# NATURE OF TONIC ACTIVITY OF THE SPINAL CORD IN RATS WITH SPASMS DUE TO ASCENDING TETANUS

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In rats with ascending tetanus and exhibiting the "universal departure station" (UDS), phenomenon stimulation of an afferent nerve to the hind limb in which the tetanus toxin was injected evokes tonic reflexes in the lumbosacral portion of the spinal cord: a long positive potential (P-wave) of the dorsal surface potential (DSP), and also tonic negative potentials in the dorsal roots and efferent discharges in the ventral roots of both halves of the spinal cord. Stimulation of the afferent nerve to the opposite hind limb evoked phasic potentials of low amplitude in these same structures. Tonic reflexes of the spinal cord were recorded also when all the dorsal roots were divided below the level of cordotomy and reflexes were evoked by stimulation of dorsal root $L_6$ on the affected side of the spinal cord. It is postulated that tonic paroxysmal activity in rats with ascending tetanus and exhibiting the UDS phenomenon is generated by interneurons of the poisoned half of the spinal cord.

In rats with ascending tetanus and exhibiting the "universal departure station" (UDS) phenomenon stimulation of a nerve on the side of injection of the tetanus toxin evokes the appearance of an unusually long positive component (P-wave) of the dorsal surface potential (DSP), and a negative potential of the dorsal roots of the spinal cord [5]. Clear correlation is observed between the changes in this enlarged P-wave of the DSP and changes in the prolonged efferent paroxysmal discharge in the ventral roots of the spinal cord [3, 5-7]. The tonic character of reflex activity is one of the most important manifestations of tetanus poisoning, and for that reason the investigation of the mechanisms of these tonic reflexes in tetanus is extremely interesting.

In this paper some aspects of the genesis of tonic reflex responses of the spinal cord are examined in rats with ascending tetanus and exhibiting the UDS phenomenon.

## EXPERIMENTAL METHOD

Experiments were carried out on 22 rats weighing 250-350 g, in which ascending tetanus and the UDS phenomenon were induced by injection of tetanus toxin in a dose of 4-6 MLD into the gastrocnemius muscle of one hind limb. To block the spread of the toxin in the blood stream, antitetanus serum was injected intravenously in a dose of 0.025 a.u.

The spinal cord was divided at the level $Th_7$-$Th_8$ 24 h before the beginning of the experiment under brief ether anesthesia. On the day of the experiment the part of the spinal cord to be studied was exposed under ether anesthesia, and the roots of the cord and peripheral nerves to the hind limbs (tibial, sural, and peroneal nerves) were dissected. The animals were immobilized by intravenous injection of D-tubocuranine (0.3-0.4 mg/kg). The difference between the ability to induce the UDS phenomenon on the side of injection of the toxin and the opposite side was seen most clearly if weak stimuli were applied to the afferent nerves; for this reason stimulation was applied in a strength of not more than four threshold units for the given

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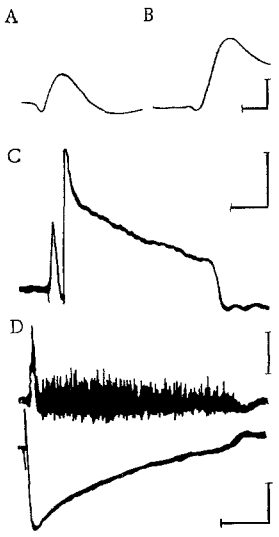


Fig. 1. Long spinal cord potentials in rats with ascending tetanus and the UDS phenomenon: A, B) DSP of $L_5$ evoked on side opposite to injection of toxin by stimulation of ipsilateral nerve (A) and nerve on "tetanus" side (B); C) the same as in A and B. Potentials recorded consecutively during single sweep and with amplification. First potential is DSP of $L_5$ evoked by stimulation of ipsilateral nerve, second potential is DSP of $L_5$ evoked by stimulation of nerve on contralateral ("tetanus") side; D) efferent discharge in ventral root $L_6$ on "tetanus" side and DSP in region of entrance of dorsal root $L_6$ into spinal cord. Calibration of amplification in A, B, and C 50 $\mu$V, in D 0.5 mV for efferent discharge and 1 mV for DSP. Time calibration in A and B 20 msec, in C 200 msec, in D 100 msec.

nerve (4T). Details of the method of stimulating the nerves and recording the potentials and of the apparatus used in the experiments were given previously [2-7].

## EXPERIMENTAL RESULTS

In the experiments of series I an attempt was made to discover whether the inflow of additional afferent impulses from the periphery is an essential condition for the formation of long tonic reflexes in animals with ascending tetanus and exhibiting the UDS phenomenon, or whether the tonic character of the observed responses is determined by intracentral processes. For this purpose the nature of the efferent discharges and DSP of the spinal cord was investigated in rats with ascending tetanus and exhibiting the UDS phenomenon, in which all the dorsal roots of the sacral, lumbar, and thoracic segments of the spinal cord were divided before the experiment began below the level of the cordotomy and reflexes were evoked by single electrical stimulation of dorsal root $L_6$.

The results of one of the eight experiments on animals with divided dorsal roots are illustrated in Fig. 1D. They show that even after total deafferentation of the distal part of the spinal cord a single electrical stimulus applied to dorsal root $L_6$ on the side of injection of the tetanus (the "tetanus" side) evokes, as before, an enlarged P-wave and an efferent discharge lasting several hundreds of milliseconds. Just as in the animals with intact dorsal roots, definite coincidence was observed between the durations of the P-wave of the DSP and the efferent discharge in the ventral root.

As has been shown previously [2, 4, 5, 7] in animals with ascending tetanus and the UDS phenomenon, dorsal root potentials and the efferent discharge evoked by stimulation of the ipsilateral nerve are considerably longer on the "tetanus" side than on the side opposite to that of injection of the toxin. The appearance of long spinal cord potentials in animals with ascending tetanus can be explained by the de-inhibiting action of tetanus toxin on interneurons responsible for the transmission of excitation to motoneurons and to primary afferents. However, the leading role in the genesis of the tonic efferent discharges on the "tetanus" side could also be played by the suppression of inhibition of the motoneurons.

To determine the site of formation of the tonic paroxysmal activity, the characteristics of reflex responses were studied in the ventral and dorsal roots on the "tetanus" and opposite sides of the spinal cord to stimulation of the contralateral nerves. The results of measurements of the amplitudes of the reflex responses evoked by stimulation of nerves of both sides at a strength of 4T are given in Table 1 and specimen records are shown in Figs. 1 and 2. It will be clear from Table 1 that the amplitude of DSPs evoked on the opposite side to injection of the toxin by stimulation of the sural nerve on the "tetanus" side was significantly higher than the amplitude of DSPs evoked by stimulation of the contralateral sural nerve on the "tetanus" side of the spinal cord.

The difference in the durations of the potentials evoked in the roots from the two sides of the spinal cord in response to stimulation of the nerve on the "tetanus" side and on the side opposite to injection of the toxin were particularly marked (4-20 times). In Fig. 1 the DSP evoked in a root on the side opposite to that of injection of the toxin by stimulation of a nerve on the "tetanus" side (see Fig. 1B) is compared with the DSP evoked by an ipsilateral stimulus (Fig. 1A) equal in duration to the DSP in a healthy rat. As Fig. 1 shows, the difference in duration of the potentials evoked in the same dorsal root by stimulation of the nerves on the "tetanus" and opposite sides was very great (Fig. 1C).

TABLE 1. Amplitude of Potentials of Dorsal and Ventral Roots of the Spinal Cord in Rats with Ascending Tetanus and the UDS Phenomenon

| Side of recording | Amplitude of DSP $L_5$ (in $\mu$V) | | | Amplitude of polysynaptic reflexes in root $L_6$ (in $\mu$V) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | side of stimulation | | | | | | | | |
| | contralateral | | | ipsilateral | | | contralateral | | |
| | $n$ | $\bar{x} \pm S_{\bar{x}}$ | $P$ | $n$ | $\bar{x} \pm S_{\bar{x}}$ | $P$ | $n$ | $\bar{x} \pm S_{\bar{x}}$ | $P$ |
| "Tetanus" | 9 | 176 ± 29 | <0,05 | 4 | 720 ± 175 | <0,05 | 4 | 66 ± 22 | <0,05 |
| Opposite | 10 | 247 ± 19 | | 4 | 169 ± 35 | | 4 | 375 ± 87 | |

Legend: n) number of observation; $\bar{x}$ arithmetic mean; $S_{\bar{x}}$) standard error; P) probability of the null hypothesis.
Note. Level of significance taken as 0.05. Difference significant if $P < 0.05$.

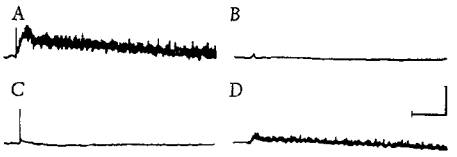


Fig. 2. Distinguishing features of ipsilateral and contralateral efferent discharges on "tetanus" and opposite sides in animals with ascending tetanus and the UDS phenomenon. A and B) efferent discharges in root $L_6$ on "tetanus" side evoked by stimulation of ipsilateral (A) and contralateral (B) tibial nerve at a strength of 4T; C and D) the same as A and B, but for efferent discharges in root $L_6$ on contralateral side to injection of toxin. Calibration of amplification 500 $\mu$V, time marker 100 msec.

It was interesting to compare the amplitudes and durations of the efferent discharges. The results of one of the observations are given in Fig. 2 and mean amplitudes of responses on the "tetanus" and opposite sides are given in Table 1. Stimulation of the ipsilateral tibial nerve evoked monosynaptic reflexes approximately equal in amplitude in the ventral roots of $L_6$ on both sides. Polysynaptic reflexes in the root on the "tetanus" side were appreciably increased, in agreement with findings obtained by other workers [8, 10].

When crossed polysynaptic reflexes were compared, the rule discovered for characteristics of DSPs evoked by stimulation of the contralateral nerves was seen more clearly still: reflex responses in ventral roots on the opposite side were appreciably larger in amplitude than the crossed reflexes on the "tetanus" side. The duration of the crossed reflexes depended on the point of entry of the afferent volley into the spinal cord. Stimulation of the afferent nerve on the "tetanus" side evoked tonic activity in motoneurons of both halves of the cord (see Fig. 1A and D), and responses evoked in the ipsilateral and contralateral ventral roots to stimulation of the afferent nerve on the side opposite to that of injection of the toxin were of short duration (see Fig. 1C and B).

## DISCUSSION

Tonic reflexes in tetanus arise at the stage of local tetanus although the tonic character of discharge of the $\alpha$-motoneurons at this stage of poisoning is evidently brought about by increased activity of the muscle receptors, reflecting increased activity of $\gamma$-motoneurons [11].

As was shown above, in animals with ascending tetanus and the UDS phenomenon a single electrical stimulus applied to the dorsal root on the "tetanus" side evoked tonic spinal reflexes even after total deafferentation of the distal part of the spinal cord. Consequently, it was shown that a characteristic feature of efferent activity in tetanus – the prolonged tonic volley – is observed in these animals after both receptors and effectors have been excluded from the reflex arc; i.e., it is central in origin.

Indirect evidence of the part of the reflex arc (in the motoneurons or interneurons) at which the tonic character of the paroxysmal efferent discharge is formed was obtained by comparing the characteristics of discharge of motoneurons on the "tetanus" and opposite sides in response to impulses reaching the motoneurons via different reflex pathways. Since stimulation of the nerve on the "tetanus" side evokes tonic activity in motoneurons in both halves of the cord while stimulation of the nerve on the opposite side gave a

bilateral phasic response, it can be concluded that the formation of the tonic character of the "tetanus" paroxysmal discharge takes place not in the motoneurons, but in the interneurons.

Evidence of the leading role of interneurons in the genesis of tonic reflexes in rats with ascending tetanus and the UDS phenomenon is also given by the results of pharmacological investigations [3, 7]: shortening of the duration of the tonic reflexes in these animals takes place after injection of pentobarbital in doses (5 mg/kg) which are known to shorten the discharge of interneurons considerably but to have no effect on exitation of motoneurons.

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