SHORT-LATENCY RETICULOSPINAL SYNAPTIC PROJECTIONS ON α-MOTOR NEURONS

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Although it has been shown that in cats the reticulospinal fibers run directly to the motor and internuncial neurons of the anterior horns of the lumbar segments [8], the most direct synaptic influences of the reticular formation on the motor neurons have not yet been studied.

The investigation described below has shown that during stimulation of the reticular structures of the brain atom synaptic responses with a very short latent period may arise in the lumbar α-motor neurons and at least some of them are the result of the monosynaptic excitation of the motor neurons by reticulospinal impulses. The paper gives details characterizing these reactions and their differences from reticulospinal excitatory influences of a polysynaptic character and also indicates certain properties of reticulospinal postsynaptic inhibition.

EXPERIMENTAL METHOD

Experiments were carried out on cats anesthetized with Nembutal (30-40 mg/kg) and immobilized with Flaxedil. Intracellular recordings were made by the use of microelectrodes filled with 0.6 M K_2SO_4 or 3 M KCl solution and connected to a bridge circuit by means of which it was possible, along with recording the potentials, to change the polarization and to measure the resistance of the cell membrane [1].

The reticular formation was stimulated by stereotaxically implanted bipolar electrodes (diameter of points 50 μ and interpolar distance 0.2-0.4 mm), to which were applied rectangular pulses of current with a duration of 0.2-0.5 msec and a strength of 0.1-0.7 mA (increased in individual cases only 1-4 mA). The position of the point of the electrode was verified histologically in each experiment. Measurements of the gradient of the electric field around the stimulating electrode in the brain tissue showed that with a current of 0.7 mA activation of the nerve cells was possible within a radius of 1.1 mm. While accepting that such a large volume of tissue could be stimulated, histological analysis demonstrated that many points could be distinguished from which the remaining direct descending projections of the brain stem to the motor neurons could not be activated. To rule out completely the possibility that these formations could be stimulated, in 8 cats (of the 14 experimental animals), the homolateral nucleus of Deiters (in three cases completely) and the contralateral red nucleus (in four cases completely were destroyed 3-7 days before the experiment (see Fig. 1). The results of the histological analysis are summarized in Fig. 2A. In most cases the stimulating electrodes were located in the medial reticular formation (the caudal reticular nucleus of the pons, the gigantocellular reticular nucleus, the ventral reticular nucleus), i.e., in regions where the largest reticular neurons undergoing degeneration after injury to the spinal cord are situated [12].

EXPERIMENTAL RESULTS

Altogether 85 motor neurons responding to reticular stimulation were investigated. All the excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials evoked by stimulation of the reticular structures were subdivided into two groups: those arising in response to a single stimulus and those arising only in response to paired or rhythmic stimuli.

As Fig. 2B-D showed, the latent period of the synaptic reactions of the second group was much longer and varied within wide limits. In addition, the reactions were characterized by an irregular, variable form and often by a mixed (IPSP and EPSP) character of the response, typical of polysynaptic potentials.

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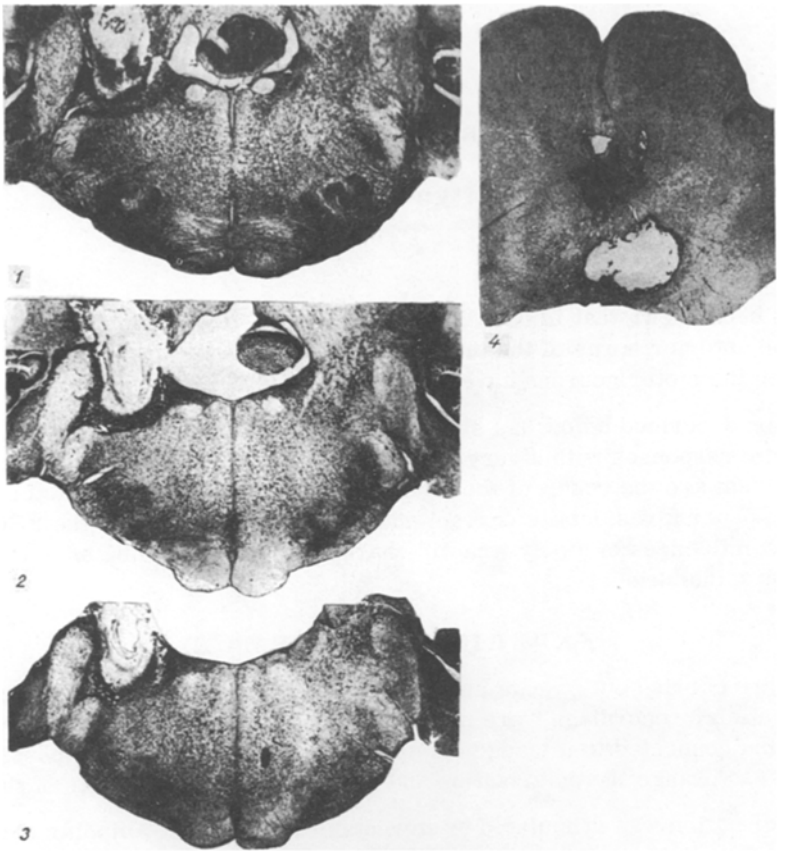

Fig. 1. Character of injury to the brain in experiments with reticulospinal stimulation. 1-3) Frontal sections through the medulla in a rostro-caudal direction, showing complete destruction of the nucleus of Deiters on the left side; 4) frontal section through the mesencephalon demonstrating total destruction of the red nucleus.

The EPSP and IPSP of the first group possessed a short latent period (amounting for the EPSP to 3.0-3.1 msec), varying comparatively little from one cell to another. Parallel recordings of the short-latency EPSP and the potentials on the dorsal surface showed that the latter appear 0.4-1.1 msec before the beginning of the intracellular reaction and, consequently, the EPSP corresponding to it may be regarded as the result of monosynaptic excitation of the motor neurons by reticulospinal impulses. Bearing in mind the extremely short latent periods of development of the dorsal wave (2.1-3.0 msec) the velocity of conduction along the fibers of the reticulospinal tract, exciting the lumbar motor neurons monosynaptically, must be 100-145 m/sec (in one case 165 m/sec), in agreement with existing data for the conduction velocity in the axons of the reticulospinal neurons [5, 13, 14]. The latency of the IPSP evoked by single reticular stimuli (about 6 msec or more) may indicate the existence of additional synaptic delays.

The short-latency EPSP was characterized by low amplitude (0.5-2.5 mV) and by a simple shape, similar to the monosynaptic EPSP of segmental origin or the "unitary" potentials of synaptic noise (Fig. 3A). They increased to a maximum in most cases during the first 1-2 msec (extreme values 0.7-3 msec). The time constant of decay, measured in 10 cells whose action potential had an amplitude of more than 55 mV was 3.87±0.74 msec (individual variations 2.85-4.9 msec). Soon after withdrawal of the microelectrode from the cell or death of the cell the EPSP disappeared or diminished sharply, without however changing their polarity. The intracellularly recorded potentials were thus not the result of distortions introduced by the extracellular electric field.

The minimal strength of the stimuli evoking short-latency EPSP was 0.1 mA. With an increase in the strength of stimulation, the amplitude of the responses rose without change of latent period or configuration of the EPSP, indicating the simple summation of homogeneous synaptic influences. With a consid-

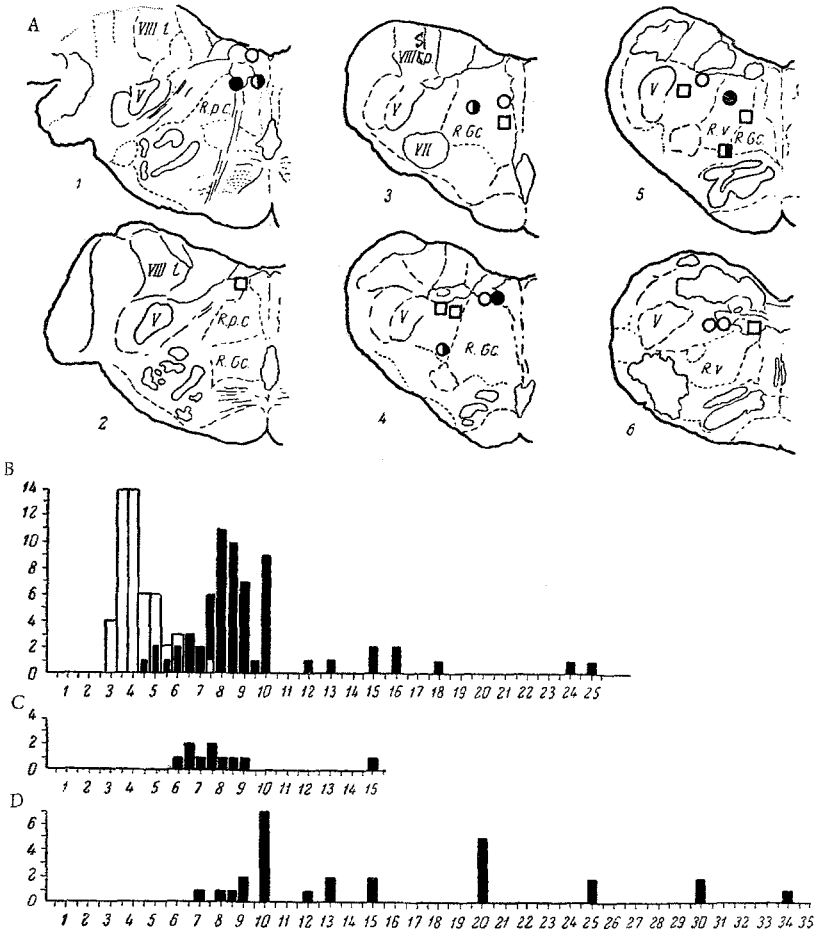

Fig. 2. Distribution of stimulated points (A) and of latent periods of the postsynaptic responses evoked by single and rhythmic stimuli (B-D). A—Sections through the pons and medulla in a rostro-caudal direction (1-6); circles — points from which short-latency responses were evoked by a single stimulus; squares — points responding only to rhythmic stimulation; half white circles and squares — experiments with total destruction of the red nucleus and the nucleus of Deiters; black circles and squares — experiments with destruction of the nucleus of Deiters only; white circles and squares — experiments on animals with an intact nervous system. VIII — lateral vestibular nucleus; R. Go — gigantocellular reticular nucleus; R.p.c. — caudal reticular nucleus of the pons; R.v. — ventral reticular nucleus; VIII sp — vestibular nucleus; VII — nucleus of nerve VII; V — nucleus of nerve V; B-D — histograms of distribution of the latent periods of the postsynaptic responses; B — latent periods of the EPSP evoked by single stimuli (white columns), and only by paired or rhythmic stimuli (black columns); C — latent periods of IPSP arising in response to single stimuli; D — to rhythmic stimuli; ordinate — number of neurons; abscissa — latent periods (in msec).

erable increase in the strength of stimulation (over 0.4-0.7 mA) later components (of polysynaptic type) began to appear (Fig. 3C). Only in one case was weaker stimulation followed by a response with a longer period and different shape by comparison with that appearing after an increase in the strength of stimulation (Fig. 3D). However, this result was probably attributable to the long distance between the stimulating electrode and the fast-conducting reticulospinal elements.

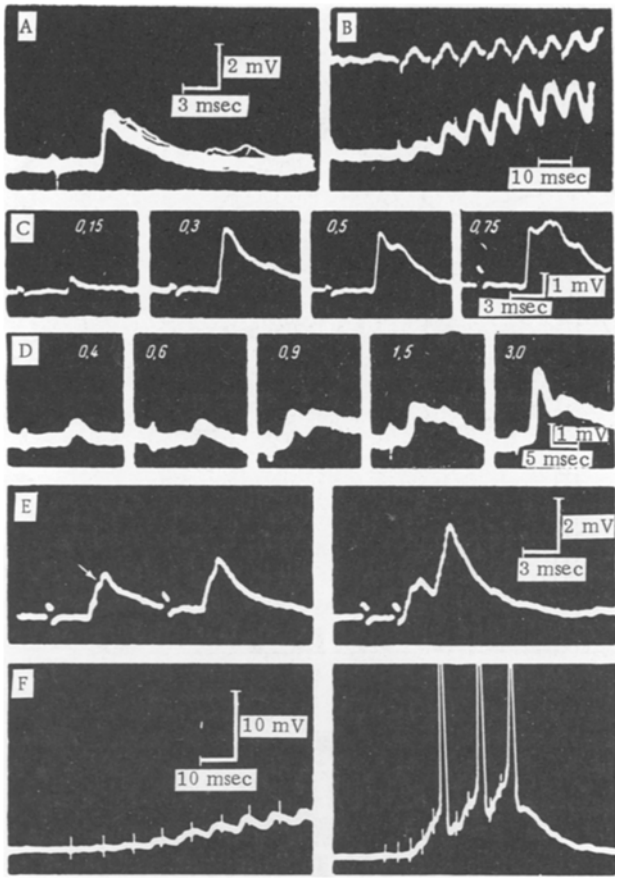

Fig. 3. Short-latency EPSP of six different motor neurons in response to single (A, C, D) and rhythmic (B, E, F) stimuli. A — Repeated superposition of 15 sweeps; B — lower beam — intracellular recording, upper beam — dorsal surface potentials; C and D — responses of two different motor neurons; the numbers denote the strength of stimulation (in mA); E — responses to period stimuli at different intervals; the arrow denotes delay in the ascending phase of the EPSP; F — responses to rhythmic stimulation of different frequency.

The short-latency EPSP were capable of reproducing without transformation very high rhythm of stimulation, up to 500-600 per sec. A sharp increase in the amplitude of the successive responses was frequently observed. The increase in EPSP was not accompanied by significant changes in the initial component of the dorsal surface potentials (Fig. 3B). This shows that the facilitation was not associated with involvement of an additional number of descending fibers. The degree of potentiation was assessed quantitatively by comparing the responses to the first and second stimuli in relation to the interval between them (Fig. 3E and Fig. 4A, B). Investigation of 24 motor neurons showed that the potentials of the second EPSP began to develop at intervals of 15-20 msec and reached a maximum at an interval of 3-5 msec, sometimes attaining 200-350%. In 17 of the 24 cells an additional wave could be distinguished at the end of the ascending phase of the EPSP or immediately after its apex, appearing 1.1-2.2 msec after the beginning of the EPSP (marked by an arrow in Fig. 3E). In most cases, the potentiation took place on account of an increase in the amplitude of this component. Because of this, the time of increase to a maximum was greater in the case of the potentiated EPSP, although the time constant of decay was unchanged by comparison with the experiments with a single stimulus. In three cells, the reticulospinal short-latency EPSP did not exhibit potentiation, while in four other cells it was very slight (120-150%), as is characteristic of monosynaptic EPSP evoked by volleys in muscle afferents of group I A [3].

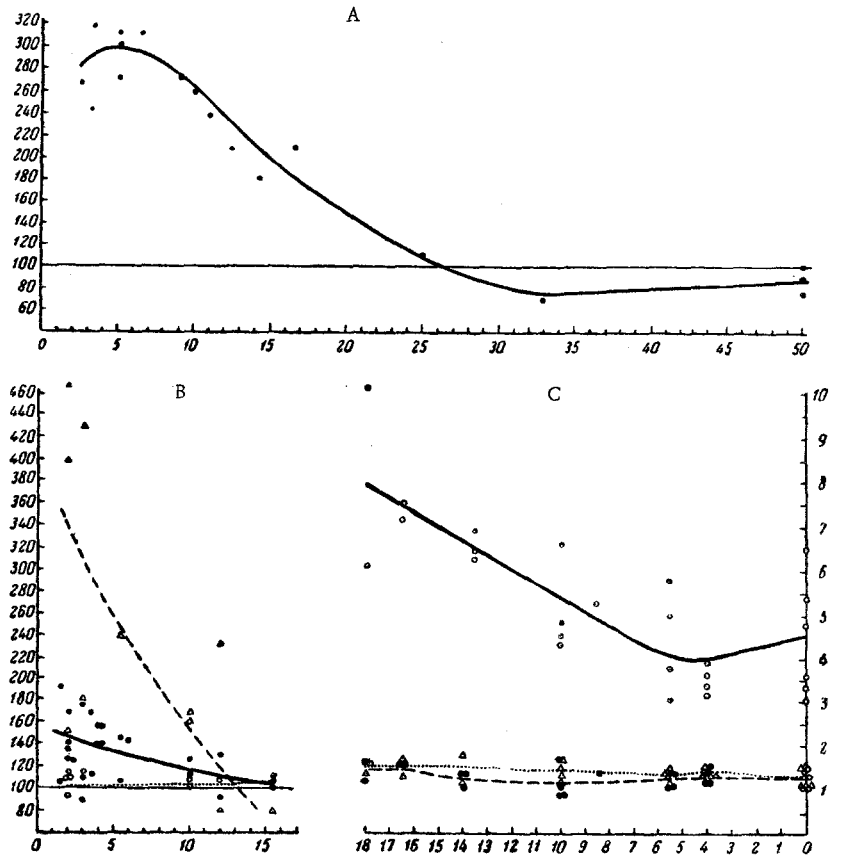

Fig. 4. Potentiation in response to paired stimuli (A, B) and effect of polarization on potentiated EPSP (C). A, B — Relationships between potentiation of the second response and the interval between stimuli; ordinate — amplitude of second EPSP (in % of first); abscissa — interval (in sec); B: the black circles denote changes in the EPSP as a whole; the white circles — in its first component; the triangles — in its second component; C — changes in EPSP evoked by paired stimuli under the action of a hyperpolarizing currnet: the black circles denote the amplitude of the response to the first stimulus; the triangles — amplitude of the first components of the response to the second stimulus; the white circles — the amplitude of the second component of the response to the second stimulus; ordinate — amplitude of EPSP (in mV); abscissa — strength of injected current (in nA).

A marked increase in the amplitude of the "relay" response through the second stimulus during reticulospinal activation was also found by Lloyd [7], when recording the focal potentials and discharges of the anterior roots.

The short-latency IPSP recorded in some cells also showed the power of undergoing considerable potentiation (200-300%) in response to paired stimuli, and in some cases they could reproduce without transformation high rhythms of stimulation (200-300 per sec).

Investigation of the effect of artificial polarization on the short latency EPSP showed that, in contrast to the polysynaptic excitatory reactions, they did not change their amplitude when a polarizing current was passed through the cell. However, the potentiated responses to the second stimulus was clearly diminished during depolarization and increased during hyperpolarization (Fig. 4C). These results show that the synapses responsible for the appearance of the short-latency responses are localized mainly on the dendritic surface of the membrane, whereas the synapses whose involvement brings about the effect of facilitation

are situated more proximally, possibly in the region of the soma. The available information is not yet sufficient to answer the question whether the synapses responsible for facilitation are formed by the more slowly conducting reticulospinal fibers or by the axons of special segmental internuncial neurons. Irrespective of the mechanism responsible for facilitation, it can be concluded that it plays an important functional role, for it increases the efficiency of reticulospinal activation to a considerable degree. During rhythmic stimulation with a frequency of 300-500 per sec, in connection with the development of facilitation in many motor neurons (24 cells), discharges appeared (Fig. 3F), their maximal frequency reaching 200 per sec. Another noteworthy feature was the considerable resemblance between the postactivation facilitation or potentiation of the short-latency reticulospinal EPSP and other direct descending synaptic influences: corticospinal in monkeys [6], rubrospinal [10], and vestibulospinal [1, 10] in cats. This mechanism evidently reflects some common fundamental property of the descending synaptic connections with the spinal neurons and it explains, at least in part, the property of the suprasegmental structures, well known from earlier investigations, of activating the motor centers mainly in response to rhythmic stimulation.

Experiments to measure the resistance during the EPSP were carried out only in the conditions of rhythmic stimulation and they did not yield equivocal results. In some cells (14) the resistance fell by 5-30%, while in others (7) it was unchanged. However, analysis of these results was difficult because of the mixed character of the reactions during rhythmic stimulation, when besides purely excitatory synaptic influences, an inhibitory fraction may be present [10].

The IPSP evoked by reticular stimulation were essentially indistinguishable from the IPSP of segmental origin in their ability to undergo transformation during an artificial change in the transmembrane polarization [4], and injection of chloride ions, and also in the decrease in the resistance of the membrane during the development of the response. The resistance of the membrane fell appreciably during development of the reticulospinal IPSP in all the cells (12) investigated, and the degree of the decrease in resistance bore a linear relationship to the amplitude of hyperpolarization.

Distortion of the reticulospinal inhibitory postsynaptic influences in response to the entry of chloride ions into the cell appeared not only during fewer postsynaptic hyperpolarization, but also during recording of mixed responses evoked by rhythmic stimulation, when the inhibitory fraction was largely masked by postsynaptic depolarization. In the last case, injection of chloride led to an increase in the amplitude of the depolarization response. It may be concluded from these findings that the synapses responsible for reticulospinal postsynaptic inhibition are located in the region of the body or the proximal portions of the dendrites.

The results described show that the widespread notion that direct reticulospinal influences reach only as far as the rostral part of the spinal cord, and are spread to the lumbar segment only through complex, polysynaptic chains of internuncial neurons [2] requires considerable correction. In fact, the reticular formation of the brain stem may control the activity of the lumbar α-motor neurons through a system of rapidly conducting fibers (with a velocity greater than that of conduction along the group I A muscle afferents) connecting it directly both with the motor neurons and also, possibly, with the internuncial cells of the lumbar segments. The results obtained are in full agreement with the latest investigation on the topography of the reticulospinal projections [8, 9, 11].

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