SYNAPTIC RESPONSES OF MEDULLA OBLONGATA NEURONS TO
STIMULATION OF THE SPINAL STEPPING ATRIA IN THE CAT

O. V. Kazennikov and G. V. Yakovleva   UDC 612.83:612.014.42

Synaptic responses of medulla oblongata (bulbar) neurons to microstimulation of
stepping points in the spinal dorsolateral funiculi were recorded in decrebrate
cats. Upon stimulation of the stepping point both in the ipsi- and contralateral
funiculi, 40% of the neurons generated synaptic responses; the remaining cells
responded to stimulation of only one stepping point. A part of the bulbar neurons
responds to stimulation of stepping points both at the C_2 and Th_{12} level. The
latent periods of the synaptic responses of the bulbar neurons to stimulation of
the stepping point at the C_2 level were in the 2-10 msec range. The data obtained
indicate that bulbar neurons, along with propriospinal neurons, can participate in
evoking locomotion upon stimulating the stepping point.

INTRODUCTION

Microstimulation of a limited bundle of fibers in the spinal dorsolateral funiculus
(DLF) of the cat leads to stepping motions of the ipsilateral hind limb [2]. It is obvious
that the occurrence of stepping is provided by excitation of spinal neurons [11, 13]. At
the same time, it is not ruled out that neurons of the medulla oblongata are excited and
participate in initiating stepping moments upon stimulation of the stepping stria of the
DLF. It is known that neurons of the medial reticular formation are activated in response
to stimulation of locomotor points of the midbrain [5, 9] and medulla oblongata [6]. In the
present work we investigated the possibility of the involvement of medulla oblongata (bulbar)
neurons in excitation after stimulating the stepping point (SP) in the DLF.

METHODS

The experiments were conducted on 18 decerebrate cats. The operation was performed under
ether anesthesia. After precollicular postmamillary decerebration, the posterior portion of
the cerebellum was removed to facilitate access to the medulla oblongata. The spinal column
was fixed in the cervical and lumbar parts; laminectomy was carried out at the C_2-C_3 and
$Th_{11}-Th_{12}$ level. An hour after the end of anesthesia, we looked for the SP by stimulating
the DLF with electrical stimuli of negative polarity with a duration of 0.4 msec and strength
up to 20 μA, which were applied with a frequency of 60/sec through a metal electrode (tungsten
wire with a diameter of 20 μm in glass insulation). To find the neurons, the SP was stimu-
lated with paired stimuli with an interval of 3 msec, strength up to 20 μA, and frequency of
2-4/sec. The responses of single bulbar neurons were recorded by glass microelectrodes with
a diameter of 5-7 μm filled with Wood's alloy. The responses of the neurons were recorded on
photographic film. A neuron was considered responding if impulses occurred in response to
presentation of at least half of the stimuli. The average latent period was determined from
20-40 responses. If a response was obtained only to paired stimulation, the latent period
read from the second stimulus.

In eight experiments we recorded the synaptic responses of bulbar neurons to stimulation
of two SPs located in the same DLF at a distance of 10-15 mm from each other at the level of
the C_2-C_3 segments. Then by means of two ophthalmic forceps we damaged the DLF between the
given SPs. After this, the responses of the neurons to stimulation of the rostral and caudal
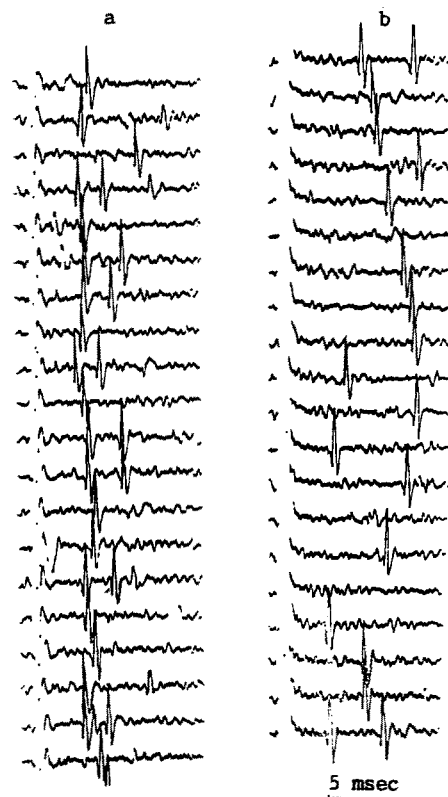SPs were recorded again.

Fig. 1. Synaptic responses of bulbar neuron to stimulation of the ipsi- (a) and contralateral (b) stepping points at the C_2 level.
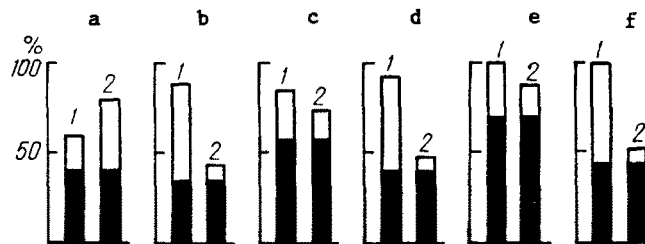

Fig. 2. Diagram of relative frequencies of bulbar neuron responses to stimulation of different stepping points: a) synaptic responses to stimulation of ipsi- (1) and contralateral (2) SPs at the C_2 level. The total number of neurons (n = 44) was taken as 100%. The proportion of neurons that responded to stimulation of both SPs is blackened; b) same as a, to stimulation of SPs at the C_2 (1) and Th_{12} (2) levels; n = 18 (100%); c and d) same as a, to stimulation of the rostral (1) and caudal (2) SPs at the C_2-C_3 levels before (c) and after (d) destruction of the DLF between them; n = 30 (for c) and 52 (for d) (100%); e and f) same as c and d, but the number of neurons synaptically responding to stimulation of the rostral SP was taken as 100%; n = 25 (for e) and 48 (for f).

In one experiment we also recorded responses to stimulation of the locomotor point in the medulla oblongata [7] (stimulation strength 20 µA, single or paired stimuli with an interval of 3 msec and frequency of 2/sec). In a part of the experiments an additional electrode was placed in the lateral funiculus for antidromic stimulation of the bulbar neurons which synaptically responded to stimulation of the SP (stimulation strength up to 80 µA).
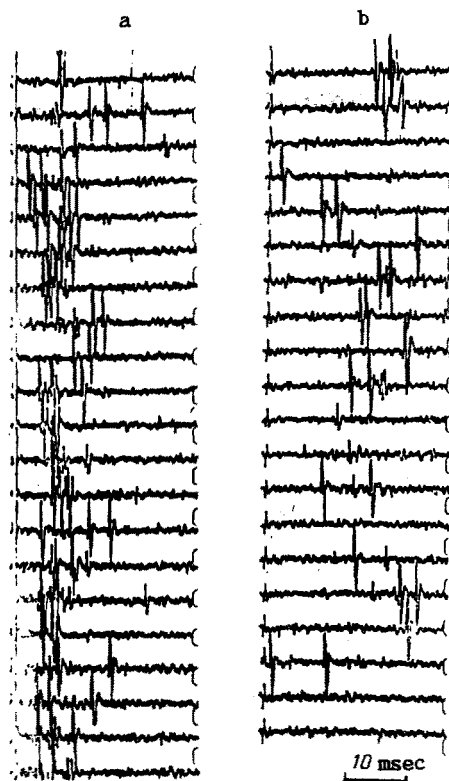
Fig. 3. Synaptic responses of bulbar neuron to stimulation of ipsilateral stepping points at the C_2 (a) and Th_{12} (b) levels. Single stimulation (10 (a) and 20 (b) μA).

The synaptic responses to stimulation of the SP were recorded in 197 bulbar neurons located 1-4 mm rostral of the obex and 0.5-2.5 mm lateral of the midline at a depth of 0.5-3.0 mm under the bottom of the IV ventricle.

RESULTS AND DISCUSSION

In the first series of experiments we investigated the responses of bulbar neurons to stimulation of the SP in the left and right DLF at the C_2 level. It was found that 40% of the neurons generated synaptic responses to stimulation both of the ipsi- and contralateral SP. Figure 1 shows the synaptic responses of the bulbar neuron to stimulation of the SP in the ipsi- (a) and contralateral (b) DLF. The spontaneous activity of the given neuron was about 6 impulses/sec. The remaining cells responded to stimulation of only one SP (Fig. 2a).

In another series of experiments we investigated the responses of neurons after stimulating the SP at the C_2 and Th_{12} levels. More than half of the cells responded only to stimulation of the SP at the C_2 level; in 1/3 of the neurons, synaptic responses occurred as a result of stimulating both SPs (Fig. 2b). Figure 3 shows the responses of the bulbar neuron to stimulation of the DLF in the cervical (a) and thoracic (b) parts.

It could be assumed that excitation of the brainstem neurons occurs analogously to excitation of spinal neurons along ascending fibers of the DLF [3]. To investigate the pathways over which brainstem neurons are excited, we destroyed the DLF between two SPs in one funiculus at the C_2-C_3 level. The synaptic responses of the bulbar neurons to stimulation of each SP were recorded before and after damaging the DLF between them. After destroying the DLF in two experiments, stepping, although feeble, could be evoked by stimulating the rostral and not only the caudal SP (compare [2]). In four experiments stepping occurred only in response to stimulation of the caudal SP, and in two experiments stepping became unstable after stimulating both SPs. Before destroying the DLF the majority of neurons responded to stimulation of both SPs and the remaining ones responded to stimulation of only the rostral or only the caudal SP (Fig. 2c). After damaging the DLF, the proportion of neurons that responded to stimulation of the caudal SP decreased (Fig. 2d). If it is assumed that the
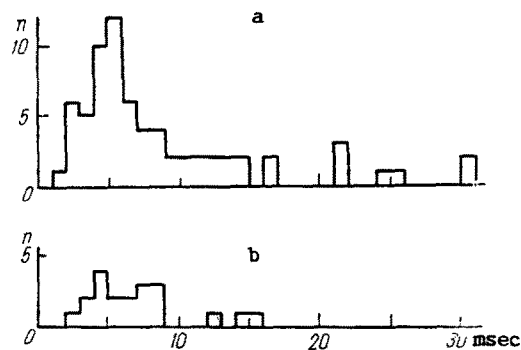
Fig. 4. Histograms of the distribution
of average latent periods of synaptic
responses (probability 0.5) of bulbar
neurons to stimulation of stepping points
of the ipsi- (a) and contralateral (b)
sides at the C_2 level. On the axis of
the abscissas is time, msec; on the axis
of the ordinates is the number of responses.

number of cells of the medulla oblongata that reacted synaptically to stimulation of the
rostral SP does not change after destroying the DLF, then the relative proportion of neurons
that generated synaptic responses with a response index (RI) $\geq$ 0.5 to stimulation of the
caudal SP will decrease by almost half (Fig. 2e, f). The latent period and its fluctuations
for synaptic responses to stimulation of the caudal SP after destruction of the DLF, as a
rule, were greater than to stimulation of the rostral SP.

The latent period of the synaptic response was determined in 69 neurons that responded
to stimulation of the ipsilateral SP (Fig. 4a) and in 20 neurons that responded to stimula-
tion of the contralateral SP (b) at the C_2 level. The latent periods of the responses (RI $\geq$
0.5) were in the same range in the case of stimulating the ipsi- and contralateral SPs. The
average latent periods of the majority of synaptic responses exceeded 4 msec; the values of
the latent periods fluctuated considerably, which indicates a polysynaptic character of
excitation. The latent periods of the synaptic responses of neurons to stimulation of the
SP at the Th_{12} level were in the 12-18 msec range. A comparison of the latent periods of
synaptic responses of bulbar neurons and spinal neurons located at the same distance from
SP shows that excitation of bulbar neurons occurs with a greater latent period [3].

In eight cells that responded to stimulation of the SP, we studied also the responses
to stimulation of the locomotor points of the medulla oblongata. Five neurons generated
synaptic responses also to this stimulation. Thus bulbar neurons can be excited as a result
of stimulating locomotor points located both in the medulla oblongata and at different levels
of the spinal cord.

In some bulbar neurons that responded synaptically to stimulation of SP in the DLF, we
recorded also antidromic responses to stimulation of the ventrolateral funiculus at the C_3
level.

The results of the experiment show that excitation not only of spinal but also of bulbar
neurons occurs as a result of stimulating the SP. The spinal neurons do not respond to
stimulation of the SP separated from them as a consequence of damaging the DLF [4], and about
half of the cells of the medulla oblongata generate synaptic responses to stimulation of the
SP also after damaging the DLF. A similar change in the synaptic responses in the spinal
cord is observed after partial damage of the DLF between to SPs in the lower thoracic segment
[4]. It is possible that the fibers excited as a result of stimulating the SP in the cervical
segment form a diffuse bundle and damage only partially affects it. The synaptic responses
of the bulbar neurons to stimulation of the SP in the DLF have a polysynaptic character (Fig.
4). After damaging the DLF, the latent period of responses to stimulation of the caudal SP
and its fluctuations increase. Therefore, it is more likely that the presynaptic volley goes
to the bulbar neurons not only over the fibers of the DLF but also after excitation of spinal
neurons sending axons to the brainstem outside of the DLF. Cells of the spinal cord with
axons giving ascending collaterals [3, 8, 12], which are located outside the DLF or pass to
the symmetric half of the spinal cord, can be neurons on which switching occurs.

On the histogram of the latent periods of synaptic responses of bulbar neurons to microstimulation of the SP (Fig. 4) there is almost no early component corresponding to monosynaptic excitation of bulbar neurons [1]. It can be assumed that excitation of the given neurons after stimulating the SP occurs over polysynaptic pathways. This is indicated also by the circumstance that the latent periods of the synaptic responses of spinal neurons [3] on average are less than that of cells of the medulla oblongata to stimulation of the SP at the same distance from the neuron.

Excitation both of bulbar and spinal neurons occurs after stimulating the SP. In animals under various experimental conditions, locomotion can be realized with various relationships of influences from the SP on the stem and spinal cord. Owing to this, destruction of the DLF can have a different effect on the effectiveness of activating the rostral and caudal SP in different preparations. Whereas mainly spinal neurons are excited as a result of stimulating the SP, after destroying the DLF at the C_2-C_3 level the threshold of evoking locomotion increases to a greater degree in the case of stimulating the rostral SP. When stimulation of the SP has the main effect on the brainstem, a greater increase of the threshold is observed in the case of stimulating the caudal SP. We showed earlier [2] that after coagulation of the middle of the three SPs at the C_2-C_3 level, stimulation of the rostral SP became ineffective in eight cases and of the caudal in two. We cannot rule out the possibility that in these two experiments, stimulation of the SP led more easily to stimulation of the bulbar and not the spinal neurons. However, in the majority of experiments the spinal neurons are excited more easily upon stimulation of the SP. Apparently, this is the main method of evoking locomotion as a result of stimulating the SP.

Bulbar neurons can participate not only in initiation of stepping but also in coordination of the work of the left and right halves of the spinal cord. They are effectively excited as a result of stimulating the SP at various levels of the spinal cord, in the ipsi- and contralateral DLF, and some of these neurons are bulbospinal. Therefore, they can be regarded as a central link in controlling stepping by analogy with brainstem neurons participating in spinobulbospinal reflexes [1, 10].

LITERATURE CITED

1. A. P. Gokin, M. V. Karpukhina, Yu. Pavlasek, and A. I. Pilyavskii, "Synaptic excitation of reticulospinal neurons during the startle response in cats anesthetized with chloralose," Neirofiziologiya, 13, No. 6, 594-603 (1981).
2. O. V. Kazennikov, M. L. Shik, and G. V. Yakovleva, "Stepping movements evoked by stimulating the spinal dorsolateral funiculus of the cat," Byull. Eksp. Biol. Med., 96, No. 8, 8-10 (1983).
3. O. V. Kazennikov, M. L. Shik, and G. V. Yakovleva, "Synaptic responses of propriospinal neurons to stimulation of the stepping stria in the dorsolateral funiculus of the cat," Neirofiziologiya, 17, No. 2, 270-278 (1985).
4. O. V. Kazennikov and M. L. Shik, "Propagation of activity along the stepping stria of the cat spinal cord," Neirofiziologiya, 20, No. 6, 763-769 (1988).
5. Sh. Mori, V. A. Selionov, and M. L. Shik, "Responses of medullary neurons to stimulation of locomotor and inhibitory points of the brainstem of the cat," Neirofiziologiya, 18, No. 4, 525-533 (1986).
6. G. N. Orlovskii, "Work of reticulospinal neurons during locomotion," Biofizika, 15, No. 4, 728-737 (1970).
7. M. L. Shik and A. S. Yagodnitsyn, "Pontobulbar locomotor stria,'" Neirofiziologiya, 9, No. 1, 95-97 (1977).
8. B. Alstermark, S. Linström, A. Lundberg, and R. Sybirska, "Integration in descending motor pathways controlling the forelimb in the cat. 8. Ascending projection to the lateral reticular nucleus from C3-C4 propriospinal neurons also projecting to forelimb motoneurons," Exp. Brain Res., 42, No. 2, 282-298 (1981).
9. E. Garcia-Rill and R. D. Skinner, "The mesencephalic locomotor region II. Projection to reticulospinal neurons," Brain Res., 411, No. 1, 13-20 (1987).
10. M. Shimamura and R. B. Livingston, "Longitudinal conduction systems serving spinal and brain stem coordination," J. Neurophysiol., 26, No. 2, 258-272 (1963).
11. C. S. Sherrington, "Flexion reflex of the limb, crossed extention reflex, and stepping and standing reflex," J. Physiol., 40, No. 1, 28-121 (1910).

12. R. D. Skinner, E. Garcia-Rill, and M. B. Jackson, "Projection of long descending prop-riospinal neurons to the brain stem," Anat. Rec. Ser. A., 199, No. 3, 239 (1981).

13. T. Yamaguchi, "Fictive stepping evoked by electrical stimulation of the white matter of the cervical cord in decerebrate cats," J. Phys. Soc. Jpn., 43, No. 6/7, 303 (1981).