The Monosynaptic Origin of the Fast Synchronous Wave in Rat Spinal Reflex

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When a hind-limb muscle nerve or a lumbar dorsal root of rats was stimulated, a fast and well-synchronized wave could be lead off from the appropriate lumbar ventral root and was named 'the fast synchronous wave'. While this fast synchronous wave resembled in shape and appearance to the cat monosynaptic reflex, it differed in some features from the latter, *i.e.*, rather long latency, absence of post-tetanic potentiation and not being most excitable of all the reflex components. To testify whether the fast synchronous wave is monosynaptic or not, and if monosynaptic, to what causes its characteristics should be attributed, electrophysiological technique and Nauta method were employed. It was concluded that the fast synchronous wave was monosynaptic and its specific features were due to the poor functional connection between primary afferents and motoneurons.

A wealth of informations about peripheral and central nervous system has been accumulated so far from the experiments on the cat. These informations, however, tend to be applied to the interpretation of the physiological or functional meaning of the nervous system of other species without serious criticism. In this connection, it is at once of interest and of use to find out inter-specific difference and community in the basic structure of the nervous system such as the spinal cord. The rat is though to be suitable for this purpose because of its handedness and availability as experimental animal in the wide field of neighboring sciences. The electrophysiological approach to the rat spinal cord has scarcely been done, and the exact and reliable data on the rat spinal reflex has not been offered. Thus, the present experiments were undertaken, bearing in mind that a specific functional characteristic peculiar to a species has a specific structural basis as background and that the mode or pattern of their integration, losing narrow peculiarity, comes to have wider meaning applicable to the nervous system of other species which in turn is helpful to build up the general scope of the functioning of the nervous system.

In the present experiments, the monosynaptic origin of the fast synchronous wave in the rat spinal reflex was confirmed, and the causal factors of some characteristics of it were discussed in connection with the specific structural basis in the rat spinal cord and peripheral nervous system.

MATERIALS AND METHODS

Sixty albino rats (Toma strain) weighing 300–500 g were used. They were anesthetized with intraperitoneal injection usually of 40 mg/kg of pentobarbital sodium (Nembutal), or in a few cases, of 4 ml/kg of 25% urethane. After tracheal cannulation, except for a few animals in which the central nervous system was left intact, the spinal cord was transected at the lower thoracic level, avoiding the arterial bifurcation at ventral surface.¹ Sometimes artificial respiration was performed at the rate of 60 per minute with 15 ml stroke volume. The anesthetic dose was intermittently added at the rate of one fourth of the initial dose to maintain the constant physiological state of the animals. After laminectomy, the dorsal and ventral roots of L4, L5 and L6 segments were identified and dissected out under the binocular microscope. The reflex potential was recorded at the central cut-end, mainly of L5 and sometimes of L4 and L6 ventral roots. The sciatic nerve in rat receives its constituent fibers mainly from L5 and partly from L4 and L6.² The peripheral branches of sciatic nerve such as tibial, common peroneal, sural, medial and lateral gastrocnemius nerves and a cutaneous branch distributing lateral surface of the thigh, were also dissected out. The skin flap of operated parts was raised and fastened to a wire ring to make a cavity which was filled with warmed liquid paraffin. The temperature of the paraffin pool was maintained by radiation of an infrared lamp, and the animal was warmed by a heating pad inserted under the stomach, when necessary. The stimulation and the recording were made mainly through platinum bipolar electrodes and sometimes through silver bipolar electrodes. For stimulation square pulse having duration of 0.1–0.2 msec was applied from an electronic stimulator (MSE-3 NIHONKOHDEN). The action potential picked up from bipolar electrodes was fed to a condenser-coupled amplifier with time constant of 0.03 sec, displayed on a two channel cathode ray oscilloscope (VC-6 NIHONKOHDEN) and photographed with a long recording camera. The sweep of CRO was triggered by the stimulator.

For intracellular recording, glass capillaries filled with 3M KCl and having 30–50 M Ω resistance, were used. They were introduced into the spinal cord from the exposed dorsal surface by a micromanipulator under the binocular microscope. A motoneuron was identified when it fired antidromically to the stimulation of the appropriate ventral root. The action potential intracellularly picked up was fed to a direct-coupled preamplifier with cathode-follower input (MZ-3B NIHONKOHDEN), displayed on the CRO and photographed with a long recording camera.

To know the distribution of primary afferent fibers in the spinal cord, and to determine the monosynaptic contact of them with motoneurons, the modified Nauta method by silver impregnation was adopted. Three adult rats were used for this experiment. Under Nembutal anaesthesia the spinal cord was exposed dorsally large enough to allow unilateral L5 dorsal root to be cut intradurally. Care was taken to make the opening of dura mater as small as possible not to impair the spinal cord and the blood circulation. After survival period of 3 to 4 days, the animals were perfused with acasia-saline and then with 10% formol-acasia-saline through the ascending aorta of the heart after the suggestion by Koenig, Groat and Windel.³ The spinal cord thus fixed was transferred into 10% formalin and divided into respective segmental blocks under the binocular microscope. Frozen sections were cut at 30 μ from the respective lumbar segments and stained with the modified Nauta silver technique. As a criterion of axonal degeneration Gleses and Nauta's description—'drop-like disintegration'—was strictly adhered.

RESULTS

In a good experimental condition, a fast well-synchronized wave could be observed in rat spinal reflex evoked by the stimulation of an appropriate dorsal root, a muscle nerve or a mixed nerve. But it was never evoked by cutaneous nerve stimulation. This is shown in Fig. 1. This fast synchronous wave was

Fig. 1. Reflex potential of the rat spinal cord. Recording was made from the central cut-end of L5 ventral root, while single stimulus was applied to the central cut-end of the ipsilateral L5 dorsal root (left one), and to the central stump of the lateral gastrocnemius nerve of the homolateral side (right one). The arrow points to the stimulus artifact.

inferred to be monosynaptic from its short latency by Nagai *et al.*⁵ However, this wave differs in some respects from cat monosynaptic reflex and even the latency itself is not so short as to leave no doubt for its monosynapticity. Closer examination is required to determine whether the fast synchronous wave of rat spinal reflex is monosynaptic or not. In Table 1, the latency—the time between stimulus artifact and the wave front of the fast synchronous wave—is indicated from experiments on 16 animals. To facilitate latency analysis, only the results from the segmental reflex were chosen, where the conduction path was

TABLE 1. *The latency of the fast synchronous wave produced by liminal and maximal shock among different animals*

Animal No.	Stimulation site—recording site	Latency when shock is liminal	Latency when shock is maximal	Difference
650724	L5DR-L5VR		1.33	
651027	L5DR-L5VR	2.00	2.00	0.00
671006	L5DR-L5VR	3.21	3.04	0.17
671013	L4DR-L4VR	2.50	2.22	0.28
671016	L4DR-L4VR	2.27	2.04	0.23
671023	L4DR-L4VR	2.31	1.76	0.55
671030	L4DR-L4VR	2.59	2.22	0.37
671030	L5DR-L5VR	2.87	2.59	0.28
671124	L5DR-L5VR	3.25	2.88	0.37
671205	L5DR-L5VR	2.00	1.90	0.10
671211	L5DR-L5VR	2.88	2.75	0.13
671216	L5DR-L5VR	2.76	2.50	0.26
671218	L5DR-L5VR	2.44	2.44	0.00
671222	L5DR-L5VR	1.85	1.85	0.00
680217	L5DR-L5VR	2.69	2.56	0.13
680304	L5DR-L5VR	2.44	2.44	0.00
680322	L5DR-L5VR	2.35	2.21	0.14
No. of cases		16	17	16
Mean		2.53	2.28	0.25

short, which enabled to avoid the error in measuring conduction time. The mean shortest latencies of the fast synchronous wave to liminal shock and to maximal shock were 2.53 msec and 2.28 msec respectively, which was rather too long to be monosynaptic, compared with the results obtained from the experiment on cat.⁶ The shortening of the latency from 2.53 msec to 2.28 msec, when the stimulus intensity was raised from liminal to maximal one, might be due to the increased synaptic drive by augmented action of the concerned afferent fibers, as well known from the experiments on cat spinal reflex.⁷ The duration of the fast synchronous wave was approximately 2 msec which is rather long, too.

In cat there has been observed a period of enhancement of responsibility after intense brief burst of presynaptic volley, *i.e.*, post-tetanic potentiation, in a simple system composed of pre- and post-elements having excitatory connection, like neuromuscular junction,⁸ sympathetic ganglia⁹ or monosynaptic reflex.¹⁰ The characteristic feature of the fast synchronous wave is the absence of post-tetanic potentiation, or more exactly the existence of post-tetanic depression. In many experiments where various combinations of frequency, duration and intensity of repetitive stimuli were applied to the central cut-end of dorsal root or to a muscle

Fig. 2. The representative record of post-tetanic depression of the fast synchronous wave. Upper inset graph: the reflex potential was picked up from the central cut-end of L5 ventral root, while stimulating pulses were delivered once every five seconds to the peroneal nerve except for during tetanic stimulation period and the amplitude change of the fast synchronous wave before and after the tetani was compared. The three rectangles mean the tetanic stimulation, the duration of which was kept same, 10 sec. Each number in the rectangle corresponds to one hundredth of the actual stimulus frequency, *i.e.*, 1 to 100 cps, 2 to 200 cps and 3 to 300 cps. Lower inset graph; in another rat, single unit discharge, which contributed to the fast synchronous wave, was lead off from a thin filament split from the central stump of L5 ventral root, while stimulating pulses were delivered once ever ten seconds to the medial branch of gastrocnemius except for during tetanic period. When the unit fires to a stimulus, a black dot is plotted at the level of 1 and when it fails, plotted at the level of 0. The rectangles mean the tetani, the duration of which remained same, 10 sec, and the frequency of which is shown by the numbers by the rectangles. Throughout the above experiment, the stimulus intensity was kept maximal. Note the frequent failure of unit discharge after tetanic stimulation.

nerve, no potentiation after tetanic stimuli was observed. Generally speaking, the after-effect of tetanic stimulation on the fast synchronous wave was depression though the degree of the depressive effect differed from case to case, and had no consistent relation with stimulus frequency and duration. In Fig. 2, the above inset record shows an example of post-tetanic depression. After tetanic stimulation depression ensues and lasts more than two minutes. Two factors can contribute to the depression: the one is the temporal dispersion of motoneuron firing and the other, the cessation of motoneuron firing. To determine which factor was responsible for the depression after tetanic stimuli, nerve filaments containing single motor unit were freed from L5 ventral root trunk for recording. The lower inset record in Fig. 2 shows the representative result of such an experiment. It should be noted that immediately after tetanic stimuli, the motor unit has a strong tendency to become silent and firing probability falls severely. At the same time, latency measurement of this motor unit reveals the considerable prolongation and instability of the latency after tetanic stimuli. So it can be concluded that the depression is attributed both to the cessation and to the temporal dispersion of motoneuron firing.

Whatever difference there may be in the fast synchronous wave from the cat monosynaptic reflex, the definition of monosynaptic reflex is only related with its path, *i.e.*, two neuron arcs and one intervening synapse. So it is essential to determine the path, through which pass the impulses destined to evoke the fast synchronous wave. In this regard, intracellular technique is quite helpful to determine the path in spinal cord. Fig. 3 represents a sampled record of the fast synchronous wave (A) and of an orthodromic spike of an impaled motoneuron which contribute to the fast synchronous wave (B). It should be noted that the interruption between EPSP and spike is fairly long. To make closer examination of the relationship between EPSP and spike, the stimulus intensity was kept at threshold (C). The EPSPs, of which latency was slightly long to be monosynaptic, were assumed to be due to the slower conduction in the peripheral afferents and the intraspinal collaterals.

This was affirmed by measuring the conduction time, both afferent and efferent, and by analyzing size distribution of dorsal and ventral root fibers, and of peripheral afferent and efferent component.² The peripheral conduction time differs considerably from animal to animal, probably owing to the experimental condition. Generally speaking, the efferent conduction is faster than the afferent one in rat peripheral nerve: the former is on an average 65–80 m/sec and the latter, 50–70 m/sec when measured at the body temperature. These values are definitely smaller than those of cat. The fact that the efferent conduction is faster than the afferent one is quite in line with the result of the fiber analysis, which shows that the efferent path contains larger fibers more profusely than the afferent one and in periphery the largest efferents are larger than the largest afferents.² Furthermore the afferent component of the rat muscular as well as cutaneous nerves almost lack the fibers more than 12μ in diameter, GI fibers in cat muscle nerves. As daughter fibers branching off from a thin stem fiber tend to be also thin, it is reasonable to

Fig. 3. The segmental compound reflex potential picked up from the L5 ventral root to the stimulation of L5 dorsal root (A), and in the same animal an intracellular record of a motoneuron discharge to the orthodromic stimulation of about the same intensity as in the upper record (B). This motoneuronal discharge was inferred to form the fast synchronous wave from its latency. The arrows indicate from left to right the entry of the stimulus, the onset of EPSP and the transitional point from EPSP to spike. Note the wide gap between the onset of EPSP and the start of spike, and the residual EPSP. A superimposed intracellular record of a motoneuron discharge from another preparation to the orthodromic stimulation maintained just at threshold intensity (C).

think that the intraspinal collaterals of primary afferent fibers of rat should be thin compared with those of cat, which contribute to the delay in the latency of monosynaptic EPSP. From above consideration, it was concluded that the EPSP, the average latency of which is 1.27 msec, was evoked by direct impulses passing through primary afferents.

Table 2 shows the other factor causing the latency prolongation. In this experiment the central cut-end of L5 dorsal root was stimulated for orthodromic excitation and that of L5 ventral root for antidromic excitation, while recording was made from individual impaled motoneurons through microelectrodes. To be noted is that the separation time between the onset of EPSP and the firing of motoneuron is considerably long, *i.e.*, 0.94 msec. This seems too long for one synaptic delay, considering analogous values from other nerve circuits. But from close examination of the relationship with the rising phase of EPSP and the onset of spike it can be safely said that the spike is monosynaptically driven. This is the main factor which contributes to the latency prolongation of the rat monosynaptic reflex. Assuming that the conduction time from motoneuron somata down to the

TABLE 2. *Numerical data from intracellular record of motoneuron discharge*

Cell No.	Latency of antidromic spike	Latency of EPSP	Latency of orthodromic spike	Latency difference between EPSP and orthodromic spike
671124-3		0.86	2.19	1.33
671124-5		1.20	2.10	0.90
671128-3	0.95	1.00		
671205-3		1.25	2.06	0.81
671205-5		1.37	2.44	1.07
671205-11		1.41	2.35	0.94
671205-12		1.47	2.41	0.94
671205-14		1.47	2.30	0.83
671208-1	0.45	1.45		
671216-1	0.25	1.12		
671216-3	0.31	1.06		
671216-5	0.19	1.00		
671216-6	0.67	1.00		
680119-1		1.50	2.12	0.62
680126-5	0.35	1.54		
680126-6	0.77	1.58		
680202-1	0.37	1.04		
680202-2	0.37	1.18		
680202-3	0.48	1.15		
680219-1	0.43	1.33	2.34	1.01
680219-2	0.81	1.19	1.71	0.52
680304-2		1.25	2.50	1.25
680304-3	0.67	1.33		
680304-4	1.20	1.33	2.13	0.80
680304-5	0.62	1.19	1.81	0.62
680305-2		1.50	1.93	0.43
680312-2		1.13	2.39	1.26
680312-3	0.98	1.48	2.26	0.78
680322-3	0.60	1.48	2.24	0.76
No. of cases	18	29	17	17
Mean	0.56	1.27	2.21	0.94

recording site at ventral root is comparable to the antidromic conduction, total reflex time of the fast synchronous wave can be calculated from the numerical data cited here, that is, the sum of 2.21 msec from the stimulation at dorsal root to the generation of spike and 0.56 msec from the generation of spike to the recording site at ventral root. Thus, 2.77 msec can be obtained as a total reflex time of the fast synchronous wave from the intracellular recording. This value is well in accordance with that obtained from the experiment on compound reflex potential, 2.53 msec in Table 1. The slight difference between both values may be due to error in sampling and measuring, and also to the fact that the results shown in Table 2 contain those from some depolarized cells.

Furthermore, to demonstrate morphologically the direct contact of intraspinal collaterals of primary afferents with motoneurons and the topographical orientation of their synapse, Nauta silver technique was employed. The degenerated axons in the spinal cord showed intermittently succeeding bead-like structure, 'drop-like disintegration' according to the description by Glees and Nauta⁴ against orange

Fig. 4. A photomicrograph of a motoneuron surrounded by degenerating primary afferent fibers, synthesized with a number of sectional photographs taken at different focus (upper). The arrow in the cross-section of the spinal cord (lower) points to the motoneuron illustrated at left. The orientation of the motoneuron in the left photomicrograph corresponds almost to the actual position in the spinal cord. The 'drop-like disintegration' can be observed profusely especially at the upper left of the motoneuron and the close examination shows the degenerating axons running obliquely from upper left to lower right.

Fig. 5. The degenerated collaterals of the dorsal root fibers, which had been sectioned beforehand, were dotted on the traced cross-section of the L5 spinal cord.

background. Using oil immersion objective the preparations were magnified 1,000 times and observed. As shown in photomicrograph in Fig. 4, the collaterals of primary afferent fibers distribute considerably densely among dendrites as well as somata of large ventral horn cells, that is, of motoneurons in all probability. The strategic orientation of synaptic contact on motoneuron surface could not be demonstrated. In Fig. 5, the degenerated primary axons were dotted on a cross section of the 5th lumbar spinal cord, the contour of which was traced on projection screen (Nikon) with 40 $\times$ magnification. The degenerated axons can be seen only in right half of the spinal cord, of which L5 dorsal root was cut beforehand. The dorsal root fibers enter spinal cord from dorsal funiculus and emerge into upper medial dorsal horn, where they spread profusely, slightly deviating laterally. Here it can be seen clearly that the axon collaterals of primary afferent fibers distribute among large ventral horn cells.

DISCUSSION

The characteristic features of rat monosynaptic reflex thus far described can be explained most smartly and simply, when it is assumed that in rat spinal cord, the functional connection of primary afferent fibers with motoneurons is scanty or weak, compared with that of cat. There are accumulated evidences in line with this hypothesis. The strongest support comes from the intracellular recording, which shows the considerable gap between the onset of EPSP and the generation of spike. This is a strong evidence which indicates the frailty of the connection between primary afferent collaterals and motoneurons. Whether this frailty is due to the paucity, or to the strategic topographic location of monosynaptic contacts, or to both can not be answered in the present state of knowledge. But there are some evidences which suggest that the primary afferent collaterals terminate mainly on remote dendrites.

In many rat motoneurons which fire monosynaptically to orthodromic stimulation, residual EPSP still persists without being wiped out by preceding spike. This is also observed by Granit *et al.*¹¹ who say 'the orthodromic spikes have not been found wholly to eliminate the post-synaptic potential'. Since monosynaptic EPSPs survive the drastic conductance change generated by soma spike, it is safe to say that the EPSPs are propagated from some place, probably remote dendrites, to soma. This is also supported by the fact that monosynaptic EPSP has a slower time course in rise and decay compared with that of cat.

Fadiga and Brookhart¹² demonstrated that in toad spinal cord, the dorsal root fibers terminate exclusively on remote dendrites of motoneurons, conforming no reduction of an antidromic spike during a developing orthodromic EPSP. It is interesting to note that in toad spinal reflex the monosynaptic EPSP evoked by dorsal root stimulation fails to fire motoneurons.

There is another evidence for the vulnerability of the monosynaptic connection in the rat spinal cord. In adult cat spinal reflex, monosynaptic volleys conveyed through the largest or the most excitable fiber group-GI fiber- exert so much

Fig. 6. The change in appearance of the L4 segmental reflex potential when the stimulus intensity was raised gradually. From top downward the stimulus intensity was increased. The upper three traces (A, B, C) were recorded at the same sweep speed and at the same gain, while the bottom one (D), at higher speed and at lower gain. Note the forward progress of the reflex potential as the increase of the stimulus intensity.

synaptic drive on motoneurons that the monosynaptic reflex is the fastest and the most excitable of all the reflex components. In rat, the monosynaptic convergence is so weak that the synaptic drive is not sufficient to fire motoneurons monosynaptically when the stimulus strength is low. This is shown in Fig. 6. Generally, L5 segmental reflex does not contain polysynaptic component much in L4 segmental reflex there can be seen considerable amount of synchronous polysynaptic components. As the stimulus intensity was augmented gradually, the latest polysynaptic component appeared first. Along with the further increase of stimulus intensity, the latest component of polysynaptic reflex, which had the lowest threshold, reached the ceiling and diminished thereafter, while the intermediate component grew more and more in amplitude to reach the ceiling in its turn. Then the fast synchronous wave emerged and gained in height while at the same time abolishing the later polysynaptic components probably through refractoriness and recurrent inhibition. It was a consistent finding throughout our experiment. This fact can be interpreted as follows: when the stimulus intensity is weak, the large part of motoneuron pool is insensitive to the monosynaptic drive and barely reaches firing level after polysynaptic convergence, and with the increase of stimulus intensity the monosynaptic drive becomes more and more effective, which leads to the gradual augmentation and forward progress of monosynaptic component and at the same time to the decrease and the dispersion of later polysynaptic components by refractoriness and recurrent inhibition. In connection with the facts mentioned above, it is quite interesting to note that

there is surprising conformity between rat and kitten monosynaptic reflex; long latency, long duration, the absence of post-tetanic potentiation or the presence of post-tetanic depression, and the drastic depression to low frequency repetitive stimulation.¹³⁻¹⁵ It is strongly suggested that in both cases the insufficient efficacy of monosynaptic contact is responsible for all phenomena.

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