DIFFERENCES BETWEEN EXCITATION PATTERNS OF FAST AND SLOW MOTOR SYSTEMS IN THE TOAD

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In the preceding papers[7)8)], it has been verified that the fast and slow motoneurons in the spinal ventral horn are under contralateral control of the central efferent pathways (*e.g.* tract. reticulo-spinalis) and fire rhythmically at different rates independently of each other. The accelerating impulses initiating at the bulbar reticular formation activated simultaneously those both motoneurons in the spinal ventral horn, being relayed at the tegmental formation, where efferent fibres started. Many experiments in mammals[14)12)13)] have shown that the spinal motoneurons are innervated contralaterally or ipsilaterally from the reticulo-spinal or the vestibulo-spinal tracts and they receive facilitatory or inhibitory actions as a direct result of algebraic summation of EPSP's and IPSP's. In the present study, differences between excitation patterns of the fast and slow motor systems activated under various conditions will be analysed by observing muscle discharges which appear to represent those motoneuron activities.

METHODS

Decerebrate toads were almost used, except intact preparations in some cases. Spinal columns were opened from the dorsal side, and spinal cord segments were designated with relation to the dorsal roots. A schematic representation of the spinal cord, out-flows of the roots, and vertebrae was shown in Fig. 1, especially drawn in detail concerning the 2nd, 3rd and 4th cord segments, because the present experiment was made exclusively on the forelimb muscles served by the brachial plexus. For the convenience of explaining experiments of cord transection, the above mentioned segments were bisected on the figure and the resulted half segments and the boundaries were named as described in Fig. 1. The transection of the spinal cord was made along these boundaries, by a rapid single stroke with a razor blade.

The operated toad was suspended in a fixation box; the vertebra was fixed upon a ceiling of the box with two steel plates, so extremities were freely movable. The ceiling, made of perspex, has a window as wide as the exposed spinal cord. For stimulation a polystyrol corted tungsten microelectrode with a bare tip of a few micron in diameter was inserted into the spinal cord from

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its dorsal surface. The tip of the electrode was guided stereotaxically towards the spinal ventral horns or the brain-stem reticular formation by means of a micromanipulator set on the author's electrode holding instrument.

Stimulating electric pulses of 4 msec duration and of 0.1–4.0 V intensity were operated synchronously with repetitive sweeps of a cathode-ray oscilloscope.

To record electrical discharges in the spinal cord, another tungsten microelectrode was employed in the same way as that for the stimulation. Muscle discharges were led unipolarily from forelimb extensor muscle (m. anconeus and m. triceps brachii), by means of a platinum electrode. The cord and muscle responses were recorded simultaneously on the screen of a dual beam cathode-ray oscilloscope through two CR-coupled amplifiers having total time constant of 1.4 msec. The experiment was performed from September to April.

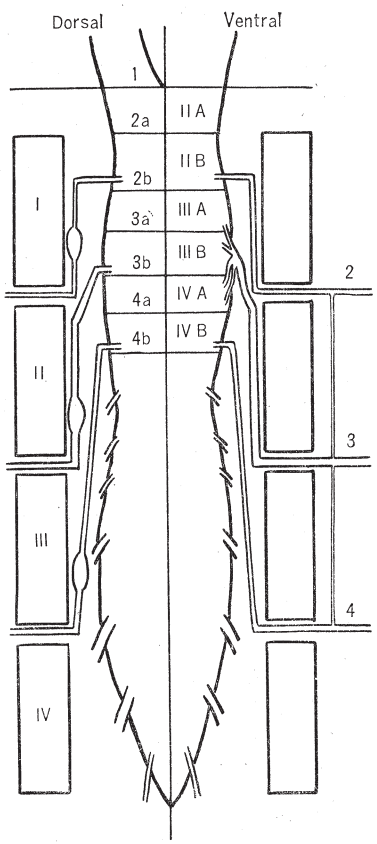

FIG.. 1 Schmeatic diagram of the spinal cord and vertebrae of the toad. I-IV: number of vertebra, 2-4: spinal nerve.

RESULTS

A. Spontaneous fast and slow muscle discharges after transection of the spinal cord

Motoneuron activities were observed in electromyographical records from the forelimb extensor muscles. When the spinal cord was separated from the brain by a complete transection at line 1 (Fig. 1), a series of only fast muscle discharges of about 500 μV in amplitude was recorded exclusively for about 2 minutes after the transection, without slow muscle discharges. The discharge pattern of the fast muscles was almost unitary, and the discharge rate attaining initially to about 20/sec decreased to half in 2.5 minutes after the section, as illustrated in Fig. 2 A and B. On the other hand, the rate of the slow muscle discharges having about 100 μV in amplitude increased gradually, and attained to the maximum rate of about 5/sec in 10 minutes. Thus the two kinds of muscle responses became reverse in a way in about 15 minutes after the transection, and the following slow muscle responses continued approximately for 30 minutes (Fig. 2 C). In Fig. 2, I, discharge rates of the fast and slow muscles were separately plotted against the time course after the cord transection at line 1. Similar courses could be observed also in cases transected at levels 2 a, 2 b, 3 a and 3 b of the spinal cord (Fig. 2, II at the level 2 b and Fig. 2, III at the level 3 b). In all these figures it may be accepted that the

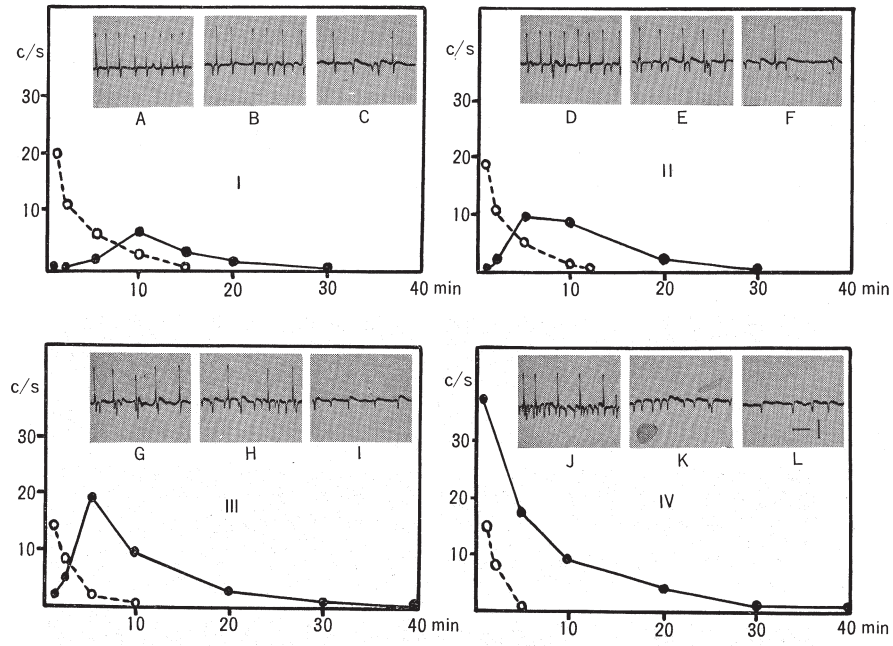

FIG. 2. Diagrams showing frequencies of spontaneous fast (dotted line) and slow (solide line) muscle responses observed after transections of the spinal cord at line 1 (I), 2 b (II), 3 b (III) and 4 b (IV). Three records interpolated in each diagram shows electromyograms in 10 minutes, 20 minutes and 30 minutes after the transection respectively. Horizontal bar: 100 msec. Vertical bar: 100 μV.

discharge rates of the fast muscles decreased along exponential curves and those of the slow muscles were characterized by gradual increase and decrease course, and also noted that the rate of the slow muscle discharges was at first exceeded by that of the fast discharges when the spinal cord was cut at levels 1 or 2, but surpassed the latter soon after the cord transection at level 3.

When the spinal cord was transected at levels 4 a or 4 b, the discharge rate of the slow muscle (about 36 impulses/sec) was higher from the beginning than that of the fast muscle (about 20 impulses/sec) and decreased along an exponential curve of half-decay time about 5 minutes (see Fig. 2, IV). After the fast muscle discharges ceased in 5 minutes, successive slow muscle discharges continued solely for about 40 minutes, as illustrated in Fig. 2, K and L.

B. Excitation patterns of the fast and slow motor systems caused by stimulation of the spinal cord

1) Electromyograms of the forelimb extensor induced by stimulation to various levels in the intact cord (undecerebrate toad).

In an undecerebrated toad, when the 2nd spinal ventral horn (area II A

and B) was stimulated by a supraliminal single pulse of 4 msec duration and of 0.3 V intensity through a tungsten microelectrode inserted into the structure, a synchronized fast muscle discharge of around 300 μV in amplitude and a few unit discharges (100 μV) of the fast muscle were always recorded from the ipsilateral forelimb extensors, as shown in Fig. 3, II, A. With increase in the stimulus strength (0.5 V), the fast muscle discharge was augmented in rate and firing duration, appearing for some time concomitantly with the repetitive slow muscle discharges of about 80 μV (Fig. 3, II B).

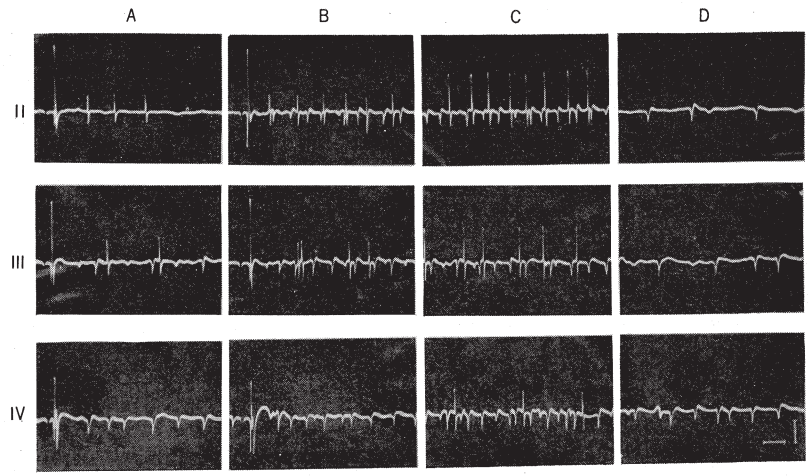

FIG. 3. Electromyographic recordings of responses of the forelimb extensor muscles provoked by stimulation to the 2nd (II), 3rd (III) and 4th (IV) spinal segment, and spontaneous responses after cessation of the stimulation in intact preparation. A, B—Recording during stimulation. Stimulus strength was 0.5 V in A and 0.7 V in B. C, D—Recordings of spontaneous muscle responses in 2 minutes and 10 minutes after cessation of the stimulation. Time: 100 msec. Scale: 100 μV.

The maximal stimulation above 0.7 V resulted in violent volleys of both fast and slow muscle discharges on both sides, causing in body some shocking movements followed by absconding (twisting) movements. During repetitive stimulations of a moderate intensity (0.5 V) at 1 c/sec synchronously with sweeps of the oscilloscope, the rates of both fast and slow muscle unit responses were accelerated, and after the stimulation for about 10 sec, there were followed long lasting after-discharges of both the fast and slow muscle responses, the former early disappeared in 10 minutes, while the latter continued for about 30 minutes (see Fig. 3, II-C, D). These time courses of the both discharges appeared to resemble those after the cord transection mentioned above.

So far as the 3rd ventral horn (area III A and B in Fig. 1) was stimulated supraliminarly or moderately, the discharge patterns of the ipsilateral forelimb extensors were almost the same as those by stimulation to the 2nd ventral

horn, except with limited fast and enhanced slow muscle discharges, as shown in Fig. 3, III, A and B. Stimulations to the 4th spinal ventral horn (area IV, A and B in Fig. 1) caused a doublet of fast and slow muscle responses, followed only by successive slow muscle discharges (Fig. 3, IV A, B).

From the above results, it may be suggested that motoneurons belonging to the slow motor system are located mostly in the 4–5th spinal segment, whereas those to the fast motor system abundantly in the 2nd–3rd.

2) Electromyograms of the forelimb extensors induced by stimulation to ventral horns at different levels of the cord bisected at line 2 b or 3 b.

In a decerebrate toad, the spinal cord was transected again at line 2 b. In this condition, no muscle discharges could be obtained by stimuli of any intensity to the 2nd spinal ventral horn. This result may reveal that the 2nd ventral root does not contribute to the brachial nerve plexus, but to the scapular or neck muscles as is well known in general.

When a supraliminal stimulus (0.3 V) was applied onto the 3rd ventral horn, which was separated from the upper central nervous structures, an initial synchronized fast and successive slow muscle discharges were always recorded as shown in Fig. 4, A. During repetitive stimulations at 2 c/sec in frequency, this typical pattern became more distinct, without accompanying slow muscle discharge. After cessation of the repetitive stimulation, unitary fast muscle discharges were accerelated transiently, reaching its maximal rate (about 10 impulses/sec) in 5 minutes later, while the rate of slow muscle responses was also enhanced in 2 minutes, and then decayed exponentially. The fast and slow muscle discharge rates during and after repetitive stimulation were plotted in respect to the mean numbers of discharges appearing for every 50 msec after stimulation in Fig. 4, E, and to their frequencies in Fig. 4, F, respectively. Similar findings were also observed on pronounced slow muscle discharges by repetitive stimulation to the 4th ventral horn in the same preparation, as illustrated in Fig. 4, B.

When a moderate stimulation (0.7 V) was delivered repeatedly at 2 c/sec in frequency to the 3rd and 4th ventral horns, some series of unitary fast muscle responses occured during and after stimulation while slow muscle discharges were suppressed compensatorily (see Fig. 4, C). From these results it was found out that the fast and slow motor systems were activated alternatively during stimulation to the spinal ventral horns, and that they were triggered to arise their own particular after-discharges after cessation of the repetitive stimulation.

The experiment was renovated under a well-controlled condition, in which the 3rd spinal segment was isolated by transections of the cord at lines 2 b and 3 b. The response induced by a single stimulation of supraliminal intensity (0.3 V) to the 3rd ventral horn were characterized by rhythmic synchronized fast muscle discharges, without any sign of slow muscle discharges, as illustrated in Fig. 4, D. During repetitive stimulations, the discharges were observable synchronously even in the contralateral extensor and flexors, as well as in the ipsilateral forelimb muscles. The 1st spike potential occured with a latency of about 3 msec and followed by the 2nd spike after an interval

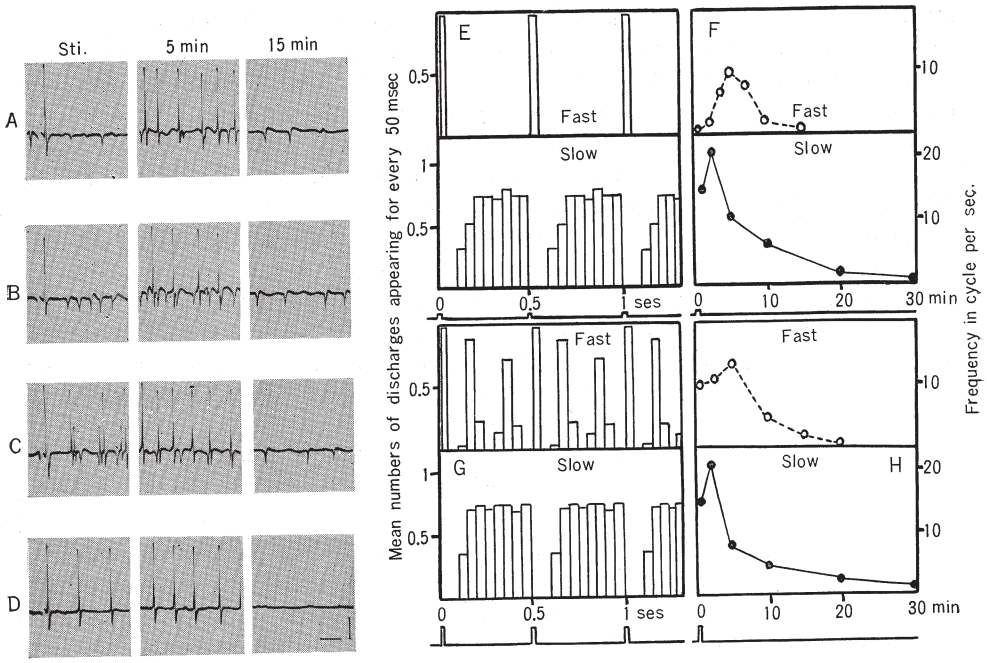

FIG. 4. Fast and slow muscle responses provoked by stimulation to III-IV spinal segments in the decerebrated or the transected preparations. A. Recordings during stimulation of 0.5 V in strength to the 3rd spinal ventral horn in a preparation removed of the upper part above the 2nd segment, and spontaneous responses in 5 and 15 minutes after cessation of the stimuli. Their frequencies of the fast and slow responses were shown in E in which those during repetitive stimuli were plotted with the mean numbers of discharges appearing for every 50 msec. and also in F the rate of spontaneous after-discharges were protted in cycle per sec with the same ordinate as that in E. B. Records during and after stimulation of 0.5 V to the 4th ventral horn. More responses than in A should be noticed. C. Records during and after stimulation of 0.7 V to the 3rd spinal ventral horn in the same preparation, and their frequencies were shown in G and H. D. Same conditions as in records C except that the 3rd spinal segment was isolated from the neighbouring segments. In these records, no slow muscle responses were obtained. Vertical bar: 100 μV. Horizontal bar: 100 msec.

of 185.3 msec in the mean. The interval times were usually shortest at the 3rd and the 4th pauses, being 152.2 msec in the mean, and thereafter restored to a constant of 178.3 msec (5.6 impulses/sec), which resembled closely the discharge rate of a single fast motoneuron demonstrated in the author's other experiments[9)8)]. The after-discharges were also devoid of slow muscle discharges and the rate of the occupying fast muscle discharges changed in the same time course as that of the above mentioned results (see Fig. 4, D).

When the stimulation was applied to the 4th ventral horn in the same preparation, which was separated from the upper central nervous systems by transection of the cord at line 3 b, no discharge from the forelimb muscles

could be recorded, though the activation of the hind limbs took place. It may be suggeted that the 4th ventral root does not innervate the forelimb muscles, rather supplying the upper abdominal muscles, and thence that the above mentioned forelimb muscle responses induced by stimulation to the 4th ventral horn were brought forth through the activities of the 3rd spinal segment mediated by slow motor impulses.

C. Electric activities of the spinal ventral horn and the forelimb muscles induced by stimulation to the mid-brain reticular formation

1) The observation in un-decerebrate preparations.

The skull and vertebral column were opened on the dorsal side, and the exposed brain was covered with liquid parafine. A stimulating microelectrode was inserted into the tegmental reticular formation by means of a micro-manipulator according to the procedures explained in the author's preceding paper[9)8)]. A recording microelectrode was inserted into the 3rd ventral horn contralateral to the site of the stimulating electrode. The cord discharges were recorded simultaneously with the electromyograms led from the forelimb extensors on the same side as the site of the recording microelectrode in the cord.

When a supraliminal stimulation (0.3 V) was applied to the tectum, a few numbers of the cord spikes and a series of slow muscle discharges continuing for about 0.5 sec were always observed (Fig. 5, B). During repetitive stimulations, successive spike discharges in the 3rd ventral horn, having 80 μV in amplitude and nearly 10 impulses/sec in frequency, occured in close correspondence to the successive slow muscle discharges in the forelimb extensors. These corresponding discharges were continued for some minutes after cessation of the repetitive stimulation, as shown in Fig. 5, C.

When the stimulations of moderate intensities (0.5–0.8 V) were applied repetitively at 0.5–1.0 c/sec frequency to the tectum, a burst discharge of large spikes in the cord was evoked independently of the rhythmic fast and slow muscle discharges. The cord discharges arose to the maximal rate at about 140 impulses/sec in 50 msec after each stimulation, and decreased rapidly with a half-decay time of 0.2 sec, as illustrated in the frequency-time relation curve of Fig. 5, A. The fast muscle discharges showed a periodic repetition at 6–12 c/sec, in which each burst consisted of 2–3 spikes at 120 impulses/sec in the maximal frequency, while slow muscle discharges continued for tens of seconds, with a constant rhythm of about 20 impulses/sec.

2) The observation in preparations removed of the spinal cord below the 4th spinal segment.

In an un-decerebrated toad, the spinal cord below the 4th segment was removed by transection of the cord at line 3 b, thence the toad assumed a hypotonic posture, showing neither movement nor tone in the hindlimb and a restricted tonic function in the forelimb muscles. A supraliminal tegmental stimulation (0.3 V) was followed by a few numbers of spike discharges in the 3rd ventral horn, resulting in the fast muscle discharges, exclusively, but not in slow muscle discharges (Fig. 5, E). With increase in the stimulus strength

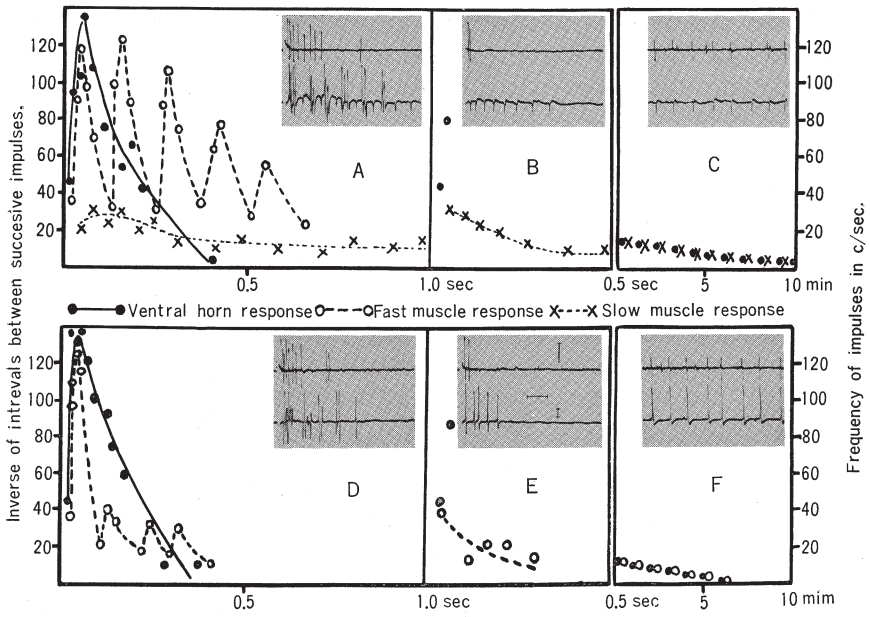

FIG. 5. Simultaneous records of responses in the 3rd spinal ventral horn (upper traces) and electromyograms in the forelimb extensors (lower trances) induced by stimulation to the brain-stem reticular formation. Vertical bar: 100 μV. Horizontal bar: 100 msec. Diagrams of frequency analysis of the responses. A, B; during stimulation of 0.8 V and 0.3 V in strength to the brain-stem in the intact preparation. C; spontaneous responses after cessation of the stimulation. D-F; same conditions as in records A-C except that the spinal cord below the 4th segment was removed. Note the absence of slow muscle responses.

(0.7 V), the discharge rate and the time course of the cord spikes changed in the same way as those in the intact preparation, and somewhat shortened periodic repetitions of fast muscle discharges were also observed, not accompanied by slow muscle discharges (Fig. 5, D).

In their after-discharges, the unit fast muscle discharges were exclusively recorded in place of the slow muscle discharges, yet no change in the firing rates (see Fig. 5, F).

CONSIDERATION AND DISSCUSSION

From all the present experiments, there were verified following four points. 1) The fast motoneurons are located mainly in the 2nd and 3rd spinal ventral horns, while the slow motoneurons relatively rich in the 4th and 5th. 2) The fast and slow motoneurons were found out to fire at their own discharge rates to various stimuli, having different half-decay times in their after-discharges; the fast motoneurons fire at about 20 impulses/sec with about 2.5 msec of half-decay time, while the latter at 12 impulses/sec with half-decay time of 5 msec, respectively. 3) There was found out an alternate excitation between the

fast and slow motoneurons; that is, predominant firings of fast motoneurons always result in restriction of slow motoneurons, and *vice versa.* 4) These two motoneurons appeared to be activated separately by different rates of descending impulses along a common spinal efferent pathway; the fast becomes detectable only when the efferent discharge rate is increased higher than 80 impulses/sec (cf. Fig. 5).

Similar findings have been observed in the author's previous report[10] with reference to the toad's respiratory center, in which the gorge respiration was shown to be caused by continuous slow motor discharges at lower frequency sent via the facial nerve from small respiratory neurons grouped in the medullar reticular formation, while the pulmonary respiration was attributable to a burst of fast motor discharges at rates higher than 80 impulses/sec which initiated at large neurons in the brain-stem reticular formation and reached submaxillar musles through the 2nd spinal ventral horn. Ishida and Mashima[7] stated that the strychnine tetanus in the frog could be elicited only by strychnization to the part of spinal cord between the 4th and the 5th intervertebrae, where was shown schematically to coincide with the 4th and the 5th spinal cord segments. Since strychinine is well known to depress the synaptic action of inhibitory interneurons, they concluded that the cell bodies of their inhibitory interneurons would be mainly located at the level between the 4th and 5th segments. The location of the inhibitory interneuron pool appears to correspond to the site of the slow motoneurons described in the present results. Actually in the present experiments, the predominant slow motor firings suppressed the fast motor excitation, and *vice versa.* The inhibitory effect of these motoneurons, however, seems to be not so strong as two types of typical inhibitions demonstrated by Eccles[2]; it may be rather considered to resemble an undecided inhibitory mechanism of synaptic transmissions in a reticulo-spinal pathway reported by Bremer[1].

It has been assumed in the present study as well as in our previous report[9] that the fast and slow motoneurons were not controlled separately by their respective efferent pathways from the particular nervous center, but they were excited individually by different rates or patterns of impulses descending along a common efferent pathway which might be recognized to coincide with the fasciculus longitudinalis medialis or the ventral tegmental fascicule designated by Herrick[5][6]. Similar assumption was also obtained on the frog's small-nerve reflexes by Kuffler *et al.*[11], who stated that the differences between the small-nerve and large-nerve reflex systems seemed to be largely quantitative; *i.e.*, small-nerve reflexes were caused by weak single or tetanic stimuli to the proximal end of severed afferent nerves, and twitches set up with increased stimulus strength or frequency. Gualtierotti[3][4] also argued that the inhibition and facilitation for spinal reflexes in the frog did not arise in different regions of the brain stem, but the difference between them was correlated only with the strength of the stimulation.

SUMMARY

Topographical and physiological properties of the fast and slow motoneurons

in the toad were studied by analizing muscle discharges of the forelimb extensors induced by stimuli to the brain-stem and the spinal cord.

1) The fast motoneurons are located mainly in the 2nd and 3rd spinal ventral horns, while the slow motoneurons relatively rich in the 4th and 5th.

2) The fast and slow motoneurons were found out to fire at their individual discharge rates to various stimuli and with distinct half-decay times in their after-discharges after cessation of the stimuli; the former having about 20 impulses/sec in frequency and about 2.5 msec in half-decay time, while the latter 12 impulses/sec and 5 msec, respectively.

3) There was found out an alternative excitation between the fast and slow motoneurons; that is, predominant firings of the fast motoneurons always resulted in restriction of slow motor activities, and *vice versa*.

4) These two types of motoneurons appeared to be activated separately by different rates of descending impulses along a common spinal efferent pathway; the fast was detectable only when the efferent discharge rate increased higher than 80 impulses/sec.

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