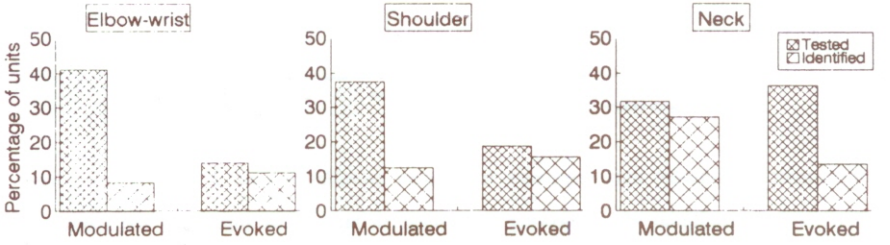

Fig. 2 – *Summary of data.*

The table summarizes the effects of the stimulation of elbow-wrist, shoulder and neck cortical areas. The analyzed units are divided into modulated, evoked and not responsive units (no response). As regards to the responsive units the number and percentage of antidromically identified units is reported.

The histograms report the distribution of the responsive (narrow cross-hatch bars) and the identified (large cross-hatch bars) units, according to the three stimulated cortical areas.

100%), lasting 50-100 msec after the train. The inhibition was usually followed by an excitatory rebound (50% increase of resting value), which could last up to 400 msec.

The stimulation parameters necessary to elicit the unit modulation were, at least partly, determined by the side of stimulation. Ipsilateral cortical areas induced more frequently, and at T values, an increase of unitary discharge, whereas inhibitions were more often induced by contralateral stimulations, which required 1.5-2 T stimulation values to clearly modulate the unit discharge. In some instances, cortical stimulation in the elbow-wrist and shoulder areas needed four shock trains at T values to obtain a unitary modulation similar to that obtained by the ipsilateral neck and back areas.

4. *Evoked-response units.* — This category includes responses evoked by cortical stimulation with fixed latency values lower than 10 msec. As shown in Fig. 2,

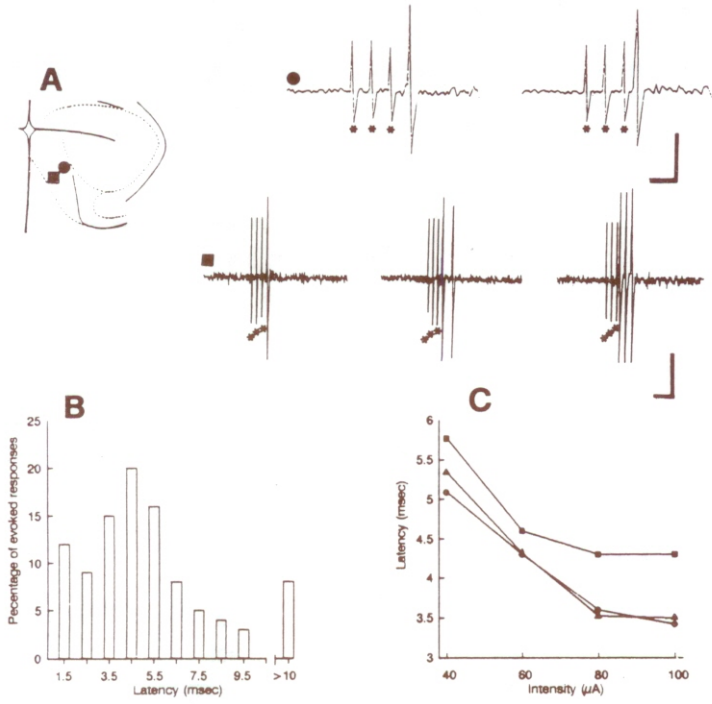

Fig. 3 – *Short-latency vestibular unit responses to cortical stimulation and latency/intensity ratio.*

A: two short-latency vestibular units showing a decrease in latency by increasing the stimulus current intensity applied to neck cortical area (filled dot) and to ipsilateral shoulder area (filled square). The neck evoked unit (dot) shows a decrease in latency at 2 T stimulation. In the neck-back unit (square) the increase of stimulus elicits at 2 T a second spike and at 3 T a third spike. Asterisks indicate stimulus artifacts. Calibration: upper unit, amplitude 1 mV, time 5 msec, lower unit: amplitude 1 mV, time 10 msec.

B: distribution of response latencies of 19 out of 34 tested LVN units, 12 of which were identified as vestibulospinal neurons.

C: plot of the latencies of three evoked LVN units (square: shoulder unit, triangles and dots: neck units) versus the stimulus intensity.

a smaller percentage of units presented evoked responses following cortical stimulation. The neck region of the explored cortex was the most effective in producing evoked responses of vestibular units (36.3%); some of these units (13.6%) were identified as vestibulospinal neurons.

Evoked responses were also observed following stimulation of the shoulder (18.5%) and elbow-wrist (13.9%) areas. In the majority of cases the evoked responses were observed following the third shock of the stimulation train, in some cases the response was evoked by the second shock. These responses were evoked mainly in silent units and the threshold for eliciting these effects generally ranged from about 60 μA to 90 μA (three shock train). The distribution of evoked response latency measured with respect to the third shock is shown in Fig. 3 B. The distribution is unimodal, with a mean at 4.6 msec ± 2.05, S.D. and a range from 1.5 to 9.5 msec. Only a few units showed latencies equal to or higher than 10 msec. Fig. 3 A illustrates the behaviour of two representative neurons to increasing stimulation values, both neurons being evoked by the stimulation of the ipsilateral neck area. The neuron on the top shows a decrease in latency from 3.5 msec to 2.4 msec, while the neuron on the bottom responded at T value with a single spike and then with two and three spikes, while the latency of the first spike decreased from 3.1 msec to 1.8 msec. Further increase in intensity did not further modify latency and discharge of these units. Fig. 3 C illustrates the ratio latency/intensity in three vestibular neurons; increasing intensity of stimulation by steps of 20 μA decreased the latency to a given value, which remained unaltered with further intensity increase.

DISCUSSION

The experiments of the present study were undertaken to investigate the effects elicited by discrete stimulations of precise sites of cortical motor areas onto LVN neurons. The selective stimulation of cortical motor areas, based on the existence of the fine somatotopic organization of the cat sensorimotor cortex (18), was carefully checked and the efficacy and specificity of stimulation was determined in each animal after implantation and upon recovery from anesthesia. The extracellular activity of LVN neurons was recorded without disrupting the physiological cerebellar connections of the vestibular system (21).

The results obtained confirm previous findings (14, 22) and provide some additional evidences. Only given cortical areas induced specific modification of LVN neuronal activity: the cortical areas controlling axial, proximal and forelimb muscles showed strict relationship, as documented by the responses at threshold stimulation values and by the possibility to obtain responses both by the ipsilateral and/or the contralateral cortical areas. The effects were characterized by long-latency modulation (excitation, inhibition followed by excitatory rebound) in the majority of units and they were mainly obtained following stimulation of the forelimb area. However, in a smaller significant group of units tested, partly identified as vestibulospinal projecting neurons, the cortical stimulation induced a short-

latency firing. In this case neck and ipsilateral shoulder areas were mainly implicated.

Among the vestibular units responding at short latency, some responded at values suggesting a monosynaptic link between cortex and the vestibular nucleus. This effect was recorded in neck and shoulder responsive units. Other units, however, responded with latencies higher than 5 msec. This finding supports the results of previous studies (11) and can be attributed to activation of corticospinal neurons which give collaterals to precerebellar structures (nucleus reticularis tegmenti pontis -NRTP, nucleus reticularis lateralis -NRL and inferior olive -IO), thus influencing the discharge of vestibular nuclear neurons through the cerebellar loop, as postulated by previous authors (7, 21).

It is of interest that short-latency responses were evoked mainly in silent units. In this instance, by increasing the intensity of stimulation, two findings occurred at vestibular unitary level: i) the reduction in latency of the response; ii) the increase in number of induced spikes. Intensity increase could lead to direct rather than trans-synaptic activation of cortical efferent cells (10), as well as to recruitment of adjacent neurons due to spread of current. In our experiments, appropriate cathodal stimulation was used to avoid trans-synaptic excitation (18). The reduction in latency observed in evoked-response units (about 2 msec) with stronger stimuli could then be attributed to direct activation of cortical efferent cells or their axons. This would also lead to an increased discharge of the precerebellar structures which would act through the cerebellar loop (6, 7) on vestibulospinal neurons determining an increase in their discharge.

In conclusion, the influence of the pericruciate area on vestibular nuclear neurons confirm the results of a previous study (22) and strongly supports the fact that the vestibular structures are involved in the postural adjustments associated with a cortically-induced distal movement. The effects described in the present work could then provide the mean of gating the central postural command as suggested by other authors (13, 16).

SUMMARY

Selected areas of sensorimotor cortex were stimulated with short trains adequate to evoke focal movements. Neurons belonging to the vestibular nuclear complex, including the lateral vestibular nucleus (LVN), were mainly affected by the stimulation of wrist-elbow, shoulder and cortical areas. The units responded to cortical stimulation either with a constant latency lower than 10 msec (short-latency units), or with variable latencies higher than 10 msec (long-latency units). Particular attention was paid to the first group of responses showing the involvement of direct and/or oligosynaptic facilitatory inputs from the cortical cells. Moreover, many of these responsive vestibular nuclear neurons were identified as projecting to the spinal cord.

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REFERENCES

1. Belenkyi, V. E., Gurfinkel, V. S. and Paltsev, E. I. On elements of control of voluntary movements. *Biofizika*, **12**: 135-141, 1967.
2. Cordo, P. J. and Nashner, L. M. Properties of postural adjustments associated with rapid arm movements. *J. Neurophysiol.*, **47**: 287-302, 1982.
3. Crenna, P., Frigo, C., Massion, J. and Pedotti, A. Forward and backward axial synergies in man. *Exp. Brain Res.*, **65**: 538-548, 1987.
4. Diener, H. C. and Dichgans, J. On the role of vestibular, visual and somatosensory information for dynamic postural control in humans. Pp. 253-262. In: Pompeiano, O. and Allum, J. H. J. (Eds.), *Progress in Brain Research*. Vol. 76. *Vestibulospinal Control of Posture and Locomotion*. Amsterdam, Elsevier, 1988.
5. Draicchio, F, Celestini, E., Gallo, M. T. and Troiani, D. Implicazione delle vie vestibolospinali negli aggiustamenti posturali. *Boll. Soc. ital. Biol. sper.*, Pisa 37° Congr., 1985.
6. Fukushima, K., Takahashi, K., Ohno, M. and Kato, M. Responses of cat vestibular neurons to stimulation of the frontal cortex. *Exp. Brain Res.*, **56**: 275-278, 1984.
7. Gahery, Y. and Pompeiano, O. Neck influences on posturokinetic responses to cortical stimulation. Pp. 181-192. In Pompeiano, O. and Allum, J. H. J. (Eds.), *Progress in Brain Research*. Vol. 76. *Vestibulospinal Control of Posture and Locomotion*. Amsterdam, Elsevier, 1988.
8. Hassler, R. und Muhs-Clement, K. Architektonischer Aufbau des sensomotorischen und parietalen Cortex der Katze. *J. Hirnforsch.*, **6**: 377-420, 1964.
9. Horak, F. B., Nashner, L. M. and Diener, H. C. Postural strategies associated with somatosensory and vestibular loss. *Exp. Brain Res.*, **82**: 167-177, 1990.
10. Jankowska, E., Padel, Y. and Tanaka, R. The mode of activation of pyramidal tract cells by intracortical stimuli. *J. Physiol., Lond.*, **249**: 617-636, 1975.
11. Keizer, K. and Kuypers, H. G. J. M. Distribution of corticospinal neurons with collaterals to lower brain stem reticular formation in cat. *Exp. Brain Res.*, **54**: 107-120, 1984.
12. Keshner, E. A., Allum, J. H. J. and Pfaltz, C. R. Postural coactivation and adaptation in the sway stabilizing responses of normals and patients with bilateral vestibular deficit. *Exp. Brain Res.*, **69**: 77-92, 1987.
13. Lestienne, F. and Droulez, J. Les problèmes sensorimoteurs en apesanteur. *Rev. Med. Fonctionelle*, **16**: 195-225, 1983.
14. Luccarini, P., Gahery, Y., Blanchet, G. and Pompeiano, O. GABA receptors in Deiters nucleus modulate posturokinetic responses to cortical stimulation in the cat. *Arch. ital. Biol.*, **130**: 127-154, 1992.
15. Massion, J. Postural changes accompanying voluntary movements. Normal and pathological aspects. *Human Neurobiol.*, **2**: 261-277, 1984.
16. Massion, J. and Viallet, F. Posture, coordination, mouvement. *Rev. Neurol., Paris*, **10**: 536-542, 1990.
17. Nardone, A. and Schieppati, M. Postural adjustments associated with voluntary contraction of leg muscles in standing man. *Exp. Brain Res.*, **69**: 469-480, 1988.
18. Nieoullon, A. and Rispal-Padel, L. Somatotopic localization in cat motor cortex. *Brain Res.*, **105**: 405-422, 1976.
19. Pompeiano, O. The role of different size vestibulospinal neurons in the static control of posture. *Arch. ital. Biol.*, **129**: 21-41, 1991.
20. Rispal-Padel, L., Troiani, D. and Harnois, C. Converging cerebellofugal inputs to the thalamus. II. Analysis and topography of thalamic EPSPs induced by convergent monosynaptic interpositus and dentate inputs. *Exp. Brain Res.*, **68**: 59-72, 1987.
21. Sarkisian, V. H. and Farnadjian, V. V. Neuronal mechanisms of the interaction

of Deiters' nucleus with some brainstem structures. *Neuroscience*, **16**: 957-968, 1985.
22. SARKISIAN, V. H. and FANARDJIAN, V. V. Neuronal mechanisms of interaction of Deiters' nucleus with the cerebral cortex. *Arch. ital. Biol.*, **130**: 113-126, 1992.