# ACTIVITY OF THE SPINAL CORD NEURONS DURING STIMULATION OF THE HYPOTHALAMUS AND HIPPOCAMPUS

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Although the hypothalamic structures play an important role in the regulation of motor acts and muscle tone [5, 6, 8, 11], the cellular mechanisms of the descending influences from the hypothalamus to the centers of the spinal cord have not yet been studied. In the present investigation the method of intracellular microelectrodes was used to study the faciltating and inhibitory effects arising in the motor and internuncial neurons of the ventral horns during stimulation of the posterior and lateral hypothalamus, and also of the hippocampus, which has descending connections with the hypothalamic formations and the structures of the mesencephalon which take part in the suprasegmental control of the spinal reflexes [7, 9, 14].

## METHOD

Experiments were conducted on 17 cats. Laminectomy, and isolation and division of the nerves and roots were performed under nembutal (35-40 mg/kg intraperitoneally) or ether anesthesia. The experiment began not less than 5-6 h after injection of nembutal (6 cats) or 2-4 h after administration of ether ceased (11 cats). The animals were immobilized with muscle relaxants (diplacin, tubocurarine) and maintained on artificial respiration.

For suprasegmental stimulation, monopolar tungsten electrodes with a diameter of 60-80 $\mu$, and insulated throughout their length except at the end, were used. The stimulating pulses were rectangular in shape, with an amplitude of 1-6 V, and they were applied at a frequency of between 0.5 and 1000 per sec (usually within the range 40-300/sec). The electrodes were inserted into the brain by means of the Horsley-Clarke stereotaxic apparatus. The position of the end of the electrode was verified histologically after each experiment by preparation of serial sections.

In all the experiments the dorsal roots were divided from $L_6$ to $S_1$ to interrupt the $\gamma$-loops. In some experiments the ventral roots also were divided, and their central segments served to identify the neurons. If the ventral roots remained intact, the nerve of the gastrocnemius muscle and the peroneal nerve were used for antidromic stimulation, which allowed the extensor and flexor motor neurons to be distinguished.

The potentials were recorded by an intracellular technique using glass capillary electrodes, the end of which was less than 1 $\mu$ in diameter. By incorporating these electrodes in a bridge circuit, the cells under investigation could be stimulated directly [2].

## RESULTS

Descending influences on the cells of the spinal cord were found mainly during stimulation of the posterior and lateral hypothalamus on the same side. During stimulation of the hippocampus, the most obvious effects were observed from the ipsilateral ventral hippocampus (although responses were also recorded during stimulation of the ipsi- and contralateral posterior hippocampus).

Stimulation of the hypothalamic structures caused both excitation and inhibition of the lumbar motor neurons. The excitatory effects were accompanied by depolarization of the postsynaptic membrane—by excitatory postsynaptic potentials (EPSP), which, on reaching the critical level, caused the generation of action potentials (AP).

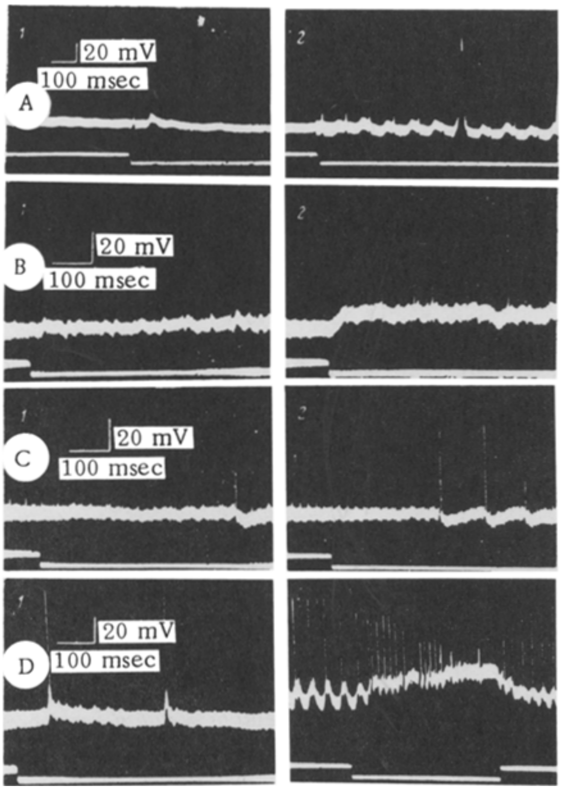


Fig. 1. Examples of responses of motor neurons to stimulation of posterior hypothalamus at different frequencies. A, B, C) Before injection of strychnine; D) after injection of strychnine in a dose of 0.2 mg/kg. Significance of curves (from top to bottom): responses of cell, marker of stimulation.

Depending on their character, the reactions to excitation could be divided into three principal types.

1. Responses resembling monosynaptic reactions (Fig. 1A). These appeared after a short latent period (7.5-6 msec), from which, taking into consideration the conduction time, it was concluded that no additional synaptic delays were present (the latent period was determined from the interval between the stimulus and the beginning of the EPSP). The duration and form of these EPSPs were similar to the corresponding parameters of the segmental monosynaptic reactions, and the critical level for generation of APs was 7-12 mV. In the course of, and after, rhythmic stimulation obvious potentiation of the EPSP was observed.

The after-hyperpolarization developing after the AP abruptly lowered the amplitude of the EPSP and limited the maximal rhythm of repetition of the AP (not more than 10-20/sec). The number of motor neurons from which responses of "monosynaptic" type could be recorded was small (6 of 127 neurons activated during stimulation of the hypothalamus).

2. Responses of polysynaptic type (Fig. 1B). The EPSPs were characterized by a long and inconstant latent period (from 7-8 to 10-15 msec), a long duration (up to 20-40 msec), and an irregular shape. If the frequency of rhythmic stimulation was high enough (40-80/sec or more), the individual EPSPs merged to form a continuous plateau of depolarization, with APs appearing at the summit, their frequency reaching 80-90/sec even when measured over relatively long time intervals (300-500 msec), while the interval between individual APs fell to 3-4 msec, corresponding approximately to 300 pulses/sec.

The results described accord well with the data obtained in experiments in which intracellular recordings were made from the motor neurons of the cat when activated through segmental polysynaptic pathways [1] or by the passage of a depolarizing current [2]. The rate of increase of depolarization depended on the frequency of stimulation: with an increase in the frequency, depolarization reached its maximum more quickly. Because postsynaptic depolarization frequently developed very gradually, the time from the beginning of depolarization to the appearance of the AP was measured in tens, and sometimes in hundreds, of milliseconds. In such cases the critical level for generation of APs rose to 15-20 mV. Having reached its maximal level, the depolarization plateau sometimes remained stable for many hundreds of milliseconds. In some cells, despite the continuing stimulation, the responses of the motor neuron were broken up into separate waves of depolarization with an amplitude of 15-20 mV and a duration of 200-500 msec, repeated at intervals of 0.5-1.0 sec. For a period of 2.5 sec after the end of stimulation such waves continued to arise "spontaneously" in the form of a prolonged after-effect.

Intensive depolarization of the motor neurons partly or completely abolished the role of the after-hyperpolarization in limiting the frequency of repetition of the AP. A change in the rate of growth and in the amplitude of postsynaptic depolarization, depending on the frequency of stimulation, provided an effective gradual control of the frequency of the rhythmic activity of the motor neuron. The graph in Fig. 2 shows that during stimulation with a frequency of 20-200/sec the relationship between the frequency of stimulation and the frequency of development of impulses in the motor neuron was linear in character, although there was no direct correlation between the number of stimuli and the number of response APs. After the frequency of discharges of the motor neuron had reached its maximal value, a further increase in the frequency of stimulation was accompanied by a gradual fall in the number of APs arising in the cell. These phenomena were seen most clearly during stimulation with a frequency higher than 500-600/sec.

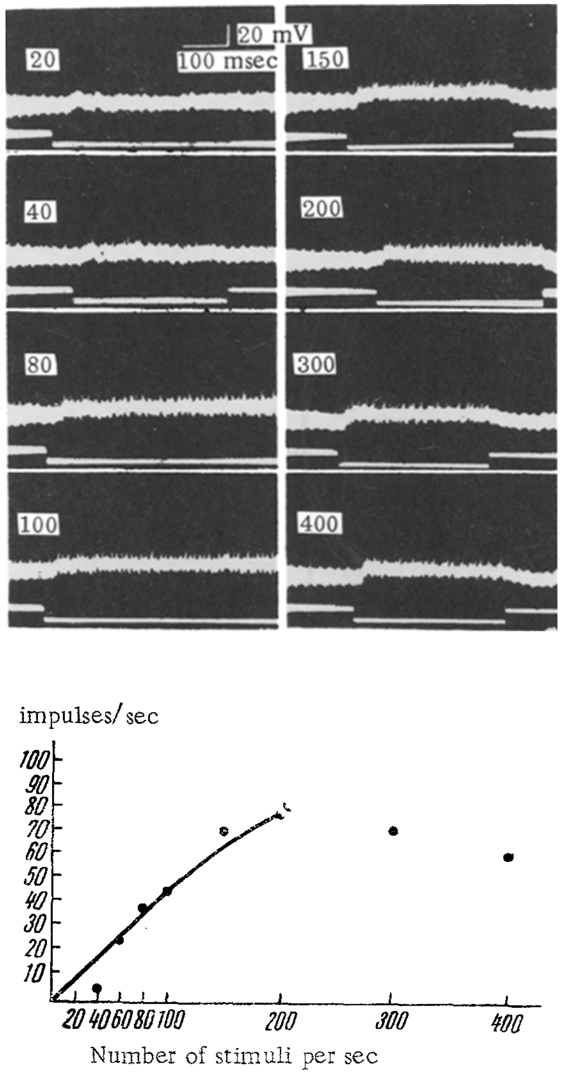


Fig. 2. Activity of the same cell during stimulation of the posterior hypothalamus with stimuli of different frequency, and graph of relationship between frequency of stimuli and response impulses of neurons. The numbers on the oscillograms denote the frequency of stimulation.

3. In response to single stimuli, and also during rhythmic stimulation, no individual EPSPs were found. Depolarization developed very slowly and was confluent. The character of the discharges of the motor neurons was very similar to that of the spontaneous rhythmic activity. The latent period of development of the AP was measured in tens or hundreds of milliseconds. On account of the very small magnitude of the postsynaptic depolarization (7-10 mV), the after-hyperpolarization potentials arising after the APs disturbed it very considerably, and the intervals between individual APs were limited by the duration of the after-hyperpolarization. Hence, an increase in the frequency of stimulation led mainly to a decrease in the latent period of the discharges (Fig. 1C), and had very little effect on their frequency.

Responses of the type described above were recorded, not only during stimulation of the hypothalamic structures, but also, characteristically, after stimulation of the hippocampus (11 motor neurons). When the stimuli applied from the hippocampus were of considerable amplitude (5-6 V), responses of polysynaptic type could also be obtained, but after a longer delay (20-30 msec) than in the case of stimulation of the hypothalamus. Nevertheless, the main part of the latent period of the AP, amounting to 0.5-1.0 sec, corresponded to the time of growth of the postsynaptic depolarization to the critical level. The postsynaptic depolarization arising during stimulation of the investigated suprasegmental structures, increased the frequency of the spontaneous rhythmic activity of the motor neurons and facilitated their responses to synaptic and direct stimulation.

Stimulation of the posterior and lateral hypothalamus and of the hippocampus was also accompanied by inhibitory reactions, manifested most commonly (in 74 motor neurons out of a total number of 89 inhibitable units) as hyperpolarization of the membrane—by inhibitory postsynaptic potentials (IPSP). These potentials were rarely evoked by single stimuli. Usually for appreciable hyperpolarization to develop, rhythmic stimulation was required. The intensity and rate of development of hyperpolarization increased with an increase in the frequency of stimulation. The most marked effects were observed during tetanization with a frequency of 100-300/sec. In spontaneously active motor neurons, with a constant initial background, a nearly linear relationship was found between the degree of the inhibitory effect and the frequency of stimulation (Fig. 3).

After the cessation of stimulation a rebound phenomenon was observed in many motor neurons. The onset of postinhibitory excitation was accompanied by postsynaptic depolarization (EPSP), the intensity of which, and also the frequency of the discharges of the motor neuron, were dependent on the frequency of the inhibitory stimuli (see Fig. 3).

Finally, in some cases (17 motor neurons), mixed reactions were recorded (EPSP and IPSP) during stimulation of the lateral or posterior hypothalamus. In the cells giving mixed responses, mainly inhibition or facilitation could be obtained by varying the frequency of stimulation. With low frequencies of stimulation (40-80/sec) facilitation was usually the predominant effect, while an increase in the frequency of stimulation to 200-300/sec abolished the facilitation and left only the hyperpolarization. These findings may be regarded as the result of activation of the

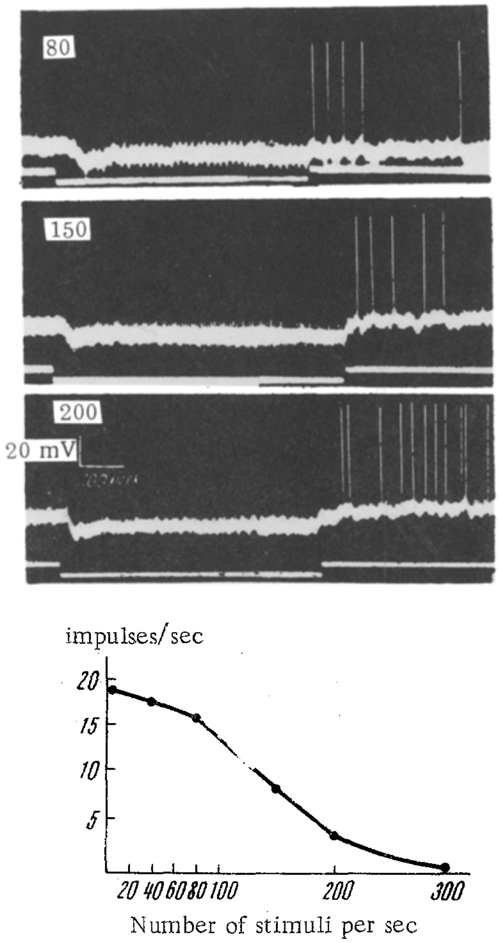


Fig. 3. Inhibition and rebound in the same motor neuron during stimulation of the lateral hypothalamus with stimuli of different frequencies, and graph showing the relationship between the frequency of the spontaneous rhythmic activity and the frequency of stimulation of the posterior hypothalamus. The numbers on the oscillograms denote the frequency of stimulation.

excitatory and inhibitory structures simultaneously by the stimulating electrode, and also of differences in the activity of these structures to respond to rhythmic stimulation.

Besides the motor cells, the internuncial neurons of the ventral horns also reacted to stimulation of the investigated suprasegmental structures. The responses of the internuncial neurons to single stimuli were, as a rule, less intensive than the responses of these same cells to afferent stimulation. Nevertheless, many internuncial cells gave long discharges of high frequency during rhythmic suprasegmental stimulation, like the long postsynaptic reactions of the motor neurons.

Stimulation of the hypothalamus, unlike stimulation of the cerebellum [3], frequently caused inhibition of the activity of the internuncial cells.

Effect of depressants and stimulants. The effects produced by stimulation of the hypothalamic structures, and especially of the hippocampus, proved highly sensitive to anesthetics. In animals anesthetized with nembutal for the operation it was difficult to find reactions of polysynaptic type even 5-6 h after administration of the drug. Injection of small doses of nembutal (3-5 mg/kg) led to a sharp reduction in the number of neurons activated during stimulation of the above-mentioned structures. The highest activity of the neurons was recorded in animals undergoing operation under ether, a few hours after termination of the anesthesia.

Strychnine had hardly any effect on the responses of monosynaptic type and caused a sharp increase in the polysynaptic reactions of the motor neurons. In the "silent" cells these reactions became identical with the reactions arising in response to stimulation of the dorsal roots or the afferent nerves. In the cells with paroxysmal activity, application of high-frequency stimuli to the hypothalamus led to intensive postsynaptic depolarization, accompanied by phenomena of cathodic depression (Fig. 1D). As in the case of stimulation of the other suprasegmental formations [3, 4], inhibition of strychnine tetanus during stimulation of the hypothalamus and hippocampus may develop without hyperpolarization of the cell as a result of a disturbance of the development of slow depolarization waves. After administration of strychnine an IPSP may appear, even in response to single stimuli applied to the hippocampus. The changes observed may be regarded as facilitation of the reactions effected through multisynaptic pathways.

The results of these experiments agree with data in respect of the descending connections of the hypothalamus and hippocampus [7, 10-12, 14, 15]. The hypothalamus is connected with the spinal cord mainly by tracts with synaptic relays in the mesencephalon and medulla, although there are indications that some of the efferent fibers composing the bundle of Schütz form monosynaptic connections with spinal elements [15, 16]. The effects obtained from the hippocampus may be transmitted to the spinal cord solely through relays situated in the hypothalamus and the mesencephalon.

The effects transmitted through multisynaptic, diffuse pathways may evoke both an intensive depolarization of the motor neurons and an even depolarization of low amplitude. It may be assumed that in the first case excitation is associated with activation of synapses terminating on the cell body, and in the second case with activation of axodendrite connections.

The results obtained show that the descending influences which were investigated, depending on the frequency of activation of the corresponding structures, may exert an effective control, capable of a wide range of adjustment, on the frequency of the rhythmic activity and on the latent period of the responses of the motor neurons, and also on the intensity and the rate of inhibition of their activity.

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