NERVE CONDUCTION PATTERNS DETERMINED ELECTRONICALLY FOR ANALYSING PERIPHERAL NERVES BEHAVIOR *IN VIVO*

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It has been stated that the nerve impulse is a wave of depolarization developed in the cell membrane, moving from the CNS to the effectors on motor nerve fibers, and from the receptors to the CNS on the sensory fibers. Moreover, it has been experimentally demonstrated that when isolated nerve cells or fibers are properly stimulated, the depolarization in the membrane occurs in all directions. Nevertheless, it is accepted that in normal biological conditions, the peripheral motor and sensory fibers conduct nerve impulses in one direction only. Or may we consider the antidromic conduction a normal fact in biology?

The main scope of this paper is not to discuss this problem, but we have to consider it, since we are trying to establish which are the patterns of nerve conduction *in vivo*, based upon electronic data, with the purpose of subsequent analysis of peripheral nerves behavior in degeneration and regeneration processes. We have been working on this matter and on its practical application in men during the last years (Erhart *et al.* [3, 4, 5, 6, 7, 8, 9, 10]). At the present, our experiments are being supported by a grant which permitted us to build the necessary system, which is basically composed of a stimulator, an electromyograph and a cathode-ray oscilloscope.

MATERIAL AND METHODS

Dogs anesthetized with Nembutal (Abbott) were operated in order to expose properly all or some of the nerves chosen for the experiment in each dog: n. ulnaris (mixed), n. hypoglossus (chiefly motor) and n. cutaneus brachii medialis (chiefly sensory). In these experiments, 42 nerves of 14 dogs were used.

The surgical procedure for the nerve exposition and the care taken to maintain each nerve, as much as possible, undisturbed in its natural connective tissue bed, to avoid nutritional deficiencies which interfere with the normal nerve biology and regenerative processes, are described and discussed in some of our former papers in the subject (Erhart & Rezze [5, 7, 8]). After the experiments, the dogs were killed.

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Bipolar nickel-chromium electrodes with 0.5 mm diameter, mounted in plastic supports and circa 1 mm apart one from the other were used. They were commanded by a charriot system in order to permit delicate and small displacements and good contacts along the nerve trunks. The connections between electrodes and stimulator or recording system were achieved by means of a selective switch-box similar to the one recommended by Fox & Kenmore [11]. All the experiments were performed in a Faraday cage.

In preliminary tests for control and in order to calibrate our electronic system properly, we have worked with neurophysiological preparations of isolated nerves: frog sciatic and dog ulnar and hypoglossal nerves. We could observe, as it was expected in such standard neurophysiological preparations, that nerve impulses flow from A to B or from B to A, indifferently, depending only on the stimulation and recording points (Fig. 1). In these preparations, the whole compound tracing of fast and slow fibers was obviously observed. Local anesthesia, xylocaine 2%, blocked the nerve impulse.

With these patterns established, the proposed experimental series were initiated. Each nerve trunk was surgically exposed (4-5 cm) but maintained undisturbed, as much as possible, on its natural connective tissue bed. In all the experiments equivalent segments of the nerve trunks were used. We have chosen short segments from which no branches spread off. Electrodes A and B, apart from each other circa 2 cm were carefully positioned as in Fig. 1. Then, the nerve trunk was stimulated with both positive and negative pulses with 50 μsec of duration, 0.8 cycles/sec of frequency, with an amplitude from zero to threshold intensity and then up to suprathreshold level, 6 to 8 Volts, in order to obtain depolarization of all the fibers which, in the nerve segment under study, were conducting from A to B and from B to A. Stimulation was indifferently applied either in the proximal (A) or distal (B) electrode, and then inverted by means of the selective switch-box of the stimulator.

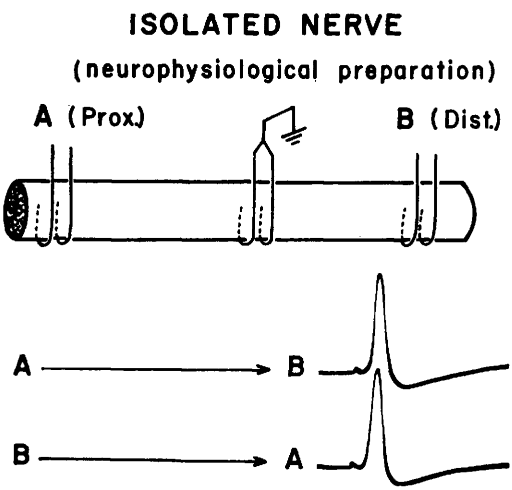

Fig. 1 — Schematic diagram of stimulating and recording electrodes and ground positioning.

Calibration of the system: *Stimulator,* especially projected, pulse width 50 μsec; frequency — 0.8 cycles/sec. *Electromyograph* TECA TE-2. Sensibility — 200 μV/division; sweep — 30 msec/division; filtre — high and 60 cycles ON. *Oscilloscope* LABO-VOLTIX (made in Brasil). Vertical sensibility — 50 mV/cm, connected to the third stage of the TECA preamplifier; sweep — 400 μsec/division.

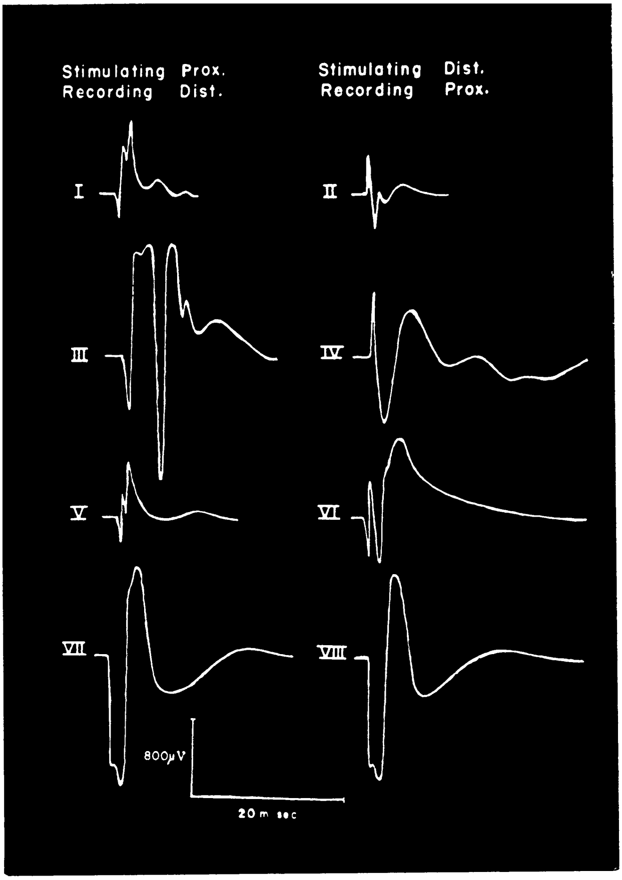

Fig. 2 — Recorded tracings selected from different experiments: I and II, ulnar nerve; III and IV, hypoglossal nerve; V and VI, medial brachial cutaneous nerve; VII and VIII, isolated hypoglossal nerve as in classical neurophysiological experiments. The stimulus artefact on account of the experimental conditions overlaps the initial slope of the tracings.

RESULTS

The nerves studied under the *in vivo* experimental conditions previously described showed the following:

a — The 13 ulnar nerves tested presented typical action potential in both distal-proximal and proximal-distal conductions. This was expected, since this nerve is composed of both motor and sensory fibers. But, it must be emphasized that these ulnar centripetal and centrifugal recorded action potentials presented, for each direction, characteristics which showed to be constant, as may be seen in Fig. 2 I and II. The amplitude and wave compounds for motor and sensory fibers of the ulnar nerve are quite different one from another.

b — The 16 hypoglossal nerves presented typical action potential in the proximal-distal conduction (Fig. 2 III). In these motor nerves, maintained undisturbed in their connective tissue bed, the recorded action potential of the distal-proximal conduction was always smaller in amplitude (Fig. 2 IV), even when slight suprathreshold and higher frequencies stimulations were used, or when the position of the electrodes were changed along the nerve trunk. We may suppose that the proprioceptive fibers of the hypoglossal nerve are responsible for this distal-proximal conduction.

c — In the 7 medial brachial cutaneous nerves, chiefly sensory, it was observed that its action potential was typical in the distal-proximal conduction (Fig. 2 VI) and had smaller amplitude in the proximal-distal conduction (Fig. 2 V). In these latter nerves we may suppose that the post-ganglionic sympathetic nerve fibers are responsible for the proximal-distal conduction.

d — All the nerves — ulnar, hypoglossal and medial brachial cutaneous — which after being worked on *in vivo*, were isolated from their natural connective beds, transsected and suspended on the electrodes, as is normally done in neurophysiological preparations, presented, in accordance with the literature, typical action potentials in both directions: proximal-distal and distal-proximal (Fig. 2 VII and VIII).

The anomalous records which were obtained from six nerves (one ulnar, three hypoglossal and two medial brachial cutaneous) severely injured by surgical manipulation, distension, excess bleeding or ischemia, were obviously not considered in these data.

DISCUSSION

In general our results, regarding the compound waves, latency, amplitude, etc., are in accord with the literature; but, as they presented the above mentioned particularities, they must be further discussed.

When a nerve maintained in its natural connective tissue bed is stimulated, the neighbouring tissues, including muscle fibers, integrate with the compound waves which appear on the scope screen. For that reason, our system included an oscilloscope to record fast events, that is, the action potentials of nerve fibers, and an electromyograph, for slow events recording, such as action potentials in muscle fibers groups.

As the behavior of nerves *in vivo* involves biochemical and metabolic processes, the surgical procedure and the electrodes positioning were such as to maintain the nerve fibers undisturbed, as much as possible, in their natural connective tissue bed. Being so, in our preparations we had a shunting effect which was partially diminished by grounding (Fig. 1). We considered it as a constant when stimulating and recording from A to B and from B to A because: *i* — if the shunting effect had really interfered in our results, we could not obtain, as we did, constancy in the recorded action potentials of the 13 ulnar, 16 hypoglossal and 7 medial brachial cutaneous nerve trunks studied. *ii* — the nerve trunk segment used was always a short one, circa 2 cm long, distance between electrodes A and B. *iii* — from these nerve trunk segments no branches spread off.

We do not intend that the data we have recorded electronically, may be considered as absolute nerve patterns. But, even so, they represent some nerve behavior which seems to be closer to biological conditions, when compared to those obtained by the experimental methods generally used in the stimulation of nerves, which constitute always an attack to nerve cell and nerve fiber integrity (1, 12, 13, 14, 15, 16). The anesthesia of the animal, by itself, will certainly interfere too on different structures, including the synaptic mechanisms. The abnormal, continuous stimulation, in high frequencies, will probably interfere also with what is considered normal nerve cell or fiber behavior. For that reason we used in our experiments *in vivo,* threshold stimulation, short duration pulses and low frequencies, to allow metabolic reestablishment of the nerve fibers being studied, as well as reposition of possible electric disturbances in the nerve trunk environment.

The results we have obtained on dog ulnar, hypoglossal and medial brachial cutaneous nerves, indicating a specific unidirectional conduction on peripheral motor and sensory fibers, although being quite different from what has ever been published on the subject up to now, seem to be supported by neurophysiological, neuroembriological and neuropathological data. Llinás and colaborators [13], as far as we know were the first to speak about "unidirectional preference". They stated: "dendritic action potentials in alligator Purkinje cells tend to have an unidirectional preference" and "probably many other nerve cells may have to be considered as highly complex units able to attain a vast number of dynamic states which would lead to the generation of a large variety of functional patterns". They talk about dendrites but, if it is accepted that the nerve impulses enter through he dendrites and leave the cell body through the axon, in these latter there must be too an unidirectional preference. Our tracings in peripheral nerves, *in vivo* conditions, showed this fact. Weiss & Edds [18] concluded that sensory nerves form no functional synapses with muscles; and Robbins [17] wrote: "if the functional potential of a sensory nerve is not the key to the origin of sensory specificity, other specifying mechanisms must be invoked to explain nerve growth to a specific location". We do agree with these authors. There must be something specific in the nerve cell body whose nature we do not know, that makes motor and sensory nerve fibers *in vivo* behave differently one from another. If Mother Nature has build these two systems (motor and sensory) embriologically, morphologically and functionally different one from another, Men will state that *in vivo* motor and sensory peripheral nerve fibers have no specific conduction! Polio lesions are chiefly related to motor neurons and those of tabes dorsalis, to sensory neurons. Moreover, nerve lesions are distinguishable one from another by the condition of motor, sensory and sympathetic functions, as revealed by clinical examination. These facts permit us to conclude that there must be something biochemical, metabolic or something else, concerning nerve-fiber structure in relation to nerve-fiber function to be investigated.

SUMMARY

Nerve conduction patterns of the ulnar, hypoglossal and medial brachial cutaneous nerves were determined and recorded electronically *in vivo* on anesthetized dogs. The recorded tracings obtained were generally found to

be in accordance with those described in the literature. However, our tracings showed a new additional fact, i.e., that motor and sensory peripheral nerve fibers have a specific unidirectional conduction (centrifugally for motor and centripetally for sensory), as long as these nerves were maintained undisturbed in their natural connective tissue bed. Although these facts correspond to what is considered normal biological behavior of nerve fibers, when in their natural environment, as far as we know, this has not been demonstrated before in neurophysiological experiments.

RESUMO

Padrões de condução de impulsos nervosos, determinados eletrônicamente, para análise de comportamento de nervos "in vivo".

Padrões de condução de impulsos nervosos foram determinados eletrônicamente, *in vivo*, nos nervos ulnar (13), hipoglosso (16) e cutâneo medial do braço (7) de cães anestesiados. Os traçados obtidos confirmaram, em linhas gerais, os descritos na literatura. Todavia, demonstraram um fato novo, adicional, isto é, que fibras motoras e sensitivas, integrantes de nervos periféricos, apresentam uma condução específica, unidirecional (centrífuga nas fibras motoras e centrípeta nas sensitivas), desde que mantidas pràticamente intactas em seus respectivos leitos conectivos naturais. Embora êste tipo de condução específica corresponda ao que se admite e aceita como comportamento normal, biológico, dessas fibras nervosas, e constitua ainda, base do exame neurológico, esta evidência nunca foi demonstrada anteriormente em experiências e preparações neurofisiológicas.

REFERENCES

1. BOWMAN, J. & COMBS, C. M. — Discharge patterns of lingual spindle afferent fibers in the hypoglossal nerve of the Rhesus monkey. Exptl. Neurol. 21:105, 1968.

2. BURGESS, P. R. & PERL, E. R. — Myelinated afferent fibers responding specifically to noxious stimulation of the skin. J. Physiol. (London) 190:541, 1967.

3. ERHART, E. A. — Normal nerve-fibres in the distal segment of nerves completely separated from the proximal stump for more than six months. Arq. Neuro-Psiquiat. (São Paulo) 20:289, 1962.

4. ERHART, E. A. & ERHART, M. B. — Normal axis-cylinders in the distal segment of nerves completely separated from the proximal stump for up to 24 years. Anat. Rec. 136:189, 1960.

5. ERHART, E. A. & REZZE, C. J. — Nerve fibres in isolated segments of dog ulnar nerve after complete brachial plexotomy and periaxilar artery sympathectomy. Arq. Neuro-Psiquiat. (São Paulo) 23:82, 1965.

6. ERHART, E. A. & REZZE, C. J. — The neuroma-like structure of the long-time severed and isolated nerve stumps. Arq. Neuro-Psiquiat. (São Paulo) 23:91, 1965.

7. ERHART, E. A. & REZZE, C. J. — Experimental data and practical results which modify the present concepts of peripheral nerve fibres degeneration and regeneration. Proc. V Internat. Congress of Neuropathol. (Zurich) p. 864-867, 1965. Published by Excerpta Medica.

8. ERHART, E. A. & REZZE, C. J. — Effect of experimental devascularization on peripheral nerves. Arq. Neuro-Psiquiat. (São Paulo) 24:7, 1966.

9. ERHART, E. A. & REZZE, C. J. — Further discussion on the newgroing nerve fibres which repopulate the distal segment of nerves completely separated from the proximal stump for more than six months. Arq. Neuro-Psiquiat. (São Paulo) 24:91, 1966.

10. ERHART, E. A.; REZZE, C. J. & BIAZOTTO, W. — The ectopic newly-formed nerve fibres which repopulate the long-time denervated and atrophic chick skeletal muscle. Arq. Neuro-Psiquiat. (São Paulo) 26:187, 1968.

11. FOX, L. J. & KENMORE, P. — The effect of ischemia on nerve conduction. Exptl. Neurol. 17:403, 1967.

12. LANDAU, M. L.; CLARE, M. H. & BISHOP, G. H. — Reconstruction of myelinated nerve tract action potentials: an arithmetic method. Exptl. Neurol. 22:480, 1968.

13. LLINÁS, R.; NICHOLSON, C. & PRECHT, W. — Preferred centripetal conduction of dendritic spikes in alligator Purkinje cells. Science, 163:184, 1969.

14. MC LEOD, J. G. & WRAY, S. H. — Conduction velocity and fibre diameter of the median and ulnar nerves of the baboon. J. Neurol. Neurosurg. Psychiat. 30:240, 1967.

15. MORIMOTO, T.; TAKATA, M. & KAWAMURA, Y. — Effect of lingual nerve stimulation on hypoglossus motoneurons. Exptl. Neurol. 22:174, 1968.

16. PAINTAL, A. S. — A comparison of the nerve impulse of mammalian non-medullated nerve fibers with those of the smallest diameter medullated fibers. J. Physiol. (London) 193:523, 1967.

17. ROBBINS, N. — Peripheral modification of sensory nerve responses after cross--regeneration. J. Physiol. (London) 192:493, 1967.

18. WEISS, P. & EDDS, M. V. Jr. — Sensory-motor nerve crosses in the rat. J. Neurophysiol. 8:173, 1945.

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