
RESPONSES OF THE RED NUCLEUS NEURONS TO STIMULATION OF THE PAW PADS OF FORELIMBS BEFORE AND AFTER CEREBELLAR LESIONS

Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland
*Department of Human Physiology, University School of Medicine, Lublin, Poland

Cerebellar cortex ablation releases deep cerebellar nuclei of monosynaptic inhibition from Purkinje cells. Therefore, it strengthens excitatory influence from Interpositus Nucleus (IN) upon Red Nucleus (RN), which results in much higher facilitation of the rubro-spinal neurons. This causes a big increase of spontaneous discharge rate, and eliminates brakes of discharges from responses generated by somatosensory stimuli. These two changes destroy content and timing of feedback information flowing through the spino-cerebello-rubro-spinal loop. This false bias of the feedback information, very important for fast postural adjustment and coordination of ongoing movements executed by central motor program, may at least in part be responsible for abnormal motor behavior evoked by cerebellar damage. Hemicerebellectomy resulted in dramatically reduced spontaneous activity and responses to limb stimulation because of severing a major input to the red nucleus from deep cerebellar nuclei. Due to direct somatosensory input to magnocellular Red Nucleus (mRN) from the spinal cord that bypassed the cerebellum, the latency of response to limb stimulation was not changed and the narrower receptive fields were still present.

Key words: red nucleus neurons; Cerebellar lesions; Response patterns.

INTRODUCTION

It is generally accepted that the two-way communication between sensory and motor systems is essential for normal motor behavior. One of the important loops subserving this communication is the spino-cerebello-rubro-spinal projection system. The feedback control of an evolving movement is provided by the cerebello-rubro-spinal loop. The loop time is approximately 20 to 30 ms (1) and it is linked to the musculature by the rubral projection to spinal motoneurons.

A comparative study of the mammalian red nucleus shows that it is composed of two parts: a caudally situated magnocellular part and a more
rostrally located parvocellular part. These two parts have different input and output connections. The parvocellular part receives main input from the ipsilateral cerebral cortex and its sole projection is to the ipsilateral inferior olive. On the other hand, the magnocellular part receives afferents mainly from the nucleus interpositus (IN) of the cerebellum (2) and gives rise to the rubrospinal tract, which projects contralaterally to several brain stem nuclei and to neurons in the spinal cord, mainly monosynaptically to the distal forelimb motoneurons (3). In agreement with various afferent and efferent connections of these two parts of the red nucleus, their functional role in motor control appears to be distinct. Microelectrode recordings from parvocellular red nucleus reveal that its neurons respond very weakly to sensory and motor stimuli, and very little is known about the participation of parvocellular red nucleus in organization of motor behavior. In contrast, microelectrode recordings made in the magnocellular red nucleus (mcRN) have shown that these units respond intensively to sensory and motor stimuli and that their motor fields are restricted to a single contralateral limb, whereas sensory receptive fields are wide and distributed bilaterally (4). The intense response of the neurons to variety of sensory and motor stimuli pointed out to the important functional role of mcRN in loops controlling limb movement. These loops interconnecting motor cortex, red nucleus and cerebellum distribute motor commands in the limb premotor network (5).

A number of hypotheses concerning the mode of cerebellar movement control have been proposed (6), and the data concerning projections from the cerebellar subcortical nucleus to the red nucleus, and also projections from the cerebral cortex and other brain stem structures are available (7). A synthesis of the combined results from behavioral and electrophysiological experiments leads to a hypothesis of the cerebellar control of movement by modulation of the proprioceptive brake (8). Little attention, however, has been given to an influence on reactivity of the mcRN neurons when the proprioceptive transmission in the spino-cerebello-rubro-spinal loop is deprived of selective inhibitory influence from cerebellar cortex. The purpose of the present study is to analyze and compare the response patterns evoked by afferent volleys generated by forelimb stimulation of mcRN neurons receiving projection from the intact nucleus interpositus with mcRN neurons getting projection from the interpositus nucleus deprived of cerebellar input, and finally when projection from interpositus nucleus has been disrupted by hemicerebellectomy. This was accomplished by an analysis of poststimulus time histograms constructed from spike trains simultaneously recorded from neurons of the normal and cerebellectomized red nucleus by acute unilateral cerebellar cortex lesion and hemicerebellectomy.
MATERIAL AND METHODS

This study was carried out on adult male cats that were purchased from authorized supplier and housed in the laboratory. All animals were premedicated with Atropine 0.2 ml and Ketamine 20 mg/kg and then kept under alpha-chloralose (initial dose 50 mg/kg). The cannulation of trachea and femoral vein were made for respiration and drug infusion, respectively. Animals were paralyzed with gallamine triethiodide and artificially respired. Throughout the experimental session, mixture of glucose/chloralose/flaxedil (5 mg/kg/h chloralose, 10 mg/kg/h flaxedil) diluted in Ringer solution was constantly infused. Body temperature was monitored and maintained at around 37°C. Expired CO₂ was monitored and kept at 4.5% by adjustments of respiratory volume. A blood pressure level was monitored and maintained within physiological limits. Before surgery, animals were mounted in a Horsley-Clarke frame that carried micromanipulators for the recording electrodes.

Electrical stimulation was applied to the paw pads of forelimbs via needle electrodes in order to test the effects induced in mcRN neurons by peripheral afferent volley before and after cerebellar lesions. For the stimulation the current pulses of 0.8—1.5 mA, 0.5 ms duration with a repetition rate of 0.5 Hz were used. Poststimulus histograms were constructed from 64 repetitions of stimulation. To determine the localization of the microelectrodes within red nuclei the focal potentials and antidromic responses of neurons were recorded. For this purpose two bipolar nickel-chrome electrodes varnished except at their tips (resistance 65—90 kOhms) were placed stereotaxically through trephine holes into spinal cord at C₂—C₃ levels. Electrodes were cemented to the bone. For stimulation, three shocks of 0.2 ms, 100/sec., and 130—160 µA were used. The criteria for antidromic activation were: short latency, ability to follow repetitive stimulation (3 pulses at 100 Hz) and collision of spontaneous with electrically evoked spikes. Stimulations were applied by means of a Grass S8 stimulator with constant current unit model CUU-1A. Mechanical stimulations such as light taps on the skin, air puffs, and joint angulation, squeezing and stretching of muscles were applied to coarsely inspect the cells receptive fields.

Conventional multiple unit recordings with metal microelectrodes (resistance: 2.5—4.5 MΩ), mounted on two separate independently driven micromanipulators were used. Two microelectrodes were fixed in each micromanipulator with the tips 350—500 µm apart. Microelectrodes were inserted at the same coordinates into the left and right mcRN. The penetrations were performed between Horsley-Clarke frontonal planes A 3.0 and A 6.0, laterally from L 1.5 to L 3.0 and vertically from H-1 to H-3.5 thus exploring the total extent of the magnocellular division of the red nucleus. It was usually possible to record impulse discharges of 2 to 3 cells from one microelectrode which were isolated well enough to be an unambiguously segregated for further processing. Impulse discharges were amplified and processed on line by a specialized analyzer (ANOPS-105) to provide computation and display of poststimulus and interval histograms. Data were also stored in an analog form on a magnetic FM/AM type recorder (RACAL STORE PLUS VL). Detailed data processing was performed off line with specialized program-STAT implemented on IBM PC computer.

Amplified analog spike waveforms stored on magnetic type have been processed with 12-bit resolution. Spikes were sorted with a software amplitude-time sensitive window discriminator enabling separation of two to three spikes on a single microelectrode. Poststimulus time histograms (PSTHs), interval histograms (IHs), and autocorrelograms (ACs) with high (bin width 0.3 ms, 150—500 ms window) and low (bin width 4 ms, 2000 ms window) time resolution were computed for all neurons. The statistical significance of firing synchronized by stimulation, indicated as a peak in the PSTH was inspected. The attenuation, augmentation and the inhibition of the firing rate provoked by limb stimulation were inspected as deviations from the mean value in the PSTH.

Two kinds of lesions were performed. In the first group, the cerebellar cortex of the right cerebellar hemisphere was extracted by aspiration. Great care was taken to remove only the cortex
and preserve the white matter containing the deep cerebellar nuclei. In the second group, the whole hemicerebellum was sucked out. Care was taken not to injure the extracerebellar structures. The operations were always confined to the right side of the cerebellum. Following ablation, all wounded places were covered with spongostan (Ferrosa, Denmark) and the animals allowed to recover from surgery for 1—2 h. At the termination of the experiment, the sites of recording were marked by electro-coagulation. Animals received an overdose of anesthetic, and the brains were perfused with 10% formaldehyde. The extent of the cerebellar lesions was verified visually and then photographed. Microelectrode placements in the red nuclei and the extent of the cortical lesions were verified histologically on frozen 50 μm thick sections stained with Nissl method. In cats with cerebellar cortex ablated, lesions were also verified with computer reconstruction from histological slices.

RESULTS

One thousand twelve cells were recorded from magnocellular red nucleus. These data were broken down as follows: 626 cells were from both red nuclei of the intact cats, and 386 cells from the same cats submitted to cerebellar lesions. From the sample of 386 neurons, 215 cells were recorded in the animals in which cerebellar cortex of the right hemisphere was removed, and 171 cells in the animals in which right-side hemicerebellum was removed. After electrophysiological identification of the recorded neurons as described in METHODS, spontaneous firing was recorded for three to five minutes, and then the response patterns to afferent volleys generated by repetitive stimulation of the paw pads were examined by means of IHs, AHs, and PSTHs. *Fig. 1.* Neurons recorded in the rostral parvocellular division (113 cells), reacting erratically to sensory stimulation, not excited antidromically from spinal cord, whose projections were not further checked, are not included in this report.

*Response patterns of mcRN neurons in the intact animals*

From the 626 units sampled from the mcRN, 475 (76%) cells were antidromically activated by stimulation of the contralateral halves of the spinal cord. The mean latency of antidromic invasion from C₂—C₃ spinal level was 1.5 ms (SD = ±0.2 ms). The remaining 151 cells (24%), which were recorded from caudal, magnocellular part of the mcNR and not activated antidromically from the spinal cord level, were classified tentatively as interneurons. The majority of the cells recorded in mcRN were spontaneously active; about 10% of cells were silent but could be activated by peripheral stimulation *Fig. 1 A-C.* Although both antidromically activated units and those not activated displayed variable spontaneous firing, the consistent differences emerged in the distribution of their firing rates (9.4—44.8 imp/s).
Fig. 1. Interval histograms (IH), autocorrelograms (AC) and poststimulus time histograms (PSTHs), showing the spontaneous discharges and typical response patterns recorded from the contralateral mRN to stimulation of forelimb in intact cat are shown. A-IH of spontaneous firing, B-AC of spontaneous firing, C-IH during forelimb stimulation, D-AC during forelimb stimulation (records from the same neuron). E to H PSTHs represents standard response patterns to forelimb stimulation recorded from four different neurons. Note the brake of discharges after phasic peak-response on PSTHs; the brake is also well seen on raster display presented below PSTHs. In Figs. E, F, neurons activated antidromically from C₂ level. In Fig. G, H, neurons, which were not antidromically activated from the spinal cord. In this and following Figs. the abbreviations are as follow: horizontal scale — time in milliseconds, the time resolution of AC histograms is 1 ms/bin, of the PSTHs histograms 4 ms/bin, vertical scale — number of occurrences per bin. Solid horizontal line indicates the mean value, dotted lines indicate the level of ±SD.
The neurons were divided into two main types, depending on whether the response to the peripheral stimulation started from excitation or inhibition of their activity Fig. 1. The first type of responses to volleys generated by electrical stimulation of the paw afferents consisted in the fast elevation of discharge rate occurring as the excitatory peak in the PSTH at different latencies relative to the onset of stimulation pulse. Cells activated antidromically from contralateral spinal cord increased their firing rate as early as 16 ms (from 13 to 18 ms) while those not driven antidromically as late as 24 ms (from 23—32 ms). The excitatory peak lasted for 52—86 ms and was followed by arrest of firing lasting for 168—450 ms. After the latter phase the rebound reaction occurred which lasted for 235—590 ms and terminated with the return to spontaneous rate of discharge Fig. 1E-F. From 475 cells driven by antidromic stimulation, 81% of cells were also activated by stimulation of the ipsilateral paw with latency that was 2 to 4 ms longer. This response was usually weaker but the sequence of events remained similar.

The second type of neuron responses to limb stimulation started with suppression of firing occurring with latency of 27 to 46 ms which lasted 114 to 150 ms. It was followed by excitatory rebound with duration of 300 to 400 ms which terminated with the return to the level of spontaneous firing rate. Large number of these cells (47 neurons) was not activated antidromically from spinal cord level. A small population of cells (7%) responded with different patterns to afferent volleys from contra- and ipsilateral paw. The responses to ipsilateral paw stimulation began with arrest of firing which showed latency from 34.4—78.0 ms, lasted 82—150 ms and then returned to spontaneous firing rate. The responses to stimulation of the contralateral paw also started with arrest of firing occurring at the same range of latencies but were followed by excitatory rebound lasting from 156 to 350 ms. Fig. 1G-F. The remaining 12% of neurons (with response patterns described above) responded only to stimulation in accordance with the contralateral projection to the red nucleus.

The majority of the neurons (75%) could be driven by mechanical stimuli of various modalities but habituated very fast to quickly repeated stimuli. They also had wide, two-side receptive fields. The modality-specific units, activated by joint rotations only (15%) exhibited excitatory receptive fields limited to one or two joints of the same limb, and often showed inhibitory receptive fields from the opposite limb or angulation. However, the reaction to touch of these units could not be excluded. The remaining 10% of units were activated or inhibited depending on the direction of joint movements. These reactions were only observed after habituation to mechanical stimuli.
Response patterns of mcRN neurons recorded after ablations of the cerebellar cortex

In these experiments, we tried to determine the extent to which the ablation of the cerebellar cortex modified the spontaneous excitatory transmission from Nucleus Interpositus to mcRN and the response patterns of mcRN units to forelimb stimulation. After completion the recording session on intact cats, each animal was subjected to the removal of the cerebellar cortex on the right side. Unilateral lesion, which left the opposite cortex intact, allowed us to consider the right-side mcRN neuron responses as the control ones. The extent of cerebellar cortex lesions is shown in Fig. 2. From 215 units recorded after cortex ablation, 175 were from left mcRN. They received projection from deep cerebellar nuclei and were deprived of input from cerebellar cortex. The remaining 40 units were from the left mcRN. In general, the characteristics of these 40 neurons, their spontaneous firing rate and their response patterns to peripheral stimuli were not influenced by ablations of contralateral cerebellar cortex. However, 24 of these units, in contrast to weaker responses evoked by stimulation of ipsilateral forelimb in animals with intact cerebellar cortex, displayed responses of equal intensity independent of stimulation of the ipsi- or contralateral limb. From 175 units recorded in the left mcRN, 87 units passed antidromic tests. In 87 of all the rubrospinal cells tested, the spontaneous firing rate was higher in comparison to units recorded simultaneously from left hand side mcNR units. These rubrospinal cells during stimulation and the first 10 minutes after termination of stimulation had a firing rate ranging from 42 to 90 imp/s. When left without stimulation for longer time they started firing with rhythmically occurring trains of spikes Fig. 2 B. The trains consisted of 12 to 30 spikes with interspike intervals of 11 to 16 ms and intertrain intervals lasting from 124 to 306 ms as measured from the first spike of each burst. Because of this rhythmic, oscillatory-like pattern of the spontaneous firing, the fluctuation of response intensity at the beginning of forelimb stimulation was pronounced. The latency of the responses to limb stimulation was not changed. However, the responses were stronger then those occurring in animals with intact cerebellar cortex. The first component of these responses lasted longer (from 87 to 124 ms) and consisted of two to four spikes with interspike intervals shorter than those occurring in burst firing during spontaneous activity Fig. 2 C. These multiple peak responses were followed by quick return to the level of spontaneous firing rate or barely pronounced attenuation of firing rate Fig. 2 C. The time course of this weak attenuation of firing was shorter than this occurring before cerebellar cortex lesion. Several minutes (3—5 min.) after termination of peripheral stimulation, the burst discharges started again Fig. 2 B. The receptive fields to mechanical stimuli were wider than before cerebellar cortex ablation and were distributed bilaterally.
Fig. 3. A — macro photograph and histological slices documenting complete ablation of the right side hemicerebellum. B — four PSTHs representing response patterns of rubro-spinal neurons recorded from RN contralateral to the removed right side of the hemicerebellum. C — control PSTHs of two different neuron responses to forelimb stimulation recorded from RN ipsilateral to extracted right hemisphere of cerebellum. C-1 response pattern of neuron, which was not excited antidromically by spinal cord stimulation. C-2 response of neuron excited antidromically by spinal cord stimulation at C₂ level.
Response patterns of mcRN neurons recorded after hemicerebellectomy

In this group, the right side hemicerebellum was removed Fig. 3. One hundred seventy one neurons from the contralateral mcRN deprived of cerebellar input were analyzed for response patterns and receptive field properties. Neurons could be divided into two groups based on their response patterns to limb stimulation. The first group, which included 120 neurons (70%), was excited antidromically by electrical stimulation of the spinal cord. Seventy-eight neurons (65%) were silent, and the remaining units displayed very low frequency of spontaneous discharge rate (from 0.5 to 1.8 imp/sec). The responses to forelimb stimulation were simple, consisted of short burst of spikes and showed latency distribution similar to that appearing in intact animals Fig. 3 B.

The remaining 51 units (30%) studied made up the second group. These units were not excited antidromically by electrical stimulation of the spinal cord at C_2 – C_3 level. They displayed spontaneous activity with rather high frequency of discharge. The responses of 18 units to limb stimulation started with attenuation of firing rate which had latency of 32 – 56 ms and lasted for 50 – 120 ms. The remaining 33 neurons showed the response pattern consisting of excitation with latency ranging from 24 to 35 ms which was followed by silent period lasting 100 to 250 ms and terminating with the return to spontaneous firing rate or to the excitatory rebound. The responses of this group of neurons resembled those presented in Fig. 1 G-H.

Neurons from the first group were less sensitive to mechanical stimuli like light touch. Responses evoked by sensory stimuli were phasic and had a form of brisk burst of spikes. Sensory receptive fields to mechanical stimuli were bilateral, very narrow, and quickly habituated; it was often difficult to define border of each of the receptive field. Responses to joint angulation were also phasic and often could be evoked from more than one joint.

DISCUSSION

The results of the present study indicate that ablation of the cerebellar cortex which eliminates inhibition exerted by cerebellar Purkinje cells upon IN neurons, also removes brakes occurring between trains of somatosensory discharges rhythmically generated by the ongoing movement in IN cells. Elimination of these brakes causes: 1) a flow of the constant stream of afferent discharges abnormally facilitating rubro-spinal neurons, 2) in this way it increases very much the sensitivity of these neurons to afferent impulses which evoke exaggerated responses, 3) this, in turn, produces a flow of false information about the actual posture, 4) and also of sensory information used
for fast posture adjustment. These new sets of misleading information disorganize motor behavior executed by central motor program.

Neurons of the cerebellar nuclei fire spontaneously both in vitro, with synaptic transmission blocked, and in vivo, in resting animals, despite ongoing inhibition from spontaneously active Purkinje neurons (9). The red nucleus receives powerful excitatory input from the contralateral interpositus nucleus of the cerebellum which converges on the same neurons as inputs from the motor cortex and other brain stem structures (10, 11, 12) In chloralose anesthetized and curarized cats mcRN units also are spontaneously active within the wide range of the discharge frequencies. The studies with intracellular recording techniques show different modes of action of cerebral and cerebellar inputs upon the RN neurons. Sensorimotor cortex exerts a monosynaptic excitatory action through cortico-rubral fibers upon the remote dendrites of RN neurons while the excitatory impulses from the nucleus interpositus of the cerebellum impinge on or near the soma (13). Excitation of cerebellar cortex by sensory stimuli produces the following sequence of events in the deep cerebellar nuclei: monosynaptic inhibition via axons of Purkinje cells, disinhibition due to basket and stellate cell inhibition of Purkinje cells, and tonic monosynaptic inhibition related to the spontaneous activity of Purkinje cells. Monosynaptic inhibition of IN neurons produces disfacilitation of mcRN neurons (10). Since the anatomical data correlate well with close functional relationship between cerebellar cortex, nucleus interpositus and the red nucleus, the influence of the cerebellar cortex upon these structures is distinctly selective (14). Ablation of the cerebellar cortex abolishes much of inhibition of the IN neurons and results in considerable increase in their spontaneous discharge rate (15). In the present study, it was shown that the ablation of cerebellar cortex also results in increase in spontaneous discharge rate (up to 94 imp/sec) of many of mcRN neurons because of exaggerated excitatory action exerted by IN neurons released from Purkinje cells inhibition. This exaggerated excitatory level of mcRN correlates well with abnormal motor behavior (16).

From the above results it can be postulated that the fluctuating level of facilitation of the mcRN cells maintained by tonic impingement of excitatory postsynaptic potentials from IN, and regulated by inhibition exerted upon IN by spontaneously active Purkinje cells, is important in maintaining the normal posture of the resting animal.

Spontaneous activity of the mcRN neurons after cerebellar cortex ablation consists in short trains of spikes occurring with frequency of 3 to 5 per/s, and displays high discharge rate of intertrain spiking with frequency up to 90 spikes/s. This type of spontaneous firing may be tentatively related to not well-defined “rubral” tremor.

Release of IN neurons from monosynaptic inhibition exerted by Purkinje cells, due to extraction of cerebellar cortex, resulted in their higher tonic
excitability. Because of this, IN neurons are more sensitive to somatosensory stimuli of the same intensity. As a result, interposito-thalamo-cortical (17) and interposito-rubral information transmission is exaggerated. The greatly increased excitatory impulses tonically impinging upon mcRN produce far-reaching changes in the behavior of mcRN neurons. The cells in this nucleus were discharging at higher frequencies up to 94 imp/s with frequently occurring bursts-like discharges showing intra-burst frequency well over 110 imp/s. In response to limb stimulation the brake of discharge occurring after first phasic component disappeared, phasic responses were much stronger, and the rebound discharge lasted longer. Exaggerated responses to somatosensory stimuli, and the removal of the brake of information flow generated by ongoing movements, transmitted through the spino-cerebello-rubral loop are, at least in part, responsible for impairment of the motor behavior. In the cat, a common pathway sends somesthetic information to the cerebellum, the red nucleus and the motor cortex. The neuronal connections between the cerebral cortex and the cerebellum are considered to be reciprocal in higher vertebrates and to form a closed loop for regulation of the limb movement (18). The strong correlation of the IN and mcRN discharges with the velocity of movement is interpreted as generated by movement-related sensory feedback (1). Because the somatosensory responses still contain strong tonic and phasic components, it can be concluded that the inhibitory input to the rubro-spinal neurons is still in action. This conclusion is supported by the result of Tsukahara (13) who on the base of intracellular recordings in the cat concluded that rubrospinal neurons receive inhibitory input from collaterals of corticospinal tract axons that activate local mcNR GABA-ergic inhibitory interneurons (19). But this rather phasic inhibition is too weak to overwhelm very high, tonic facilitation of rubro-spinal neurons evoked by constant stream of excitatory afferent impulses from IN released from Purkinje inhibition.

The neural connections between the cerebral cortex and the cerebellum were considered to be reciprocal and to form a closed loop for regulation of the limb movements. Ablation of the cerebellar cortex disrupts reciprocal connections in this loop. This in the first step eliminates inhibition of IN neurons and in consequence the evoked abnormal facilitation of rubro-spinal cells. High excitation of mcNR neurons produces false, exaggerated information generated by ongoing movements, which disorganize posture and movements coordination.

Hemicerebellectomy eliminates the main stream of afferent impulses from deep cerebellar nuclei to rubro-spinal neurons. Severing this major source of tonic facilitation dramatically reduced the excitability of rubro-spinal neurons to afferent stimuli. It, also, considerably lowered the rate of spontaneous firing, sensitivity to peripheral stimuli, and decreased the size of their receptive fields.
The response to electrical stimulation of forelimb consisted only of short phasic response whose latency did not change. This response is evoked by volleys from direct spino-rubral pathways that bypass the cerebellum (20).

REFERENCES


Received: March 20, 2001
Accepted: July 9, 2001

Author's address: Prof. Remigiusz Tarnecki, Nencki Institute of Experimental Biology, 3, Pasteur Street, 02-093 Warszawa, Tel.: 0-22 822-27-26, Fax.: 0-22 822-53-42.
E-mail: rem@nenciki.gov.pl