EXCITABILITY OF THE MEMBRANE OF LUMBER MOTOR NEURONS AND NATURAL SLEEP IN THE CAT

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Amplitude of the monosynaptic reflex is smaller during so-called activated sleep than during slow wave sleep in the cat spinal cord with or without the reflex control through the fusimotor loop^{7,8,10}. A question may arise as to whether any inhibitory mechanisms, pre- and post-synaptic, are operating during the former state. Intracellular recording of the motor neuron in the chronic state would give a crucial answer to this question. This seemed technically infeasible. However, as a substitute, the excitability of the motoneuronal membrane and the primary afferent nerve terminal may be tested by the local stimulation of the spinal cord. In a preliminary study, size of the antidromic focal potential during the two sleep states was studied and its definite change was not revealed¹⁷.

It is attempted in this paper to observe the excitability change of the motor neuron at a cellular level. A method was used to stimulate the cord so that a limited number of lumbar motor neurons could be excited directly and to record from the hindlimb muscles the evoked single motor unit activities. It will be shown that during the activated sleep the motoneuronal membrane becomes less excitable. Preliminary reports have appeared^{11,17}.

METHODS

Eight adult cats were used. Recording electrodes were implanted aseptically in the skull for EEG, neck muscle for EMG and sciatic nerve¹⁰.

For the cord stimulation one or two copper wires (90 μ in diam.) covered with a polyurethane film of 15 μ thick except the cut end were thrustured blindly into the ventral part of the spinal cord at the L5-S1 level in a dorso-ventral direction through a punctured hole of the pia. They were fixed by stitching to the dorsal surface of the pia at the more caudal level. As a reference for both stimulation and recording, a polyurethane-coated copper wire (150 μ in diam.) was thrustured into the dorsal part of the cord at the S2 level. Its tip was exposed for 1-2 mm. Dorsal roots were cut

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bilaterally from L5 to S2. This procedure helped to reduce body movements. After the electrodes were implanted, the dura mater was tightly sutured by the threads used for ophthalmological surgery (Elp eyeless needles, Toyohara Co., Tokyo). Experiments were done for 1-4 days after the implantation. Activities of flexors of the ankle and knee joints (tibialis anterior, extensor digitorum longus and biceps femoris) and ankle and knee extensors (soleus, gastrocnemius, probably plantaris, and quadriceps) were evoked by the cord stimulation and were recorded by two syringe needles (diam. 1 mm) insulated with polyurethane except the tip and separated by 1-3 mm from each other. As it was not always possible to identify the name of ankle muscles, they were simply classified as ankle extensors or ankle flexors. In most of the cats the knee flexors and extensors were denervated.

In the chronic state an appropriate single shock to the cord (0.1-0.3 msec, less than 9 V; either anodally or cathodally) elicited a 'single' motor unit activity of polyphasic shape in all-or-none manner. To assess the direct excitation of the motor neuron by the cord stimulation, the laminectomy was performed under pentobarbital anesthesia after the chronic experiment, and the ventral root (L6, L7, or S1) was mounted for stimulation at the cord entry. The shortest latency of a given muscle activity thereby induced was compared with the latency of the 'single' motor unit which had been obtained by the cord stimulation in the chronic state. When the former was shorter than the latter by less than 0.4 msec, the motor unit activity was considered as resulted from the direct excitation of the motor neuron. The value of 0.4 msec will not leave a doubt that a single motor unit is excited transsynaptically (Cf. 16). Alternatively the following measurements could be done in the chronic state. Both the EMG mass response and the antidromic field potential of motor neurons were produced by supramaximal stimulation of the sciatic nerve. When sum of the both responses was shorter by less than 0.4 msec than the latency of the single motor unit by the cord stimulation, the single unit was similarly considered as directly excited.

The same single motor unit activity could be recorded for several hours without any distortion of the potential shape.

Intensities of the cord stimulation to evoke the same motor unit were compared between the slow wave sleep and the following activated sleep. The threshold voltages were determined in the slow wave sleep state. It is represented by the arithmetic means between the highest voltage evoking no motor unit activity by successive 10 trials of approximately 1 cps stimulation and the lowest voltage evoking 10 responses to the same repetitive stimulation. The threshold change during the activated sleep was studied. Although stimulus intensity is not represented in terms of the current, it was always monitored on the oscilloscopic screen as the voltage drop across the small resistor (1 K, 100, or 50 Ω) which was inserted in series with the output of the ISU of the stimulator (NIHON KOHDEN MSE 3, output impedance of the ISU was less than 400 Ω). This recording was used to confirm that the threshold increase during activated sleep to be described, is at least in part, not due to the impedance increase of the cord tissue as a whole between two intraspinal electrodes.

The experiment was completed by passing d.c. current between the two cord electrodes for checking the electrode position. Then, the cord was fixed by 9% formaline and stained with Kluver-Barerra method. An example of the stained spinal cord is illustrated in Fig. 2.

Recording equipment and experimental environment for the animal were the same as previously described¹⁰⁾.

FIG. 1. Excitability test of an ankle flexor motor unit during natural sleep. Polygraphic recordings: from top to bottom, neck EMG (neck), EEG of somatosensory area (MC), eye movement (EYES), and evoked single motor unit activity from one of ankle flexors (FLEX). Left ventral quadrant of the spinal cord at L6 level was stimulated cathodally by a pulse of 0.1 msec duration. (Site of the tip of the active electrode is seen in Fig. 2). Oscilloscopic traces at the bottom illustrate samples of motor unit responses evoked by 1 cps stimulation for 10 sec (upper traces of A, C, E, L, N and P), together with the simultaneously recorded current intensity of the shock (lower traces). Mass responses in the muscle (S) and in the cord (not illustrated) were recorded by the supramaximal stimulation of the sciatic nerve at the hamstring nerve branching, the latencies were 1.8 msec and 1.0 msec respectively. Sweep speed in S is two times and amplitude sensitivity is 1/5 of the calibrated marks.

The stimulus voltage in each excitability test was represented in multiples of threshold below the polygraph. In bracket is shown the firing index (F.I., number of responses/number of trials) during 10 repetitive shock trains by 1 cps. The muscle activities of burst type as seen in the P and J responses in FLEX trace probably are not the twitch-like activity which is typical of the activated sleep. Because such pattern is seen in the control state (B). This cause is not known.

RESULTS

FIG. 1 illustrates, together with polygraphic recordings of neck EMG, EEG and eye movement, an example of threshold changes of the single motor unit of an ankle flexor elicited by the cord stimulation during a transition from the slow wave sleep to the activated sleep. The EMG unit responses were recorded on the ink-writer in the fourth trace of the polygraph. Some of them are shown in the bottom (A, C, E, L, N, P). This unit responded with 3.1 msec latency from the cord and satisfied the criteria of the directly excited activity (Methods). Shocks of varied intensity were given and the firing index (F.I.) of the motor unit was calculated from 10 responses evoked by 1 cps stimulation for 10 sec. During the slow wave sleep, as seen to the left of FIG. 1, shocks were given in decreasing order of intensity. The unit was

FIG. 2. Spinal cord section to show the electrode position used to evoke the response illustrated in FIG. 1. Tip locates at the medial portion of the left motor neuron pool (L6). Extreme distortion of the implanted side of the cord is due to the inadequate suturing of the dura during the preliminary operation.

always evoked in A and was not at all in E. In B, C, and D, some of shocks failed to evoke the response. From the voltages in A and E the defined threshold was calculated as 3.32 V, which is approximately identical with the voltage used in C. Numbers shown below the polygraph are the shock intensities relative to the threshold of the slow wave sleep state. Numbers in

FIG. 3. Threshold increase of a motor neuron which innervates a single ankle extensor motor unit during a transition from slow wave (A and B) to activated sleep (D, E and F). Stimulus intensity of the cord is illustrated in the number at bottom left in each figure. Latency of the motor unit was 2.80 msec. C1: mass EMG response recorded through the same electrode as used for the unit recording at the same position within the muscle after the activated sleep episode. Sciatic nerve was stimulated supramaximally at a point where the hamstring nerve gave off (latency 1.65 msec). C2: antidromic focal potential of the motor neuron evoked by the supramaximal sciatic nerve stimulation, similar stimulus as C1 but recorded 2 days after the experiment. Sweep speed for C1 and C2 is two times faster than the indicated calibration.

brackets show the F.I.'s for respective trials. In the right half of the polygraph, the tonic neck EMG activity became almost silent, EEG showed low voltage fast wave pattern and eyes began to move around; thus the activated sleep developed. During the activated sleep even the shock 14-16% higher than the control threshold during the slow wave sleep failed to evoke the unit responses (L, Q).

FIG. 3 illustrates another example of the threshold increase of a motor unit which belongs to the ankle extensor (latency from the cord stimulus, 2.80 msec). During the slow wave state, the response was not evoked in A (2.08 V) and was always evoked in B at slightly high intensity (2.18 V). Thus, the calculated threshold intensity is 2.13 V. Responses in D, E, and F were

TABLE 1.

Summary of 15 motor units whereby threshold increase during activated sleep were observed. Parentheses of (3) represent latency of the EMG recorded by the electrodes picking up the unit response and latency of antidromic negative response of the motor neuron by the stimulation of the same peripheral nerve.

(1) No. of motor unit	(2) Unit latency	(3) Mass EMG latency from VR	(4) Difference (2)-(3)	(5) Threshold at slow wave sleep	(6) Threshold increase at activated sleep
(ankle extensor)					
1	3.0 msec	2.6 msec	0.4 msec	6.1V	33%
2	2.8	2.7	0.1	4.6	4
3	2.6	2.4	0.2	2.8	18
4	2.6	2.4	0.2	3.2	11
5	2.8	(1.0+1.6)	0.2	2.1	10
6	2.9	(1.0+1.7)	0.2	2.1	5
7	2.7	2.5	0.2	1.9	6
	(2.77)	(2.53)	(0.21)	(3.25)	(12.4=m)
(ankle flexor)					
8	3.0	2.7	0.3	2.7	11
9	2.8	2.7	0.1	3.2	10
10	2.7	2.7	0	2.2	12
11	2.7	2.7	0	2.5	6
12	3.1	(1.0+1.8)	0.3	3.3	17
	(2.86)	(2.72)	(0.14)	(2.78)	(11.2=m)
(knee flexor-Biceps femoris)					
13	2.5	2.2	0.3	8.1	14
(knee extensor-Quadriceps femoris)					
14	1.6	1.6	0	3.5	40
15	1.8	1.6	0.2	3.9	22
	(1.7)	(1.6)	(0.1)	(3.7)	(31=m)

FIG. 4. Directly evoked EMG responses and rapid eye movement. From top to bottom; ink-writing recordings of neck EMG, somato-motor EEG, eye movement and EMG response of the ankle flexor. The EMG was evoked by cord stimulation at intensity of $1.25 \times$ threshold of the direct elicitation of the EMG during slow wave sleep at a repetition rate of once a second. At the right is shown the oscilloscopic trace of the EMG obtained from responses underlined below the fourth trace. Its latency was 2.95 msec.

recorded during the activated sleep state. In D, the same intensity as in B did not evoke the response. In E at slightly higher intensity (2.38 V), the unit responded to two of ten shocks. In two responses slight difference of the latency is noted (0.2 msec). The response is always evoked in F at higher intensity (2.44 V).

TABLE 1 summarizes the data of 15 motor units.

The average threshold increase during activated sleep was about 15%. No units were found which showed the threshold decrease during the activated sleep. When animals were awakened from the activated sleep, the threshold became higher or lower, depending probably on flexion or extension postures of the limb. When the animal returned to the slow wave sleep, the threshold returned to the value within the range of 15% of the preceding control (8 units). In 2 units were observed two successive episodes of the activated sleep during which the threshold increases were of similar order.

It has been reported that the monosynaptic reflex was transiently depressed during the rapid eye movement^{7,8}. However, constant threshold increase or decrease which closely associated with the rapid eye movement were not found in this study (FIG. 4).

DISCUSSION

During the activated sleep compared to the slow wave sleep, the lumbar motor neuron was less excitable in both flexors and extensors, as far as tested by the threshold change of the single motor unit evoked by the direct excitation of the motor neuron.

According to ÅSTRÖM and PORTER, by the local stimulation of the lumbar motoneuron pool in the cat, the axonal part of the spinal motor neuron was more easily excited than the soma or dendrites^{1,15}. On the contrary, LORENTE DE NÓ interpreted that in rabbit abducens the motor neuron soma had a lower threshold than the axon. In view of LORENTE DE NÓ's data, ÅSTRÖM interpreted his results as that the soma was probably destroyed by the penetration of electrode. In this paper it was impossible to infer which part of the post-synaptic structures were excited by the cord stimulation. However, the threshold increase during the activated sleep appears to reflect, more or less, the hyperpolarization of the motor neuron membrane. Probably it is caused by the IPSP^{2,4}) or subsidence of the EPSP.

As one factor of the latter case the presynaptic inhibition may be nominated. There is evidence that within the lateral geniculate body the optic tract fibers are depolarized during the activated sleep, indicating the pre-synaptic inhibitory influences thereupon to reduce the incoming volleys⁹.

However, evidence of the depolarization of primary afferent terminals in the spinal cord could not be available during the activated sleep¹⁶. Appli-

cation of sufficient picrotoxin which would block the presynaptic inhibition in the monosynaptic pathway did not block the MSR depression in the activated sleep¹⁶⁾.

Therefore, presynaptic inhibitory mechanism appears as not involved to depressed the monosynaptic pathway during activated sleep.

SUMMARY

Excitability of the single motor neuron was tested by stimulating directly the lumbar cord in unrestrained and unanesthetized cats, and was compared between sleeps with slow wave and spindle burst in EEG (slow wave sleep) and sleep with low voltage fast wave in EEG (activated sleep). During the latter, the threshold to elicit the single motor unit by the direct excitation of the motor neuron increased in both hind-limb flexors and extensors.

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