Extrapyramidal Pathways with Monosynaptic Effects upon Primate α-Motoneurons

According to present concepts[1], a direct cerebromotoneuronal linkage is a unique feature of the pyramidal tract in primates. However, there is now evidence from several subprimate species to show that spinal motoneurons receive monosynaptic input from brain stem structures[2-4]. A present study was undertaken to compare the properties of corticospinal and brain-stem-spinal projections in the monkey.

Material and method. Experiments were performed on 16 rhesus monkeys weighing 1.5–2.3 kg, anesthetized with pentobarbital sodium. Laminectomy was accomplished at appropriate segmental levels to permit intracellular recording from motoneurons by means of glass microelectrodes. Hind limb motoneurons were identified by stimulation of various limb nerves and ventral roots. Several monopolar or bipolar stimulating electrodes insulated except at the tips were inserted stereotaxically into the brain stem. The leg area of the precentral motor cortex was stimulated unifocally with surface anodal pulses through movable silver ball electrodes. In 3 monkeys the motor cortex was removed by suction 14–15 days before recording. The pyramids were acutely cut in a group of 4 monkeys after exposure of the upper medulla from the ventral side. In 5 experiments acute sections of the spinal cord were performed. The location of the brain stem electrodes and the pyramidal and spinal sections was checked histologically. The synaptic potentials and potentials of the cord dorsum surface were recorded photographically and by averaging of 32–64 successive responses using a digital computer.

Results and discussion. Single shock stimulation of the magnocellular region of the contralateral red nucleus, the medial reticular formation of the lower pons and medulla and ipsilateral Deiters' nucleus produced tract volleys recorded from the dorsal surface of the lumbar cord. The conduction velocity of the rubrospinal volley determined from the peak of the positive potential varied from 51.5 to 92.2 m/sec (mean 69.2 m/sec), that of the reticulospinal and vestibulospinal volleys was 59.0–87.9 m/sec (mean 75.9 m/sec) and that of the direct pyramidal volleys 57–76 m/sec (mean 65.2 m/sec). Acute sections of the spinal cord of pyramidotomized monkeys have shown (Figure 1 A) that rubrospinal and reticulospinal volleys are mediated by the separate descending pathways.

Single reticulospinal and vestibulospinal volleys elicited monosynaptic EPSPs in motoneurons of proximal

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[2] A. I. SHAPOVALOV, in *Nobel Symposium 1: Muscular Afferents and Motor Control* (Ed. R. GRANIT; Almqvist and Wiksell, Stockholm 1966), p. 331.
[3] S. LUND and O. POMPEIANO, Acta physiol. scand. *73*, 1 (1968).
[4] A. I. SHAPOVALOV and N. R. GUREVITCH, Brain Res. *21*, 249 (1970).

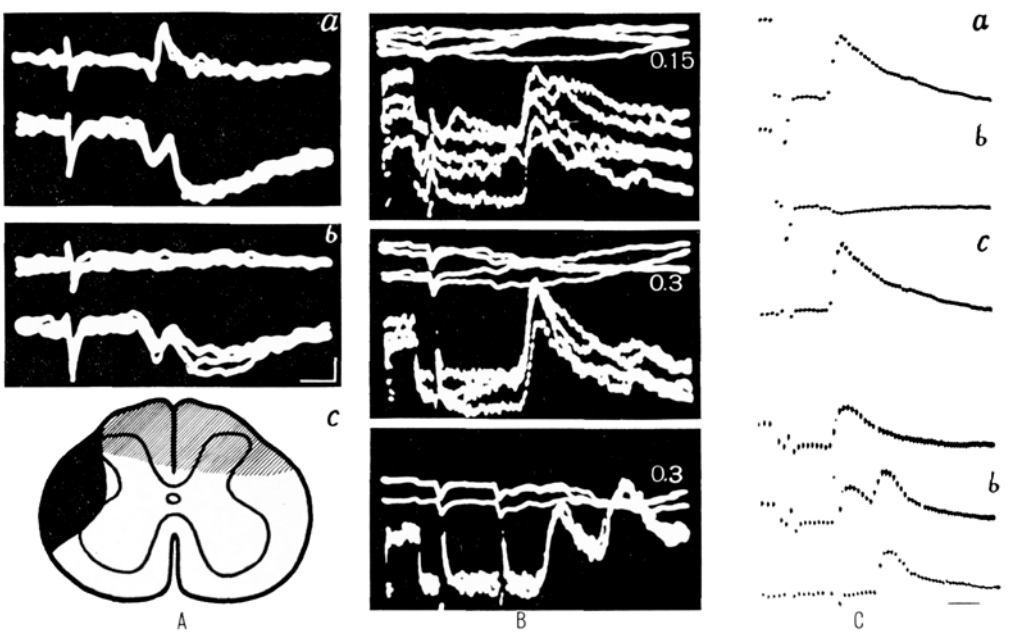

Fig. 1. Cord dorsum potentials (A) and monosynaptic reticulomotoneuronal EPSPs in monkeys with intact brain (C), with destroyed motor cortex (B) and after pyramidotomy (D). A) Simultaneous recording of cord dorsum potentials evoked by rubral (upper trace) and reticular (lower trace) stimulation of pyramidotomized monkey after bilateral section of dorsal funiculi (a) and after unilateral section of lateral funiculus (b), showing that reticulospinal fibers are located in the ventrolateral funiculi; schematic representation of spinal cord sections (c). Time: 1 msec, ampl.: 20 V. B) A popliteus lateralis motoneurone of the monkey after contralateral chronic lesion involving leg area of the motor cortex. The upper traces – cord dorsum potential, the lower traces – EPSPs evoked by shocks applied to the nucleus reticularis pontis caudalis. Calibration pulses 1 mV, 1 msec. The strength of the stimulating current (mA) is noted beside each record. C) A hamstring motoneurone of the monkey with intact brain. Averaged records of intracellular EPSPs (a) evoked by shocks applied to fasciculus longitudinalis medialis at intensity 0.2 mA and of the focal potentials recorded just outside the cell (b) and the result of algebraic summation of the other 2 records (c). Calibration pulse 2 mV (subtracted by computer in c). D) A hamstring motoneurone of pyramidotomized monkey. Averaged records of EPSPs evoked by single (a) and double (b) shocks applied to fasciculus longitudinalis medialis at intensity 0.3 mA and the result of subtraction of the single response from the double (c) showing the absence of demonstrable frequency potentiation. Calibration pulse 1 mV (subtracted by computer in c). Time 2 msec for B) and C).

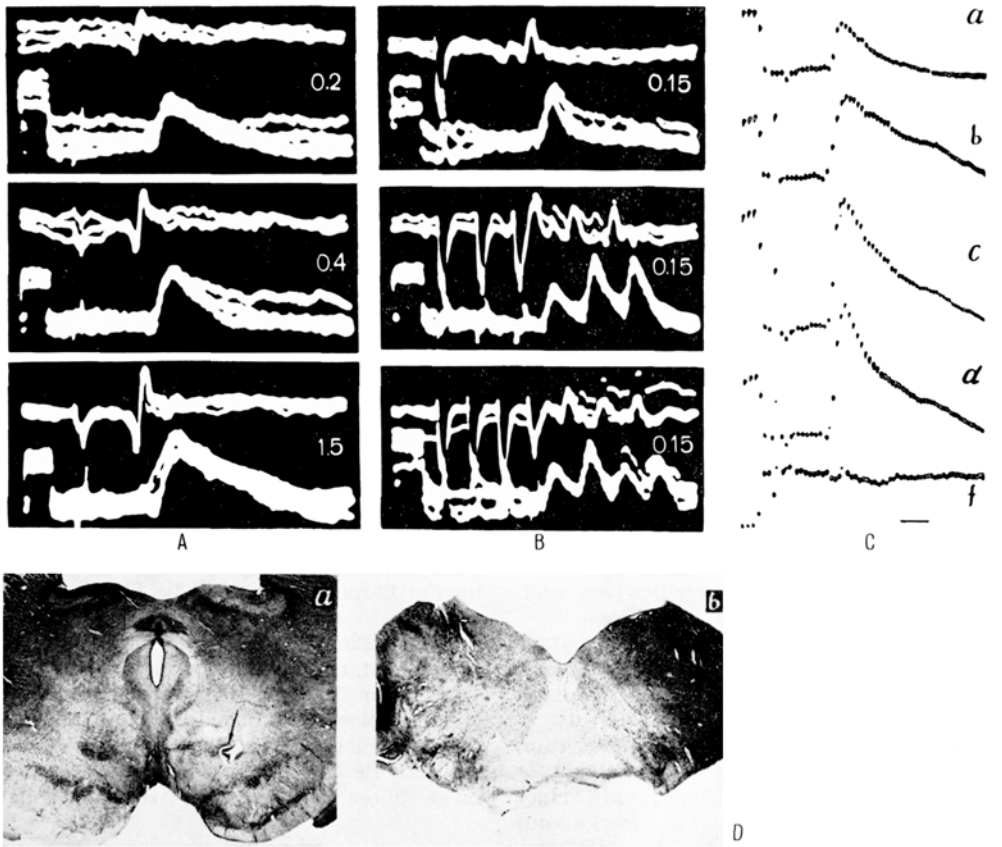

Fig. 2. Monosynaptic rubromotoneuronal EPSPs in monkeys with destroyed motor cortex (A), after pyramidotomy (B) and with intact brain (C). A) Responses evoked by rubrospinal volleys in a plantar motoneurone of the monkey after chronic bilateral lesion involving leg area of the motor cortex. B) Responses evoked by rubrospinal volleys in a peroneal motoneurone of the pyramidotomized monkey. The strength of stimulating current (mA) is noted beside each record. The upper traces are records of the cord dorsum potential, the lower traces are intracellular records. A squire wave calibration pulse 1 mV in height and 1 msec in duration precedes the EPSP. C) A flexor digitorum longus motoneurone of the monkey with intact brain. Averaged records of EPSPs evoked by rubrospinal (a) and pyramidal (b) volleys separately and then together simultaneously (d), their algebraic sum (c) and the difference between the algebraic and motoneuronal sum (f). Calibration pulse 1 mV (doubled by computer in c), time: 2 msec. D) Location of stimulating electrode in the red nucleus (a) and pyramidal section (b) of the same animal as in B).

muscle groups which were located more ventro-medially than those monosynaptically activated by corticospinal or rubrospinal volleys. Similar reticulomotoneuronal EPSPs were observed in monkeys with intact brain and after chronic cortical lesions or after acute pyramidal section (Figure 1, B–D). The amplitudes of the monosynaptic EPSPs from the reticulospinal tract varied from 0.2 to 2.1 mV. The threshold for evoking monosynaptic EPSP was below 0.1–0.05 mA. Monosynaptic EPSPs reveal moderate or no potentiation of response to the second of the 2 reticular stimuli (Figure 1, B and D). These findings contrast with the striking potentiation noted at the cortico-motoneuronal junction of baboon[5], but agree with observations on reticulo-motoneuronal junction of subprimates[4,6].

Figure 2A shows that stimulation of the red nucleus in the monkey with chronic bilateral destruction of the motor cortex produces an EPSP in a plantar motoneurone after a segmental latency of 0.5 msec, wich indicates that the linkage is monosynaptic. The threshold for evoking monosynaptic rubromotoneuronal EPSPs was frequently below 0.1–0.05 mA. When the intensity of the stimulus was increased the EPSP grew progressively until it reached a maximal amplitude of 0.2–1.7 mV. Similar patterns of rubromotoneuronal EPSPs were found in pyramidotomized monkey (Figure 2B) and in animals with intact brain (Figure 2C), hence there is no evidence that effects described are mediated by the collaterals of corticospinal fibers. Location of stimulating site and of pyramidal section are shown in Figure 2D. The monosynaptic EPSPs followed rate of stimulation in excess of 800/sec, revealing a moderate frequency potentiation. The records of Figure 2C are tracings of the averaged EPSPs elicited by rubrospinal and pyramidal volleys. The linearity of the observed summation of monosynaptic actions demonstrates the absence of occlusion which shows that each effect is mediated by the separate descending pathway. Moreover, this linearity suggests the spatial segregation of corresponding synaptic inputs on the motoneuronal membrane.

It appears from several lines of evidence in the present study that fast-conducting fibers running in the rubrospinal, reticulospinal and vestibulospinal pathways form monosynaptic articulations with primate segmental α-motoneurons. The direct connection between fast corticopyramidal cells and α-motoneurons is supposed

[5] C. G. Phillips and R. Porter, in *Progress in Brain Research* (Eds. J. C. Eccles and J. P. Shade; Elsevier publishing Co., Amsterdam 1964), vol. 12, p. 222.

[6] A. I. Shapovalov, J. Neurophysiol. *32*, 948 (1969).

to be especially advantageous for the fine control of discrete movements of distal muscles of extremities. On the other hand, it may be suggested on analogy with the corticomotoneuronal connections that monosynaptic brain-stem-motoneuronal linkage is also essential for cerebral control of movements.

Выводы. В опытах на обезьянах, включая животных с перерезкой бульбарных пирамид, или с хроническим повреждением моторной коры, показано, что быстропроводящие волокна руброспинального, ретикулоспинального и вестибулоспинального трактов вызывают моносинаптические ВПСП в поясничных альфа-мотонейронах.

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Resorption von Serumproteinen in die Haemolymphe bei Larven von *Hypoderma bovis* (De Geer) (Diptera, Hypodermatidae)

Proteine werden in der Regel bis in Aminosäuren zerlegt, bevor sie aus dem Darm resorbiert werden. Nur in wenigen Fällen scheint bei Insekten ein völliger Abbau der Proteine nicht stattzufinden[1,2]. Ein Vergleich der Elektropherogramme der Haemolymphe von *Hypoderma bovis* (De Geer) mit dem Serum ihrer Wirtstiere legte die Vermutung nahe, dass auch Dassellarven in der Lage sind, Proteine direkt in die Haemolymphe aufzunehmen.

Material und Methoden. Für die Untersuchungen standen Larven des dritten Stadiums von *Hypoderma bovis*, die den Häuten gerade geschlachteter Rinder entnommen waren, zur Verfügung.

Die elektrophoretische Auftrennung von Rinderserum, Haemolymphe, zerquetschtem Ovarium und Fettkörper sowie Darminhalt von Dassellarven wurde mit einer Mikrozonenelektrophorese-Apparatur (Fa. Beckmann) vorgenommen. Gleiche Mengen wurden auf die vorgepufferte Zelluloseazetatfolie aufgetragen. Die Trennung dauerte 15 min bei 250 mV konst. und pH 8,6 (Barbitursäure-Puffer). Die Anfärbung erfolgte mit Poinceau-S. Zur photometrischen Auswertung stand ein Analytrol (Fa. Beckmann) zur Verfügung.

Mit ^{131}Jod markiertes Albumin (Originallösung 1:10 verdünnt mit physiologischer Ringerlösung) wurde mit etwas Bindegewebe aus der Cutis des Rindes an zehn *Hypoderma*-Larven verfüttert, die während des Versuchs bei 36–37 °C einzeln in Glasröhrchen gehalten wurden. Nach 24 h wurde den Tieren Haemolymphe und Mitteldarminhalt entnommen und in zwei Serien auf die Elektrophoresefolie aufgetragen. Nach der Auftrennung wurde die Folie halbiert, eine Serie wie üblich angefärbt, die andere zur Autoradiographie mit einem Röntgenfilm in Kontakt gebracht.

Ergebnisse. Das Elektropherogramm der Haemolymphe von Dassellarven zeigt 3 deutliche Proteinfraktionen, die mit 1, 2 und 3 in der Reihenfolge ihrer Wandergeschwindigkeit benannt wurden (Figur 1). Im Vergleich zum Elektropherogramm des Rinderserums fällt zunächst auf, dass Fraktion 1 die gleiche Wandergeschwindigkeit hat wie die Albuminfraktion des Rinderserums. Fraktion 2 stimmt in der Wandergeschwindigkeit mit α-Globulin des Rinderserums überein. Die dritte Fraktion ähnelt in etwa der γ-Globulinfraktion des Rinderserums, ist jedoch nicht so deutlich vorhanden. Der Vergleich der Haemolymphenproteine von *Hypoderma bovis* mit den Serumproteinen des Rindes stützt sich hier lediglich auf ihre elektrophoretischen Eigenschaften. Vergleicht man die prozentualen Anteile von Albumin und α-Globulin des Rinderserums mit den ähnlichen Haemolymphenfraktionen 1 und 2 von *Hypoderma*, ergibt sich folgendes Verhältnis:

$$\frac{\text{Albumin}}{\alpha\text{-Globulin}} = \frac{76{,}92}{23{,}08}; \quad \frac{\text{Haemolymphenfraktion 1}}{\text{Haemolymphenfraktion 2}} = \frac{15{,}04}{84{,}96}.$$

Das Mengenverhältnis ist also in etwa umgekehrt.

Im Gegensatz zu anderen vergleichbaren Fliegen werden bei *Hypoderma* die Ovarien schon während der Larvalentwicklung angelegt[3]. Die elektrophoretische Untersuchung von zerquetschten Ovarien zeigte das gleiche Proteinspektrum wie von Haemolymphe (Figur 1). Auch im Fettkörper liessen sich die Fraktionen 1 und 2 nachweisen.

Soll tatsächlich eine Resorption von Serumproteinen stattfinden, müssen diese auch im Mitteldarm vorhanden sein. Figur 2 zeigt die Elektropherogramme aus 3 aufeinanderfolgenden Mitteldarm- und 2 Enddarmabschnitten desselben Tieres. Die Gesamtproteinmenge nimmt im Verlaufe der Darmpassage ab. Besonders deutlich ist im gesamten Darmtrakt die Albumin-ähnliche Fraktion 1

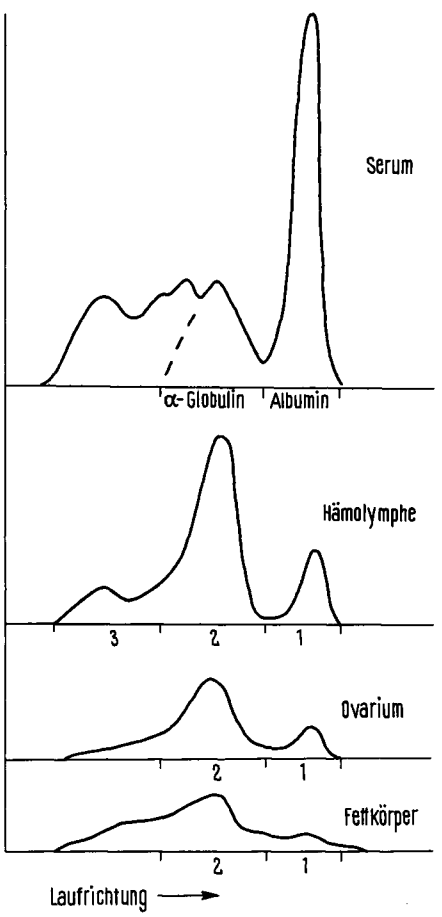

Fig. 1. Elektropherogramm von Rinderserum sowie Haemolymphe, Ovarium und Fettkörper von *Hypoderma bovis*.