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NEUROPHYSIOLOGICAL ASPECTS OF THE AGING OF THE PERIPHERAL NERVOUS SYSTEM

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NEUROPHYSIOLOGICAL ASPECTS OF THE AGING OF THE PERIPHERAL NERVOUS SYSTEM

Premise

The issue of growing aging in the world, and in particular in Western countries, in relation to the lengthening of the average life span favored by modern biotechnologies, is an increasingly crucial topic. Recent statistics show that the number of centenarians is increasingly on the rise, as well as those who reach an advanced age, between 80 and 90 years, so as to give rise to a defined category of "longevity outliers". For this reason, the study of the mechanisms that regulate the processes related to aging in the scientific field becomes increasingly stimulating, even if it is often not easy, as one of the most debated dogmas in this area is the definition of the boundaries between physiological processes. related to aging and those related to the development of pathology. However, from the latest studies, what has emerged, in relation to the nervous system, is that the process related to aging has a basis, certainly physiological, represented by neuronal plasticity and redundancy. These properties of neuronal cells, which were initially believed to be a prerogative of young cells, have been much considered in recent decades and the conclusion is being drawn that these characteristics of neuronal cells persist even in old age.

Introduction

The peripheral nervous system (PNS) is made up of all the nerve structures found outside the pial membrane of the spinal cord and brain stem. The structures that must therefore be considered are the roots of the spinal nerves: the posterior ones are sensitive (or afferent) and are constituted by the axonal extensions of the cells of the spinal sensory and cranial ganglia; the anterior ones are motor (or efferent) and consist of axons emerging from the anterior and lateral horns and from the motor nuclei of the brain stem. The larger fibers go directly into the muscle fibers, while the smaller ones go into the sympathetic or parasympathetic ganglia. From these emerge the axons that end in smooth muscle, heart and glands and thus constitute the autonomic system.

The myelinated nerve fibers are covered by a nerve sheath that takes the name of myelin, which has a different composition in the PNS being made up of Schwann cells, compared to the CNS, where it is made up of oligodendrocytes. Unmyelinated fibers, quantitatively greater than myelinated fibers in peripheral nerves, also originate from dorsal root cells and ganglia of the vegetative nervous system.

When a pathological process occurs, each type of affected cell (whose axon constitutes the nerve trunk) shows a specific vulnerability to different pathological processes and, if it is destroyed, secondary degeneration of the axons and myelin sheaths. Some toxic agents, for example, selectively damage Schwann cells or the membranes that form myelin sheaths and cause demyelination of peripheral nerves leaving axons relatively intact, or

there may be specific damage to the axon by acting on the cell body, on the assembly or on the complex axonal transport apparatus (1).

From the anatomopathological point of view, three main pathological processes affecting the peripheral nerves are distinguished: 1) segmental demyelination, in which the axon is spared and there is a focal degeneration of the myelin sheath with disappearance of the sheath for segments of variable length; 2) wallerian degeneration, in which degeneration of both the axon and myelin occurs distal to the axon breakpoint; 3) axonal degeneration, in which the axon is affected from the most distal to the most proximal site (disto-proximal or dying back neuropathy), with dissolution of myelin which occurs in parallel with the axonal alteration. Finally, some diseases primarily affect the cell body rather than the axon and cause motor or sensory neuropathy to the extent that the pathological process affects either the cells of the anterior horns or the cells of the sensory ganglia, respectively. As for the recovery of function, this can be rapid, as occurs in segmental demyelination, because the axon is intact but "uncovered" and must only be remyelinated, or it can be very slow, as occurs in wallerian degeneration in which it can take months or even years because in this case it is the axon that must regenerate at a rate commonly believed to be 1mm / day.

The typical symptoms of PNS diseases can be motor, sensory, reflex, vegetative and trophic type. If there is weakness or paralysis, we are faced with a deficit of motor function which in polyneuropathies generally has a symmetrical distribution; nutritional and metabolic neuropathies have a more common picture, mainly distal and axonal: in this case the

pathological changes start from the most distal portions of the largest and longest nerves and advance along the affected fibers towards the respective cell bodies. If the sensory deficit prevails, as occurs in many toxic and metabolic neuropathies, there is a reduction or abolition of one or more sensitivities (tactile, painful, thermal, vibratory, proprioceptive) each being able to be affected exclusively or clearly prevalently compared to the others. In polyneuropathies, the sensitivity at the level of the distal segments of the limbs is symmetrically affected, with prevalent involvement of the lower ones. Only in extreme cases, when the neuropathy worsens, does the sensory deficit extend from the distal to the proximal portions of the limbs and sometimes also to the anterior abdominal region, chest and face. Sensory symptoms most commonly reported by patients are described as "stinging and needling" sensations, stabbing, pinching, itching, tingling, electric shock or anesthetic sensations. In some neuropathies there is pain that is described as burning, stinging, sharp, cutting, which sometimes resembles the stabbing pain of the dorsal tabe.

Another important symptomatic category is represented by vegetative disorders, of which anhidrosis and orthostatic hypotension are the most frequent: they are observed more frequently in amyloidosis and in some others hereditary polyneuropathies of small fibers, particularly in the diabetic ones. Other vegetative symptoms are: pupillary reactivity, the absence of sweating, impotence and sphincter disorders.

The reduction or absence of osteotendinous reflexes as generic signs of peripheral nerve diseases and deformations of the hands, feet and spine,

especially when the disease begins in childhood (as in polio), due to considerable muscle weakness and the uncontested action of the opposing muscles during bone formation. Finally, the alterations in trophism are due to denervation in case of interruption of the motor nerve trunks which are very often associated with analgesia of the distal districts, which makes them susceptible to burns, bedsores and other forms of traumatic injury that can lead to loss of substance in the fingers, sometimes with true mutilations (without pain) or ulcers of the sole of the foot (plantar malperforating), as in the case of ulcer-mutilating sensitive acropathies.

Hints of neurophysiology

Neurographic studies are dedicated to the functional evaluation of the peripheral nerves. The study of motor conduction velocity (MCV) is the most used method to evaluate the morphological and functional integrity of the Motor Unit and to study the anatomo-functional circuit consisting of the axon and all the innervated muscle fibers . It generates a response called M wave or cMAP (compound muscle action potential) or more simply MAP.

The criteria for defining the quality of a compound action potential are based on the analysis of the morphology of the potential itself. Any cMAP, derived with a bipolar technique, has a biphasic morphology, where the negative peak (directed above the isoelectric) and the positive peak (directed in the opposite direction) are an expression of the presence of the depolarization wave respectively below the active electrode and under the reference electrode. The parameters of this potential are represented by the onset latency, the peak latency, the amplitude of the negative peak or from

negative peak to positive peak, the duration of the potential expressed by the area underlying the negative peak (fig. 1) :

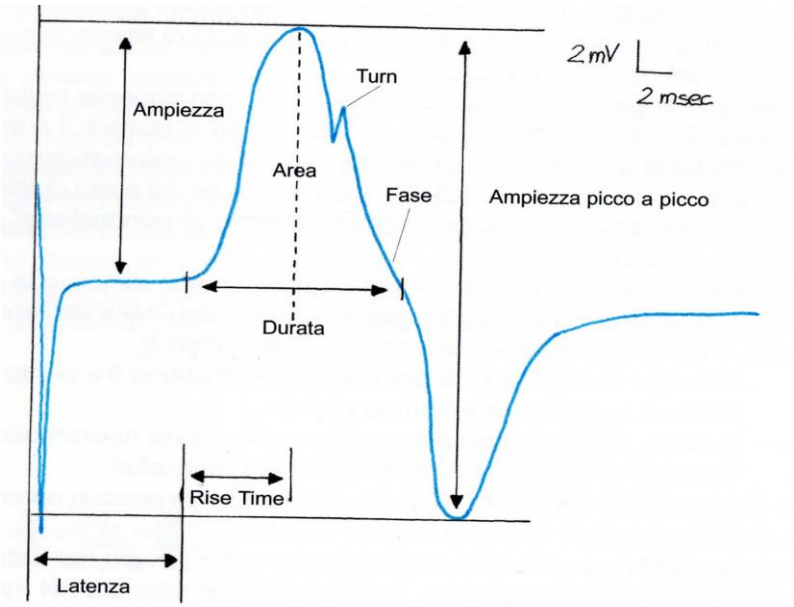


Fig. 1

Latency is the time elapsing between the delivery of the stimulus and the appearance of the response, and is an expression of 3 distinct phenomena:

1. the time required for the passage of the stimulus between the stimulation point and the neuromuscular synapse;
2. the time required for crossing the neuromuscular junction;
3. the time required for the activation of muscle fibers.

Latency is generally measured at the onet, but in some cases the latency at the peak can also be considered. The amplitude of the potential can be measured from the isoelectric to the negative peak or from the negative peak to the positive peak. A reduction in amplitude may indicate the loss

of a certain amount of axons or the presence of a partial conduction block of the action potential. The duration is expressed by the time that elapses from the onset of the response to that in which the negative phase crosses the isoelectric again; it indicates the synchronization of the fibers and increases in all cases of slowing of conduction (the most typical case is the presence of temporal dispersion in demyelination). The area is the surface under the curve of the negative component of the response; it expresses the number of activated motor fibers. The difference between the area of the responses obtained from the distal and proximal stimulation point is an expression of the so-called conduction or dispersion block (2)

Beyond the M wave, nerve stimulation can activate additional responses, called "late" responses, as they have a higher latency than that of M, which provide useful information on proximal segments in the evaluation of neuromuscular diseases.

They can be distinguished on the basis of their latency, the change in latency by modifying the stimulation sites, the change in response to different stimulation intensities (see table 1). They are represented by the F wave (recurrent response), the H reflex (reflex response), the axonic response (recurrent response) and the A wave (recurrent response) (3)

CHARACTERISTICS OF LATE RESPONSES				
	Configuration	Latency	Frequency to consecutive stimuli (%)	Stimulation intensity
F wave	*Variable*	*Variable (5-10ms)*	*30-100*	*Above the threshold*
H reflex	*Dependent stimulus*	*Constant*	*100*	*Low*
A wave	*Constant*	*Little variable (<1.5ms)*	*30-100*	*Above the threshold*
Axon reflex	*Constant*	*Constant*	*100*	*Very low*

Tab. 1

The F wave is evoked by the antidromic excitation of all the motor axons of a nerve, directed to the spinal cord, with the activation of a small part of the axons of the anterior horn cells, and with orthodromic action potentials directed to the muscle. The name “F” wave dates back to the earliest recordings that were performed in the small muscles of the foot (foot) (4). By definition, the F wave is a compound action potential evoked by supramaximal antidromic stimulation of a motor nerve. In contrast to the H reflex, the F waves are more evident with high stimulation intensity.

	H REFLEX	F WAVE
Response	Reflected	Recurrent
Afferent fibers	Ia (large diameter, fast conduction)	α motor neuron
Stimulus	Mild	High
Amplitude	Variable (to the intensity of the stimulus)	Small
Morphology	Variable	Variable

Tab. 2

The consequence of an inconsistent antidromic motor neuron activation (also due to the collision between afferent and efferent stimuli) is the appearance of inconstant motor action potentials during stimulation; therefore some F waves may not appear in every stimulation, be variable in morphology and have a low amplitude (5).

The latency, amplitude, morphology and persistence are the main parameters of F waves.

The **amplitude** of the potential is normally less than 5% of the maximum of the M wave recorded by the same muscle: this depends on the small number of cells of the anterior horn that are activated in response to the stimulus. The F waves can be recorded by each muscle and the latency varies with the variation of the stimulation sites, shortening with more proximal stimulations (latency = (M + F) -1/2).

Latency (minimum, maximum and average): the most used measurement for the F wave is the minimum latency. This is the shortest latency (of the initial deflection - both negative and positive -) of all recorded F waves. Most neurophysiology laboratories define the upper normal limits for the minimum latency of the F wave for different height levels.

To evaluate unilateral signs or symptoms, it is useful to compare the minimum latency of the F wave of both sides.

The following values are the upper limits of nerve-specific asymmetries

(fixed at an average difference plus 2 standard errors):

median: 2.3 ms; ulnar: 2.7 ms; peroneal: 3.5 ms; tibial: 3.5 ms (6).

Chronodispersion is the difference between the shortest and longest of the F wave latencies; it is an expression of the variability of the conduction velocity of the axons of the nerve under examination. The change in latency in a train of 10/20 stimulations is typically around 5-10 ms (7,8)

Amplitude (both absolute and the ratio of F wave - M wave): the amplitude of the F wave is very variable; however, it may be useful to relate the average amplitude of the F wave with the maximum cMAP (F wave/cMAP X 100, F/M ratio) in order to obtain an estimate of the proportion of a pool of motor neurons activated by antidromic stimulation; values greater than 5% are common in upper motor neuron diseases. Given the great variability of the F waves, it is preferable to use the average amplitude, rather than the maximum, for the calculation of the ratio. Both the amplitude and the duration are related to the caliber and number of fibers involved in each stimulation (9,10)

Persistence of the F wave

Persistence is the percentage of stimuli that produce F waves and refers to the antidromic excitability of a particular motor neuron pool. In some normal series, the persistence for the peroneal nerve is 5%, so even the absence of the fibular F wave must be interpreted cautiously because it can fall within the normal range.

The persistence for the median, ulnar and tibial nerves is usually much higher, close to 100% for the last 2 nerves. The most frequent alteration of this parameter is its reduction which often reflects a conduction block in some fibers, as in the case of Guillain-Barrè syndrome in which the F waves, if present, can result in increased latency or have a low persistence, even if the peripheral neurographic studies are still normal (11).

A high persistence (80-100%), on the other hand, occurs in lesions of the upper motor neuron, especially when spasticity is present. The persistence of the F wave is also increased when recording from a non-relaxed muscle. Even very slight contractions increase the ease with which F-waves are evoked.

Clinical applications

The most frequent practical application of this type of late response consists in determining the slowing of proximal conduction in polyneuropathies, especially in those with acquired demyelination, where they can be more or less lengthened or even absent. The F wave study is considered the safest and most reliable conduction study for the analysis of patients with polyneuropathies; in acute and chronic demyelinating ones

this may be the only conduction anomaly. The most frequently detected anomalies consist of an abnormal chronodispersion or an altered persistence (25-50% of the nerves examined). Chronodispersion tends to be greater in demyelination than in axonal lesions. The evaluation of the quantity of identical responses in each pulse train is also important: a higher percentage was observed in patients suffering from neurogenic muscular atrophy, for example with amyotrophic lateral sclerosis or with cervical spondylosis, in agreement with a marked axonal loss. In diabetic patients, minimal F-wave latency is often lengthened even when symptoms and clinical signs of neuropathy are mild or absent altogether (12).

In some cases, conventional neurographic studies, through the study of the cMAP, may not be able to diagnosing the type of neuropathy in patients with chronic neuropathies, for example when the conduction speeds are increased or the amplitudes are low; in such cases the presence of F-waves with normal or slightly delayed latencies points to an axonal rather than demyelinating process (13).

Their use is more controversial in radiculopathies. It is unlikely that the minimum latency will be lengthened because the slowing down through the short segment of nerve that is potentially demyelinated will be diluted by a very long stretch of normally conducting nerve. In any case, F waves are much less sensitive than EMG studies when evaluating motor involvement in radiculopathies, although they remain, however, of extreme relevance in other conditions with proximal focal lesions such as Guillain-Barre syndrome, or in patients with stenosis of the spinal canal or multilevel root lesions, in which an increase in chronodispersion may be

highlighted, in particular with the dynamic test in standing position. An increased ratio of the average amplitude of the F wave and cMAP is a frequent finding in spasticity and can be easily seen in patients with upper motor neuron syndrome; in such cases, the average amplitude of the F wave is greater than 5% of the M wave and often more than 10%, and there is also a prolongation of latency and duration. Several studies in patients with upper motor neuron syndrome show that their motor neurons discharge more frequently than normal.

H REFLEX

The H reflex (from Paul Hoffman who in 1918 described it by stimulating the tibial nerve (14) is the simplest of the spinal reflexes. It is a motor action potential obtained from low intensity orthodromic stimulation (a few mAmp) of the sensitive fibers, rapid conduction of group Ia afferents from the fusal muscle terminations, which contract synapses at the medullary level, activate the motor neuron pool and return orthromically through motor fibers. The response is obtained from a medullary monosynaptic reflex that is evoked, in healthy adults, in the gastrocnemius and radial flexor carpal muscles. In this respect, the H reflex is similar to the phasic myotatic reflex produced by the stretching of the muscles but is distinguished from it because it does not involve the activation of the muscle spindle; this may explain the presence of the H reflex in the gastrocnemius muscle in the absence of the Achilles reflex and vice versa. Failure to demonstrate a reflex response can be attributed to afferent, efferent, or low central excitability. In fact, increasing the intensity of the

stimulus increases the central inhibition and the H reflex is inhibited in favor of the maximal direct response (M) (see fig. 2)

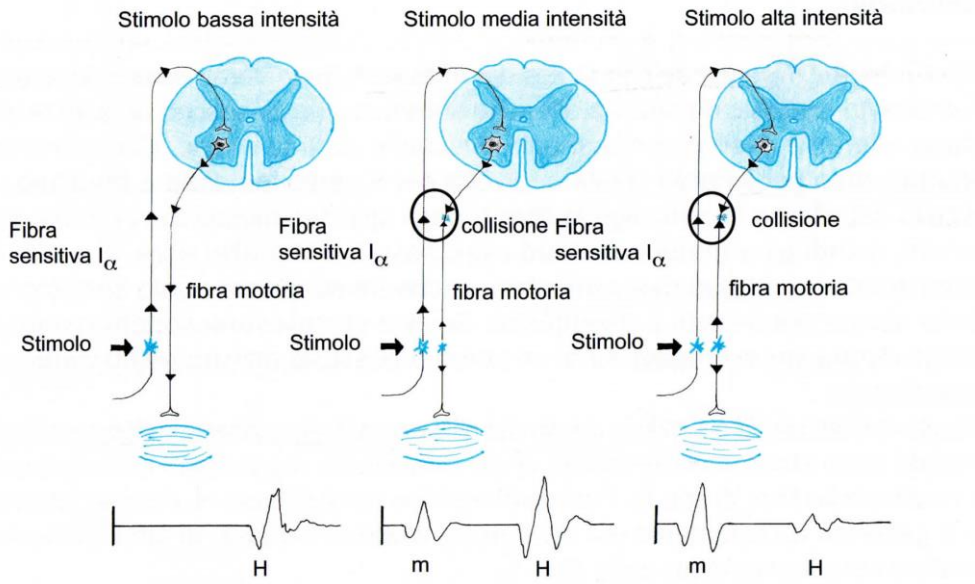


Fig.2

This can be explained by medullary-generated inhibitory mechanisms in which Renshaw cells are probably involved, activated by supramaximal antidromic stimulation. Several studies, also with electromyography recording, demonstrate the activation of inhibitory synapses with stimuli of increasing intensity. The H reflex is enhanced by maneuvers that increase motor neuron excitability, such as the “Jendrassik maneuver” or clenching of the teeth or fists.

Clinical applications

The study of the H reflex is very useful in polyneuropathies and can also be found altered in mild neuropathies or in the early stages of polyneuropathies; it allows to highlight any lesions involving the proximal nerve fibers and could be altered even when the studies in the distal districts are normal. For example, its absence is an initial feature of some acute polyneuropathies such as Guillain-Barrè syndrome (11).

A fairly frequent example is the alteration found in S1 radiculopathies, both in the impairment of the anterior and posterior root. On the other hand, the H reflex can also be used in the diagnosis of upper motor neuron diseases if it is particularly represented; for example, its finding in the anterior tibial muscle or in the small muscles of the hand, in which it is not normally present, can be a useful clinical comparison for the diagnosis of central nervous system dysfunctions, as well as an increase in the H / M ratio (normal value = 0.7).

The H reflex is found acutely depressed after an acute spinal cord injury, while an increase in the H / M ratio is typically observed a few weeks or months after a cerebrovascular injury associated with first motor neuron syndrome (hypertonia, reflex vivacity, plantar extensor responses); this revealed altered Renshaw cell activation patterns, consistent with the loss of supraspinal inhibitory control (15-19).

Axon Reflex And A Wave

A wave, initially called "axon reflex" by Fullerton and Gilleat, was described in 1965 as a response with constant shape and latency; today we know of other types of A waves, more frequently observed in routine conduction studies, differing in various characteristics. (20) However, as mentioned above, during routine F wave studies, the type of delayed response is observed more frequently it is a response that, although similar in some respects, does not meet the criteria of the axon reflex for several reasons: because it does not disappear with a greater intensity of stimulation, as in the case of the H reflex, it is found more frequently in the lower limbs, it appears already in the initial stages of a neuropathy to the point that they are also considered as predictors of the development of subsequent neuropathy in asymptomatic patients, has a persistence lower than that of the axon reflex (around 40%), has a small variation in latency (<1, 5 msec.) In the series of stimulations: this response is generally referred to as A-wave. It can be easily differentiated from both the F wave and the H reflex. Unlike the F wave, it has a constant morphology, a less amplitude and a much smaller latency variation than that of F (<1.5 ms) (21); the latency is generally shorter than F wave but it can also be the same, so as to be included in it, or even higher. Unlike the H reflex, the amplitude of the A wave does not change with the change in the intensity of stimulation. The A waves can be found exceptionally also in healthy subjects, or rather, in subjects in which it is not possible to trace a specific pathological condition; in such cases they occurred exclusively in the stimulation of the tibial nerve.

The A waves, when present, are typical in one third of patients with peripheral neurological symptoms, while in normal patients both on clinical examination and in the remaining neurophysiological study, the frequency of A wave detection reaches up to 9% and in such cases both the tibial nerve and the peroneal nerve are involved (22).

Commonly, however, in the literature it is reported that 2/3 of patients with polyneuropathies have A waves in at least one nerve even though there does not seem to be any correlation between the number of A waves or the number of nerves presenting A waves and the duration or severity of the disease; most of these neuropathies are demyelinating and this is confirmed by the fact that A waves are a common sign at the beginning of the course of an inflammatory demyelinating polyneuropathy (such as Guillain-Barrè syndrome), in which case they may even precede the other neurophysiological abnormalities or be present in the absence of the typical F wave alterations (11).

In addition to polyneuropathies, A waves can also be found in patients with proximal lesions, for example in cervical or lumbar radiculopathies, while they are less frequent in plexopathies and distal lesions. There are also A waves that can derive from a hyperexcitability of a proximal segment of the nerve that causes a reflection of the afferent nerve impulse, as if they were repetitive discharges of an axon, and others that are generated by an ephaptic transmission between contiguous axons or from a myo-axonal ephapsis (fig. 3) (23,24).

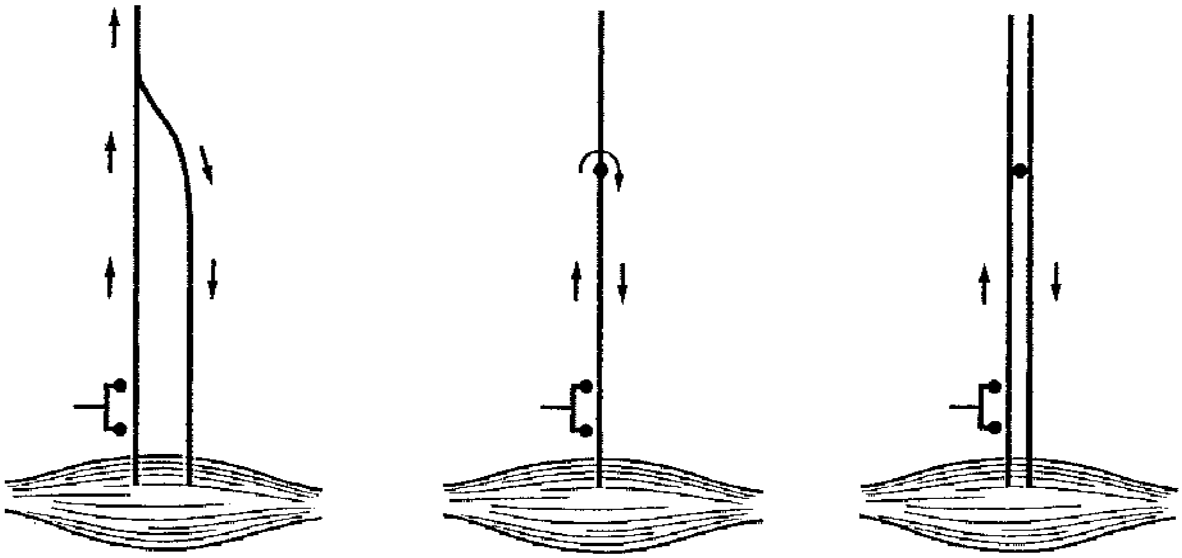


Fig. 3

Fig. 3: Possible sources of generation of wave A; left to right: a recurrent impulse through an axonal branch (similar to a true axon reflex), a reflex impulse at a proximal lesion of the myelin sheath, a reflex impulse through an ephaptic transmission between 2 axons.

In the first case, similar to what happens in the true axon reflex, the impulse, during its antidromic path directed towards the motor root, meets the point from which the "sprouted" collateral fiber starts; therefore there will be an inversion of the direction of the current which will excite the collateral fiber thus moving towards the muscle and generating the small action potential which will be much earlier,with respect to response F, the closer the branching point of the collateral fiber is to the stimulation point.

The other possibility is that the impulse during its antidromic path encounters a lesion of the myelin sheath, probably due to a focal demyelinating process, and that at this point a reflection of the stimulus is created which therefore will return to orthromically excite the muscle and therefore to generate the small potential which, also in this case, will have

a much earlier latency than the F response the closer the demyelination point is to the stimulation point. It can also be hypothesized that these demyelination points can be multiple and that therefore the reflection of the impulse does not occur in a single moment but in several moments, thus generating different A waves within the same series of stimulation, at varying latencies, a case typically present in Guillain-Barrè syndrome.

The last possibility is that the impulse reflection point is not an alteration of the stimulated axon itself, but that it depends on a short circuit due to an ephaptic transmission of the impulse between 2 contiguous axons. This possibility seems to derive from the ephaptic excitation of an axon through a contiguous motor unit.

Unlike the true axon reflex, not only do they not disappear, but even increase with increasing stimulus intensity, and their latency remains constant. The latency was variable as in most cases it found itself in an intermediate position between the M and the F, in other cases it appeared very close to the M (almost as if it were one of its late component) (12), in still others it appeared very close to F wave, to the point of being included, or even more rarely of latency even higher than that of F (12). The tibial nerve is the one in which the highest percentage of A waves are found in neuropathic subjects and the only one in subjects without neuropathy (12), while the number of A waves is small in the upper limbs, consistent with the frequency of neuropathic lesions (25-27).

NEUROPHYSIOLOGICAL ASPECTS OF THE AGING OF THE PERIPHERAL NERVOUS SYSTEM

In consideration of the extensive scientific documentation already acquired in the field of pathophysiology of the central nervous system, the research on the effects of aging on the peripheral nervous system has been oriented, poorly evaluated in the current international scientific literature.

With the aging process there are numerous processes and developments which organs and tissues undergo. The peripheral nerves do not escape these inevitable modifications.

The electroneurographic method allows an accurate exploration of the peripheral nervous system, allowing the study of the motor and sensory conduction speeds, of any alterations in late responses, the evaluation of the proximal roots of the motor nerves, the presence / absence of function, the various degrees of impaired motor and / or sensory function, the lesional site (proximal or distal), the type of distribution (asymmetrical, symmetrical, bilateral,multifocal) and clinical evolution (acute, subacute or chronic), progressive or regressive, obviously with valuable repercussions in terms of diagnostic and prognostic evaluations.

MATERIALS AND METHODS

The present research was conceived as a retrospective observational study aimed at the search for alterations in the peripheral nervous system in subjects over 65 years of age, without documentable alterations, commonly considered by the international scientific literature to be responsible for neuropathic lesions. The etiological diagnosis of neuropathies is sometimes extremely difficult, so much so that invasive procedures such as nerve biopsy are simetimes required (Brain 1996) (28).

During the activity of the Neurophysiopathology Unit aimed at the study of outpatient or inpatients at the Polyclinic of our University, between 1998 and 2016, among the approximately 55,000 patients undergoing neurophysiopathological evaluation, we selected 84 patients who demonstrated the absence of alterations that could be responsible for neuropathy, such as underlying or latent diabetes, alterations in renal or thyroid function, inflammatory bowel diseases, rheumatoid diseases, vasculitis or other autoimmune diseases, Vit. B12 deficiency, radiculopathies or neoplastic diseases (see. tab. 3).

Exclusion criteria:

- familiarity for hereditary neuropathy
- dystyroidism
- alcoholism
- Vitamin B12 deficiency
- inflammatory bowel diseases
- hemo-lymphoproliferative diseases

- neoplastic pathologies

- metabolic diseases (diabetes, nephropathies ...)

- vasculitis and rheumatological diseases (Wegener's granulomatosis, Churg-Straus syndrome, SLE, rheumatoid arthritis, Sjogren's S.)

- infectious neuropathies (HIV, borreliosis)

- granulomatous diseases (sarcoidosis, leprosy)

- hereditary forms (HNPP)

- atypical dysimmune diseases (i.e. Lewis Sumner syndrome, MMN)

- treatment, in the last year, with potentially neurotoxic drugs.

Table. 3

The patients consisted of 36 men and 48 women, aged between 65 and 94, who had not undergone treatment with drugs or neurotoxic substances in the 12 months prior to inclusion in our case series (2). The cohort of selected cases was divided into 3 distinct pools for 3 age groups:

1. patients aged between 65 and 74 years (n.32, 18F and 14M)
2. patients aged between 75 and 84 years (n.30, 16 F and 14 M)
3. patients aged 85 to 94 years (n. 22, 12 F and 10 M)

They were all subjected to the static-dynamic clinical evaluation, in search of all the sensory-motor and reflective functions: in particular, symptoms and signs typical of peripheral nervous system impairment were sought, such as hypoesthesia, paraesthesia, tingling, needles sensation, electric shocks, symptoms of localized or widespread reduction in muscle strength, in the absence of concomitant pathology leading to neuropathy and, to this end, for each of them, the usual tests aimed at excluding the presence of

the aforementioned pathologies, including ultrasound or neuroradiological evaluations where necessary.

All underwent the neurophysiological study, with research of sensory/motor neurography, including the search for late waves (F and A waves); if necessary, including electromyographic examination.

We examined 2 nerves of the upper limbs, median and ulnar, and 3 of the lower limbs, peroneal, tibial and sural, with complete neurography and inclusive of the search for late responses, in particular F and A waves. A total of 840 nerves (84x5x2) were therefore examined.

The nerve conduction speeds were studied with the use of surface electrodes with standard techniques. The nerves were stimulated with impulses lasting 0.2 ms, with a distal cathode, with an intensity such as to ensure the evocation of maximal cMAP (Fig. 4) (Peripheral Neuropathies- D. Cazzato, 2016).

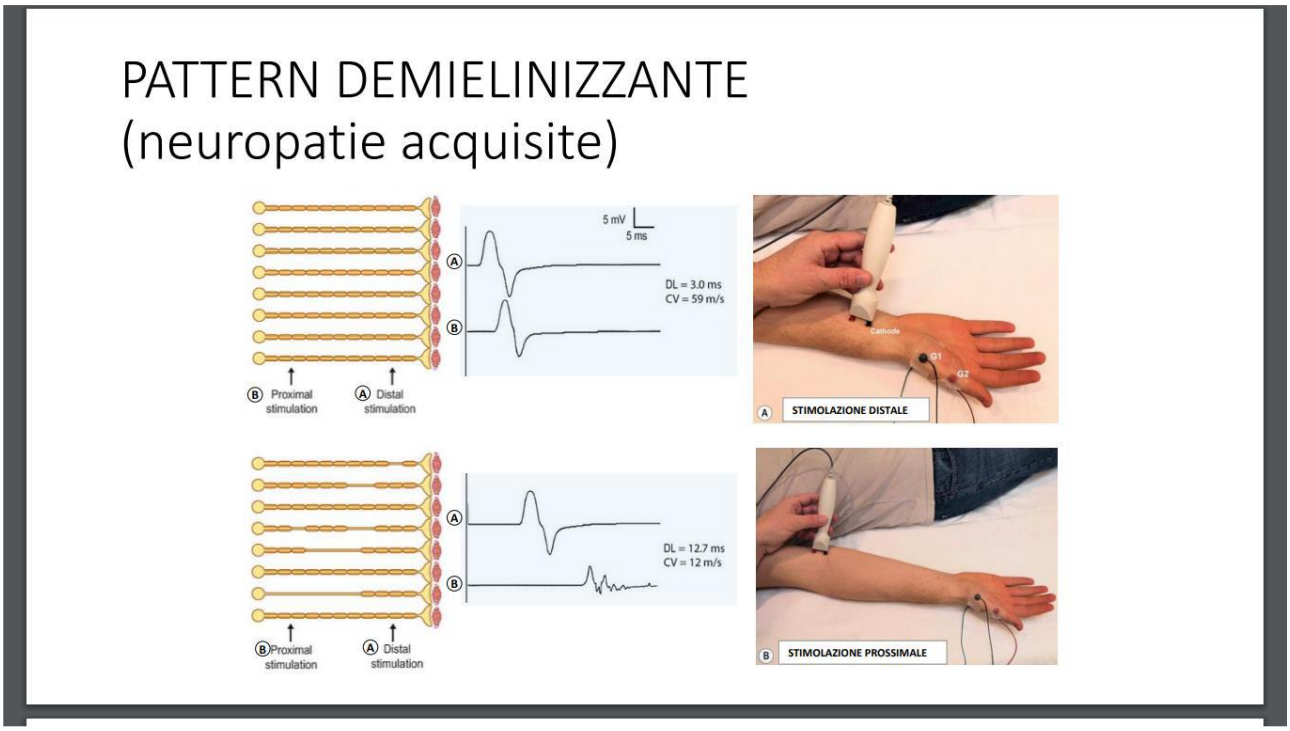


Fig. 4

The search for F waves was evoked with 10 supra-maximal stimuli delivered at the rate of 1 / sec. To differentiate the late responses from each other, occasional variations in the location or intensity of stimulation were used to differentiate between F waves, axon responses or A waves, as illustrated above. The criteria followed to differentiate the F and A waves were, according to the guidelines codified in the literature, the following (23):

F Waves

• Variable amplitude and morphology of the potential

• Variations in latency between 5 and 10 msec.

• Variable persistence in a train of 10 stimuli

• Sweep setting 10 ms / div., for the lower limbs, 5 ms / div., for the upper limbs

A Waves

• Amplitude and morphology of the stable potential

• Changes in latency <1.5 ms

• Persistence in at least 30% of the stimuli

• Sweep setting at 10 ms / div., for the lower limbs, 5 ms / div., for the upper limbs

The electromyographic examination was performed exclusively in patients with clinical symptoms or signs of denervation (muscle atrophy,

spontaneous activity with fasciculations) with a search for positive sharp waves and / or fibrillations.

RESULTS

The neurophysiological examination showed different results in the various nerves examined:

1. prevalent alterations relative to sural n. (125/168 nerves examined, 74%) which presented more frequently its absence (71 nerves), minus frequently a slowing of the conduction velocity (38), more rarely the reduction of amplitude (16 nerves),(see fig. 5,6)

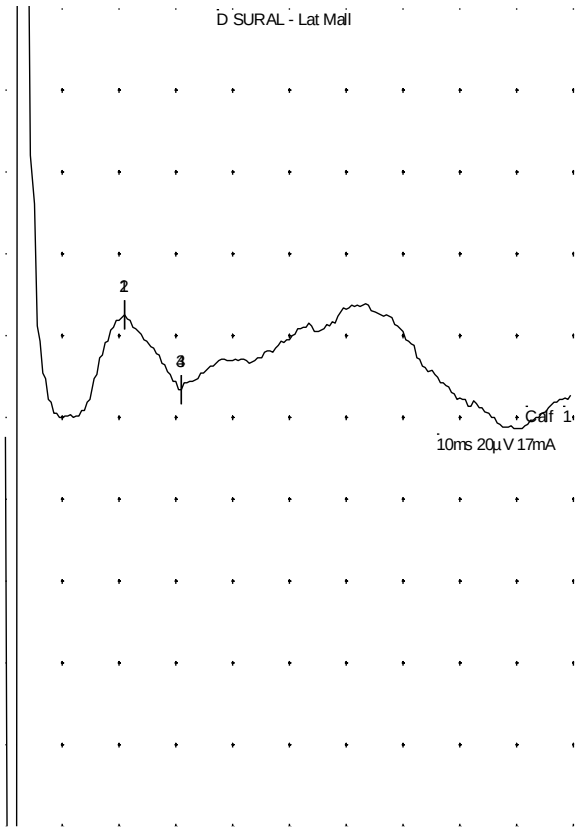


Fig. 5 Neurography of normal sural nerve

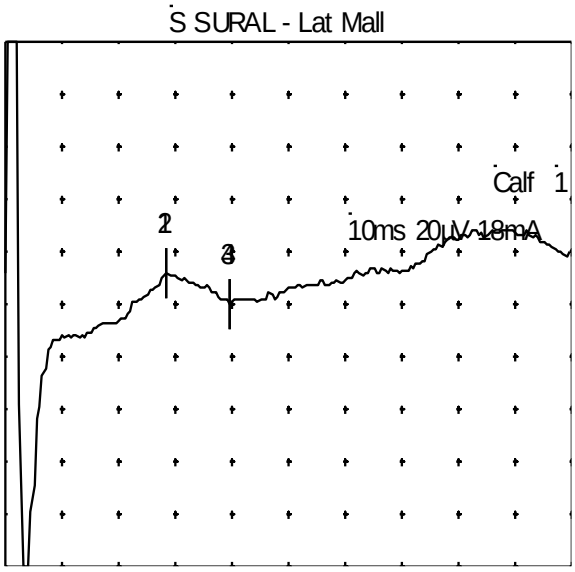
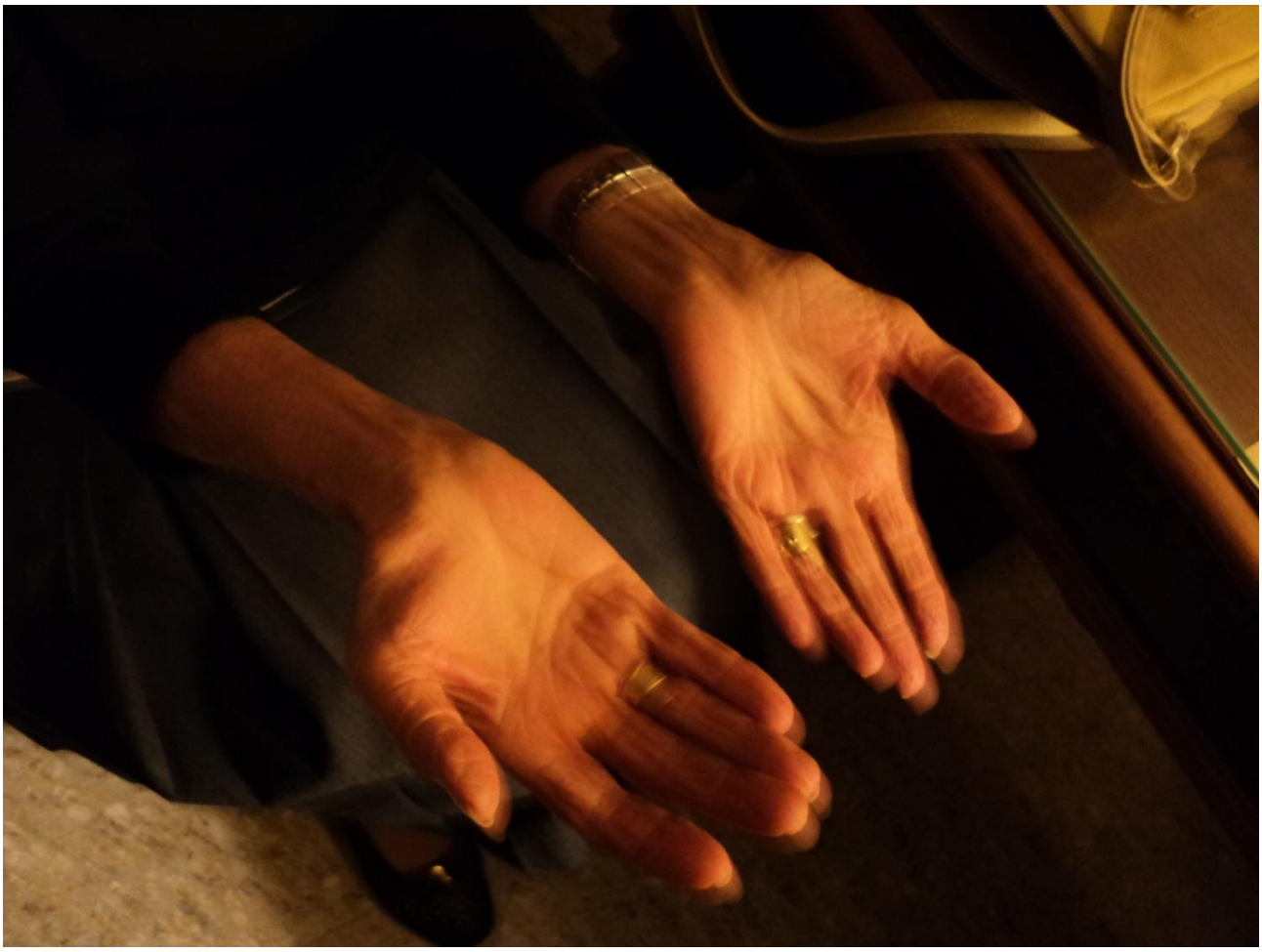


Fig. 6 Neurography of sural nerve with slight increase in latency and amplitude reduction.

2. after the sural nerve, the second nerve most affected by the alterations was the median nerve (109/168, 64%) who predominantly presented a reduction in sensory / motor conduction velocity and / or an increase in distal latency (95 nerves); only rarely (14 nerves) a reduction in amplitude was observed with signs of denervation (muscle atrophy, fasciculations / fibrillations on the abductor pollicis brevis muscle) (see fig. 7-10)



Fig, 7 Atrophy of median nerves (right> left).

Presence of marked hypotrophy of the abductor pollicis brevis muscle right> left, in the context of physiological mild diffuse hypotrophy of the muscles of the hands, with modest weakness of individual muscles examined. It should be reported that the patient, a person of high cultural level, a high school teacher, marveled at the reported objectivity, commenting that she had never noticed it before.

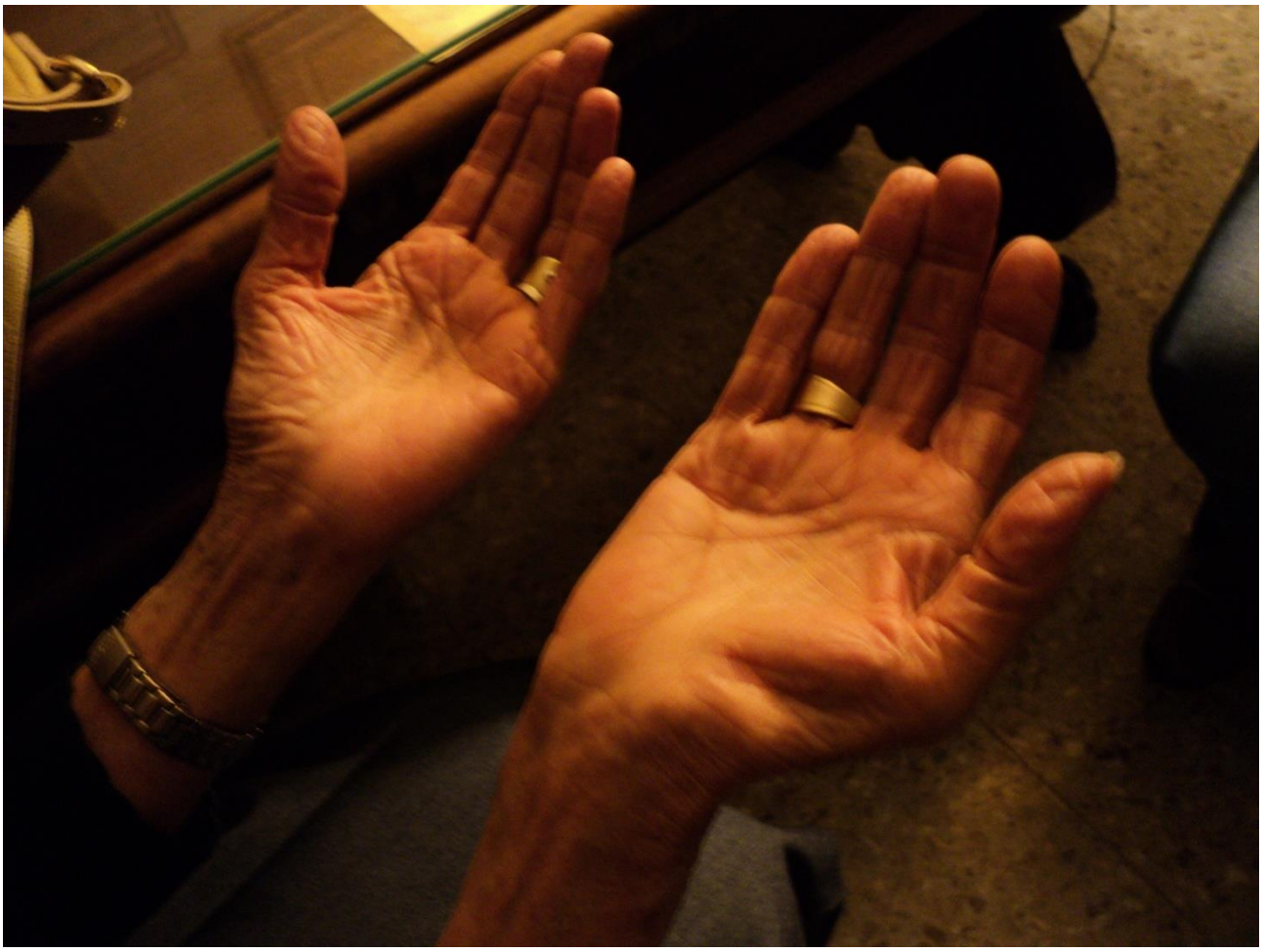


Fig 8

Same case as the previous figure

Neurophysiological evaluation of the median nerve:

Right median nerve

Nervo / Posizioni	Rec. Site	Lat ms	Amp mV	Amp.2-4 mV	Dur.1-3 ms	Area 1-3 mVms	Dist cm	Vel m/s
D MEDIAN - APB								
1. Wrist	APB	3,75	7,8	11,8	8,20	33,7		
2. Elbow	APB	8,45	7,8	11,5	8,75	34,9	25,5	54,3

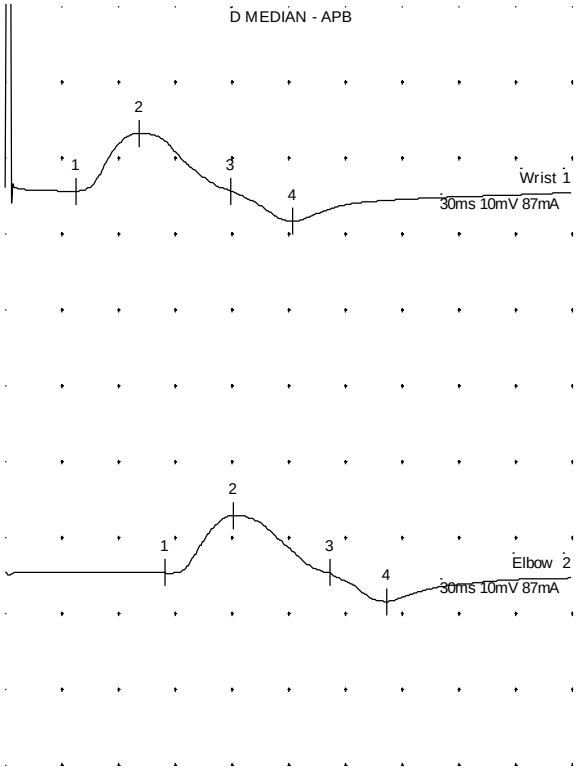


Fig 9

Neurography of the median nerve

Motor NCS

Nervo / Posizioni	Rec. Site	Lat ms	Amp mV	Amp.2-4 mV	Dur.1-3 ms	Area 1-3 mVms	Dist cm	Vel m/s
D MEDIAN - APB								
1. Wrist	APB	3,95	10,1	15,2	6,70	39,5		
2. Elbow	APB	7,50	10,1	15,2	6,60	38,4	20	56,3
S MEDIAN - APB								
1. Wrist	APB	3,35	6,8	11,0	7,50	29,5		
2. Elbow	APB	6,90	5,9	9,5	7,75	26,3	19	53,5

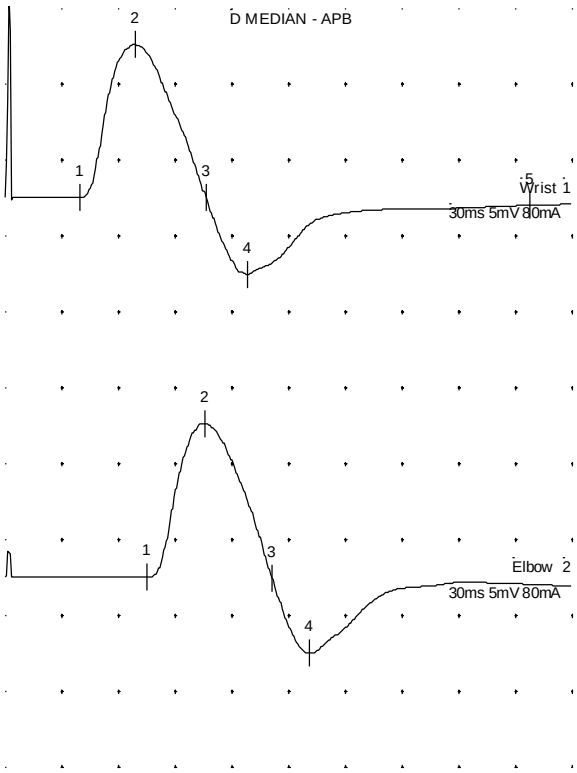


Fig.10

Pathological neurography of right median nerve (increased latency, 3.95 msec., n. v. <3.6 msec.). Normal amplitude and morphology.

3. the III nerve altered in frequency was the tibial nerve (101 out of 168, 60%), with a prevalent reduction in motor conduction speed, more rarely (24 nerves) reductions in amplitude with signs of distal denervation (muscle atrophy, fasciculations / fibrillations especially in the gastrocnemius) (see fig. 11-13):

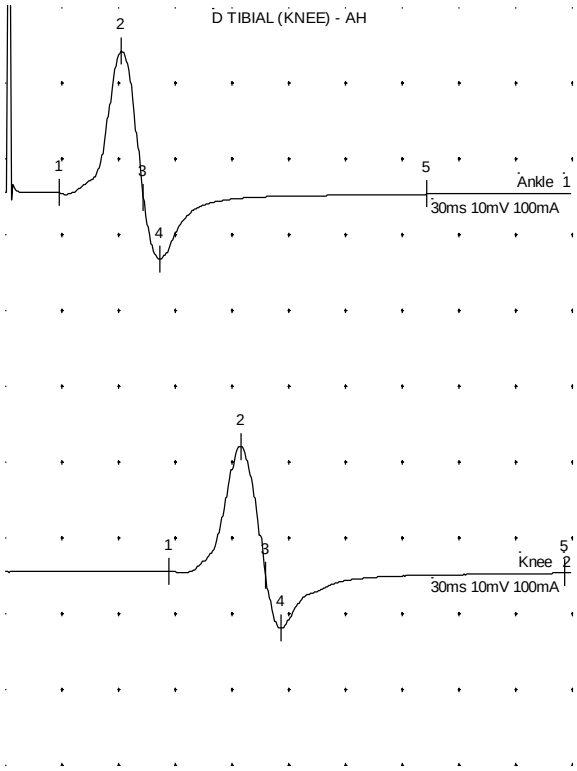


Fig. 11 Neurography of normal tibial nerve

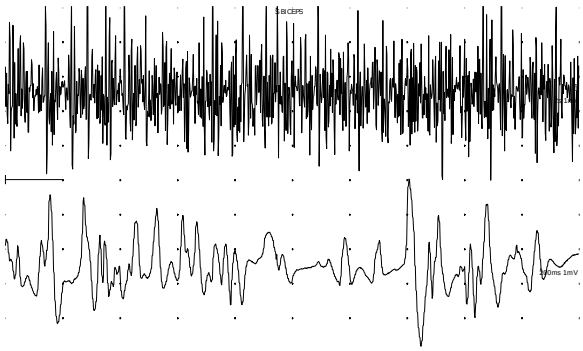


Fig. 12 Normal EMG of tibial n. detected on medial gastrocnemius m. at maximal effort (rich transition with potentials of normal amplitude e morphology)

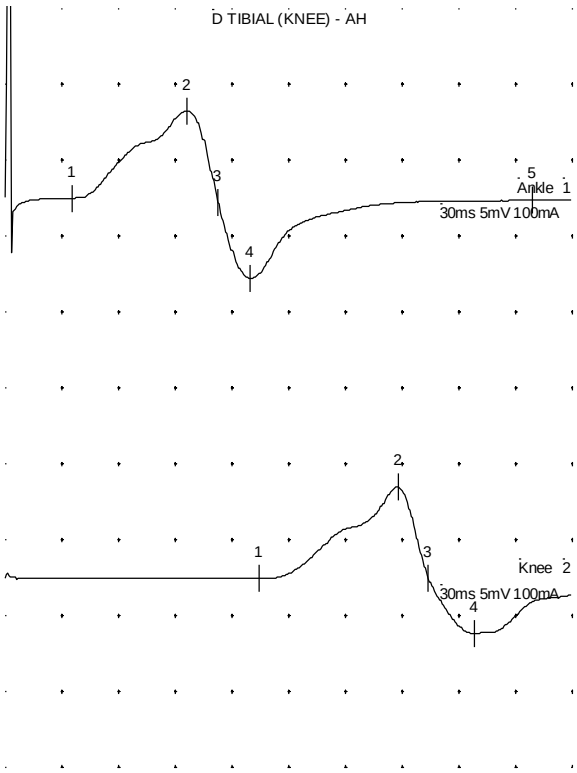


Fig. 13 Neurography of tibial n.with slight increase in latency

4. the peroneal nerve was compromised in 74 of the 168 cases examined (44%), with signs of demyelination and consequent reduction of conduction speed; in 15 cases there was denervation (fasciculation / fibrillation) with the presence of functional impotence (paresis) in the m. tibialis anterior (see fig. 14-16)

Nervo / Posizioni	Rec. Site	Lat ms	Amp mV	Amp.2-4 mV	Dur.1-3 ms	Area 1-3 mVms	Dist cm	Vel m/s
D COMM PERONEAL - EDB								
1. Ankle	EDB	2,85	6,0	9,0	4,50	12,3		
2. FibH	EDB	10,20	5,4	6,9	5,40	11,7	31	42,2

3. PopF-FibH	EDB	12,70	4,5	6,1	5,70	10,7	11	44,0

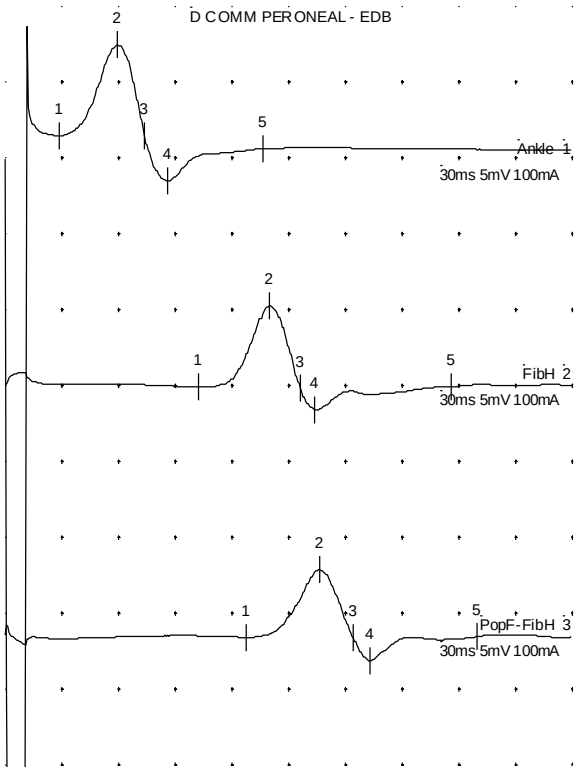


Fig. 14 Normal neurography of peroneal n., with stimulation of the ankle, below and above the head of the fibula

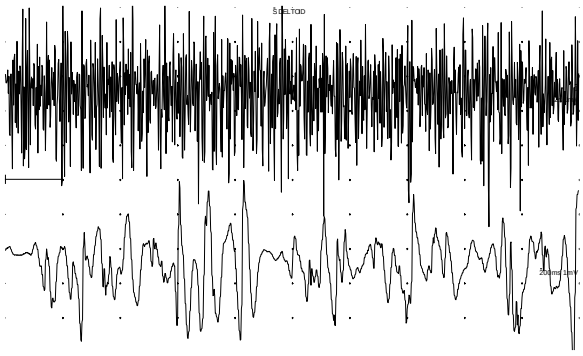


Fig. 15 Normal EMG of anterior tibial muscle at the maximum effort

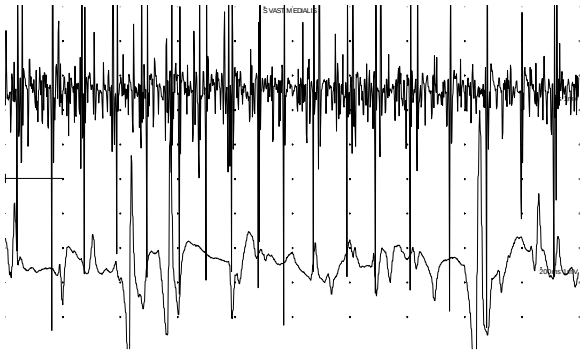


Fig. 16 Pathological EMG of anterior tibial muscle at the maximum effort (poor recruitment with amplitude potentials increased and altered morphology due to the presence of polyphasia, chronic denervation with collateral sprouting and reinnervation, with motor units enlarged)

5. the ulnar nerve was the least altered with evidence of alterations in 35 of 168 nerves (20%), with a prevalence of demyelinating alterations (reduction in sensory / motor conduction speed, increase in distal latency); in a few cases (n.7) denervation with fasciculations / fibrillations and muscle atrophy was observed in the hypotenar eminence or in the interosseous (see Fig. 17-19).

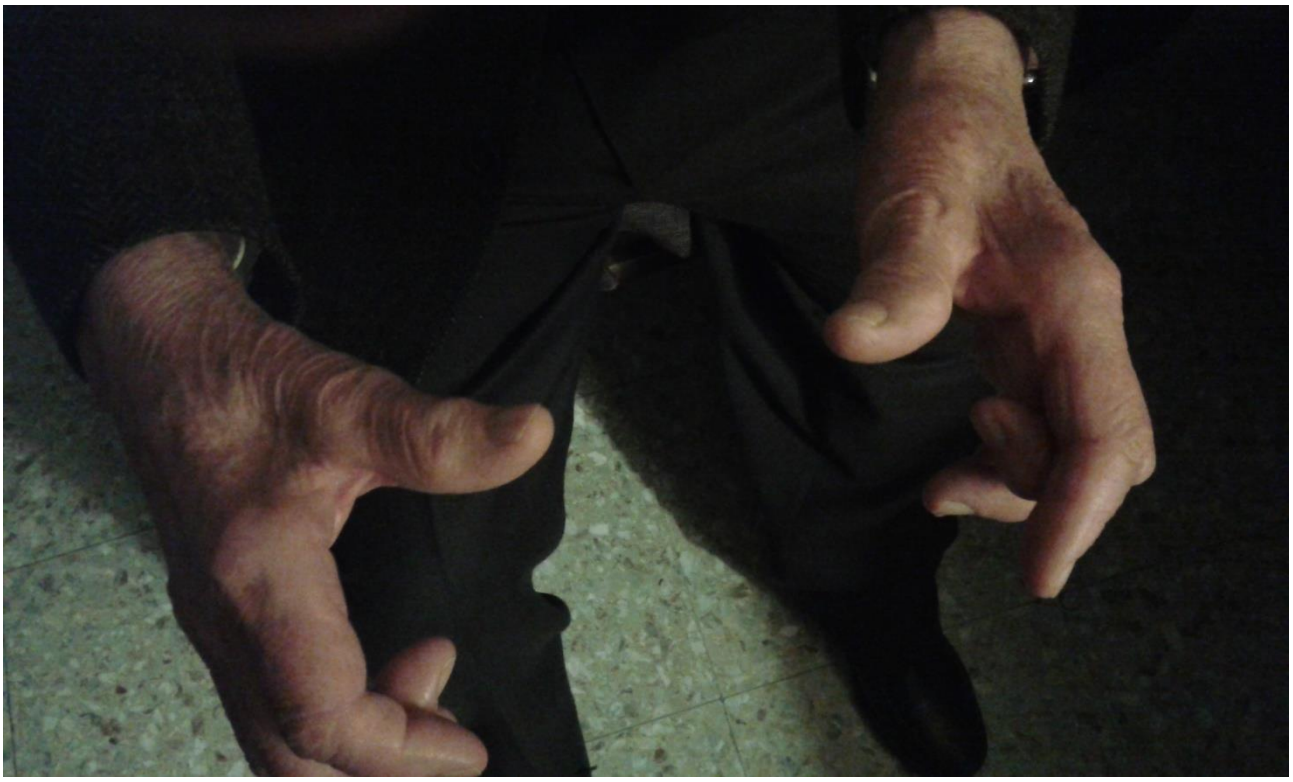


Fig. 17 Bilateral neuropathy of the ulnar nerve with signs of denervation and atrophy of the first dorsal interossei muscles.

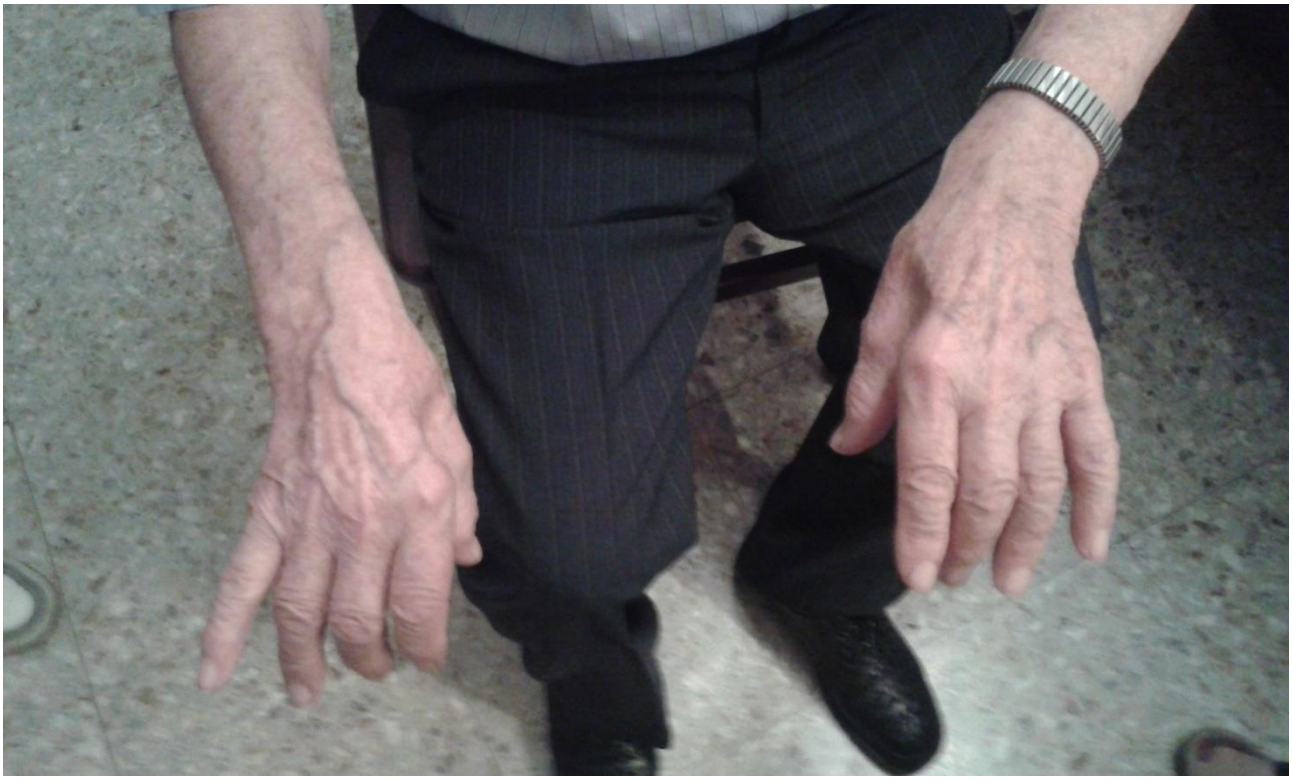


Fig. 18 Axonal suffering of ulnar nerves (bilateral axonal ulnar neuropathy).

Presence of marked hypotrophy of the dorsal interossei muscles of both hands, in a context of widespread hypotrophy and weakness of the remaining hand muscles, in particular of the first dorsal interossei muscles

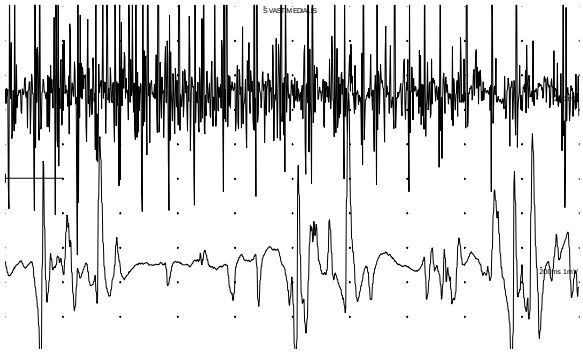
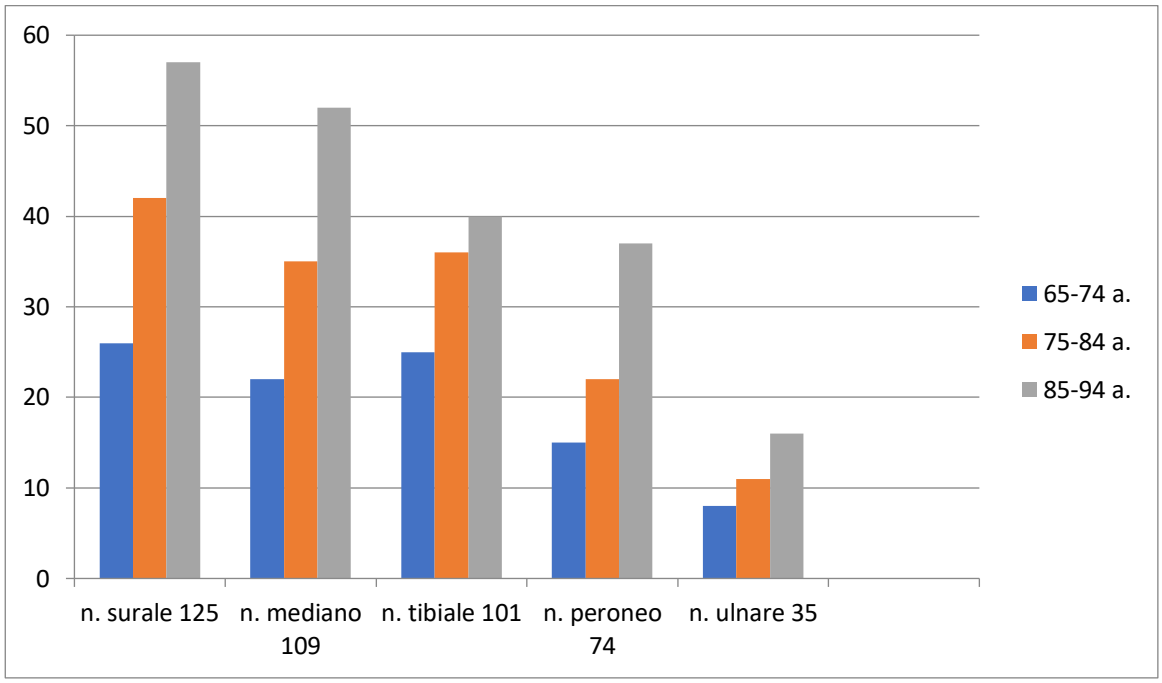


Fig. 19

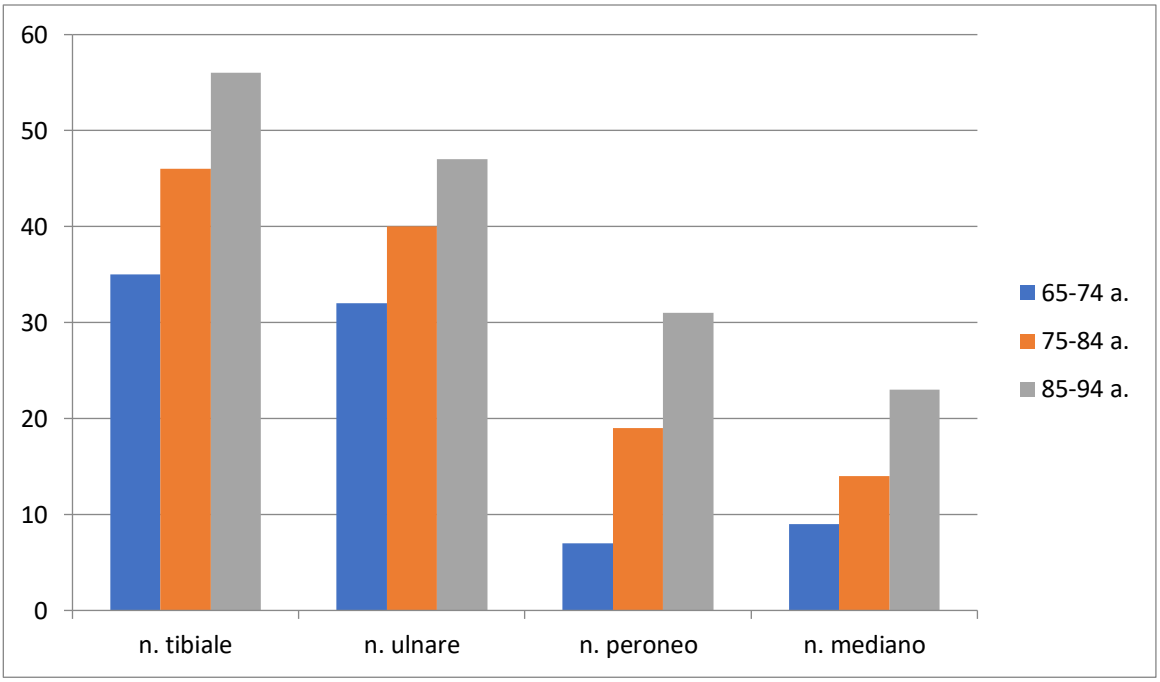
Pathological EMG due to the presence of reduced recruitment (poor transition), with the presence of potentials of increased amplitude (larger motor units due to the presence of denervation and collateral sprouting with recruitment of muscle fibers already of other motor units), with amplitude and duration increased of the potentials of the abductor muscle of the fifth finger.

Cumulatively the altered nerves were 444 out of the total 840 examined (52%) (see table 4).



Tab. 4

Number of altered nerves, individually or in association, chronocorrelated in all 3 age groups considered: qualitative and quantitative nerve alterations increased with advancing age.



Onde A

Tab. 5

Tab. 5

Percentage of A waves for each nerve examined, by age cluster.

A-waves represent an inconstant neurophysiological parameter in peripheral nerve diseases, often ignored and / or not recognized in neurophysiology laboratories. They are very valuable as their recognition and description in the final report contributes to the diagnosis of the nature of the nerve injury, as they are expressive of demyelinating disease.

The search for late waves (F and A waves), during the neurophysiological evaluation, contribute to detect frequent alterations in the presence, persistence, symmetry, latency and morphology of F waves in the nerves with demyelinating alterations, with recurrent appearance of A waves

(pathological), consistent with the presence of demyelinating lesions, in various combinations and locations with respect to F waves (see table 5).

A typical example of normal F waves can be obtained from the 2 nerves, tibial and ulnar nerve, which classically present F waves at supramaximal stimulation, with 100% persistence (see fig. 20-25).

The A waves can occur in various ways and localizations, usually interposed between the M and the F wave, but not necessarily and not only, in fact the latency was variable: in most cases it was found in an intermediate position between the M and the F, in other cases it appeared very close to the M (almost as if it were a late component), in still others it appeared very close to the F, to the point of being included or even more rarely of latency even higher than that of the F, in exceptional cases both before and after the F wave (see fig 34). as we can see from the examples that are illustrated below (see fig. 26-35):

F wave of normal ulnar n.:

Nervo	Lat. F min ms	Lat. F max ms	LatF media ms	Amp. F min mV	Amp. F max mV	Amp. F media mV	Lat. M min ms	Lat. M max ms	LatM media ms	Amp. M min. mV	Amp. M max mV	AmpM media mV	% F %
D ULNAR	29,30	30,15	29,75	0,1	0,2	0,1	2,45	2,85	2,63	4,9	5,6	5,3	100

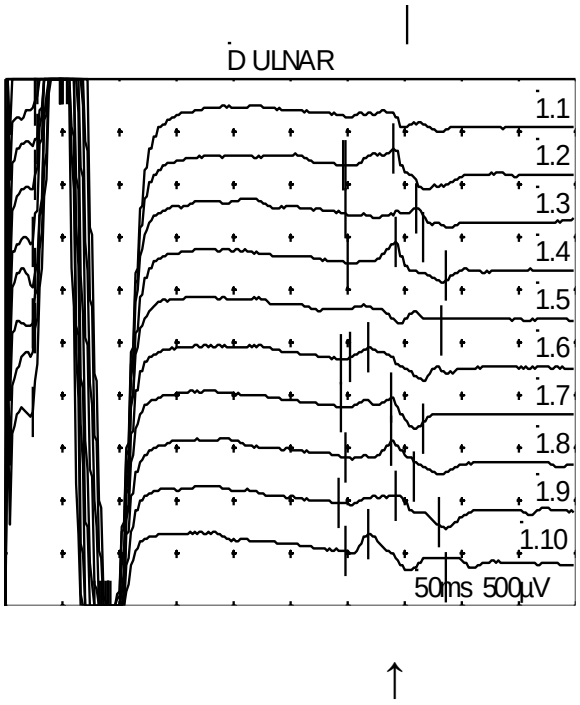


Fig. 20. Normal F wave (↑) of ulnar n. evoked in abductor of the fifth finger n. (normal latency, symmetrical with the contralateral finding, with normally variable morphology, with 100% persistence, with physiological fluctuation of the latency of appearance, usually greater than 1.5 msec)

Normal F wave of tibial n.:

Nervo	Lat. F min ms	Lat. F max ms	LatF media ms	Amp. F min mV	Amp. F max mV	Amp. F media mV	Lat. M min ms	Lat. M max ms	LatM media ms	Amp. M min. mV	Amp. M max mV	AmpM media mV	% F %
D TIBIAL (KNEE)	51,10	53,05	52,08	0,1	0,6	0,3	4,60	4,85	4,77	6,6	6,7	6,7	100

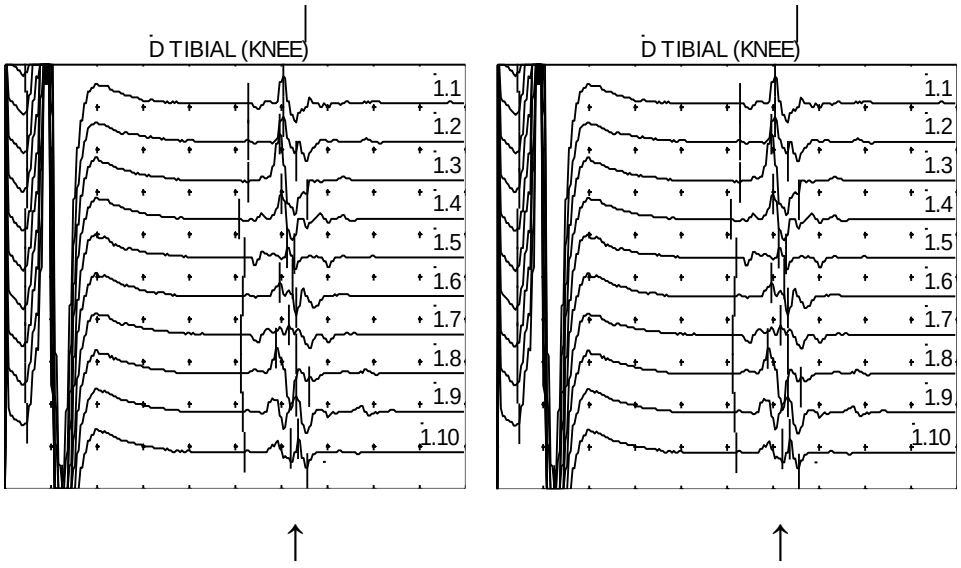


Fig. 21 Normal F waves (↑) of tibial n. evoked in medial gastrocnemius m. (normal latency, with 100% persistence, with physiological morphology and jitter of the onset latency usually greater than 1.5 msec)

Nervo	Lat. F min ms	Lat. F max ms	LatF media ms	Amp. F min mV	Amp. F max mV	Amp. F media mV	Lat. M min ms	Lat. M max ms	LatM media ms	Amp. M min. mV	Amp. M max mV	AmpM media mV	% F %
D TIBIAL (KNEE)	51,10	53,05	52,08	0,1	0,6	0,3	4,60	4,85	4,77	6,6	6,7	6,7	100

Normal F waves of tibial nn.:

Nervo	Lat. F min ms	Lat. F max ms	LatF media ms	Amp. F min mV	Amp. F max mV	Amp. F media mV	Lat. M min ms	Lat. M max ms	LatM media ms	Amp. M min. mV	Amp. M max mV	AmpM media mV	% F %
D TIBIAL (KNEE)	42,15	43,60	42,54	0,2	0,3	0,2	1,55	3,30	2,74	7,2	8,0	7,5	100
S TIBIAL (KNEE)	42,70	43,75	43,37	0,1	0,4	0,2	1,90	3,80	3,39	5,7	7,1	6,7	100

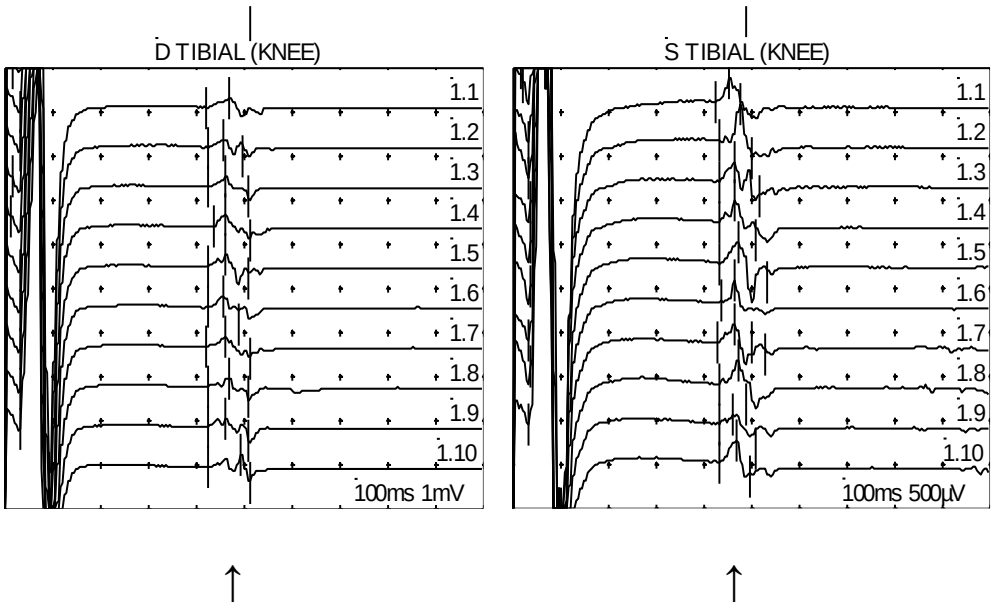


Fig. 22,23 Normal F (↑) waves of tibial n. with 100% persistence

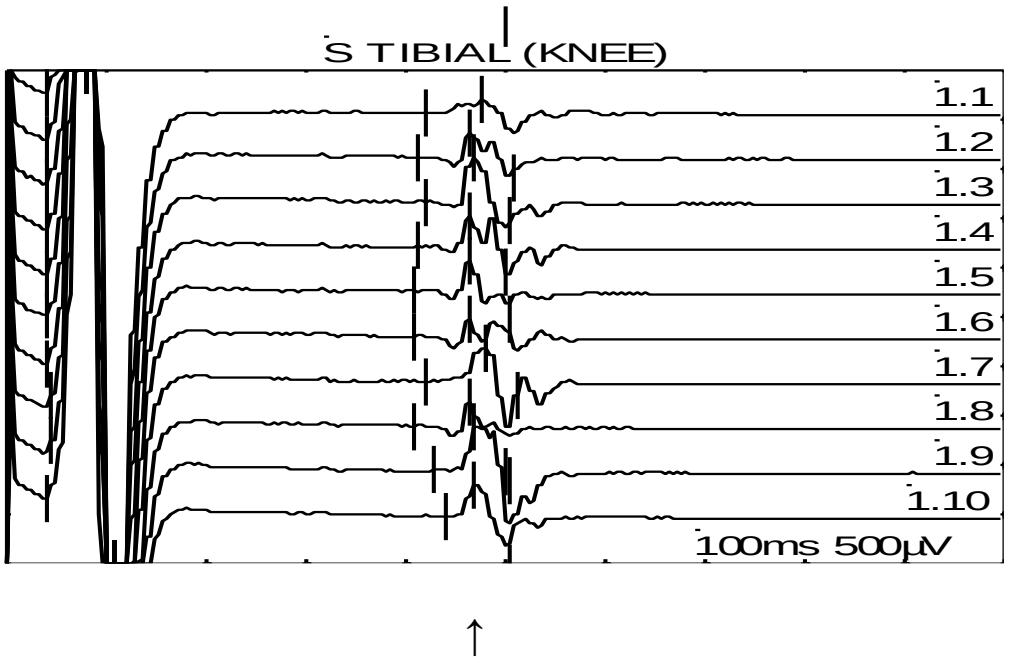


Fig.24 Normal F (↑) waves of tibial n. with 100% persistence

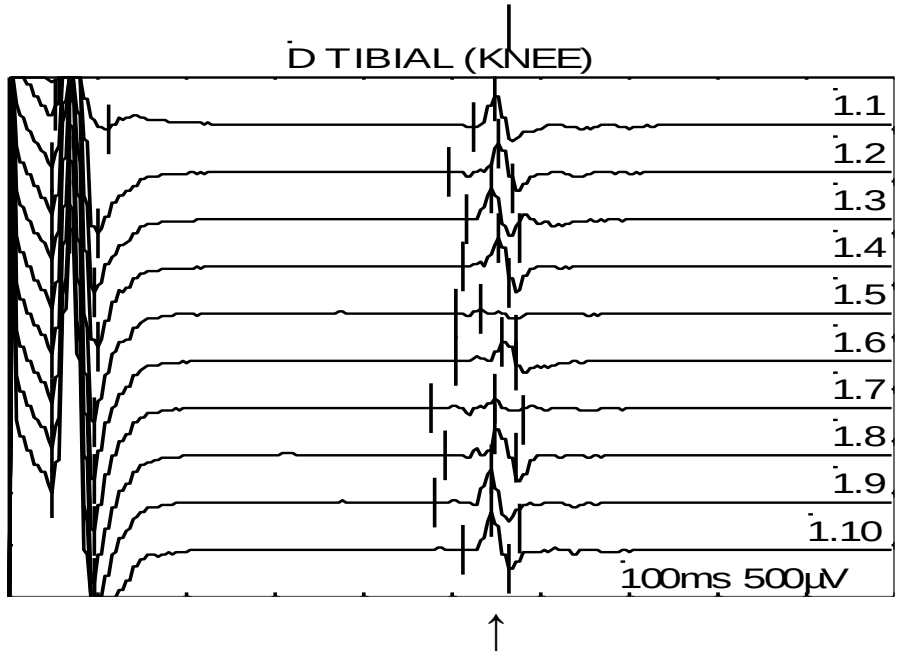


Fig.25 F waves (↑) of normal tibial n. with 100% persistence

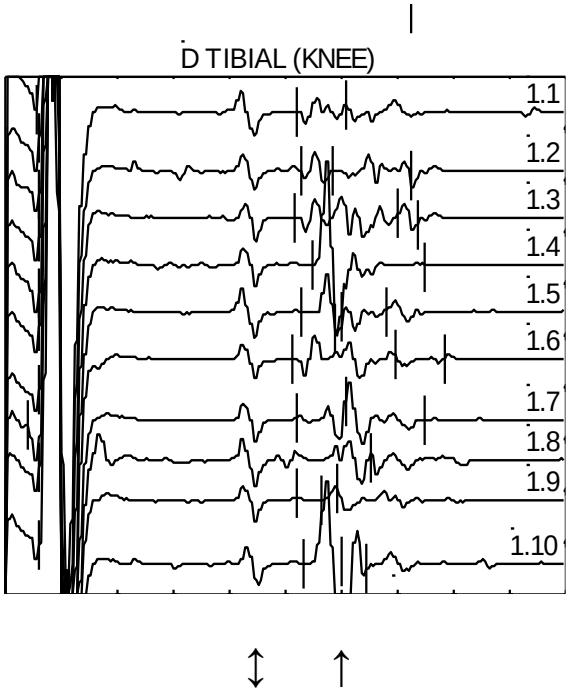


Fig.26 A waves (↕) pre-F (↑), with 100% persistence, with constant latency and identical morphology; the F waves present with significant temporal dispersion, consistent with the pathophysiological significance of the A waves (demyelinating disease of the nerve to which both belong).

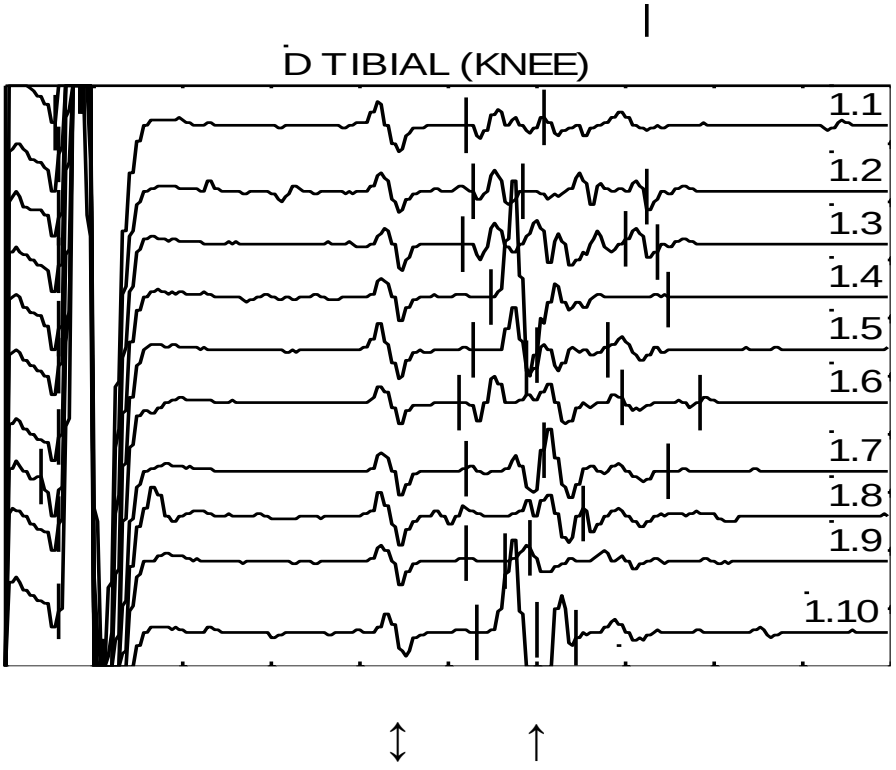


Fig.27 A waves (↕) pre-F waves (↑), with 100% persistence, with constant latency and identical morphology; the F waves present with significant temporal dispersion, consistent with the pathophysiological significance of demyelination of the A waves

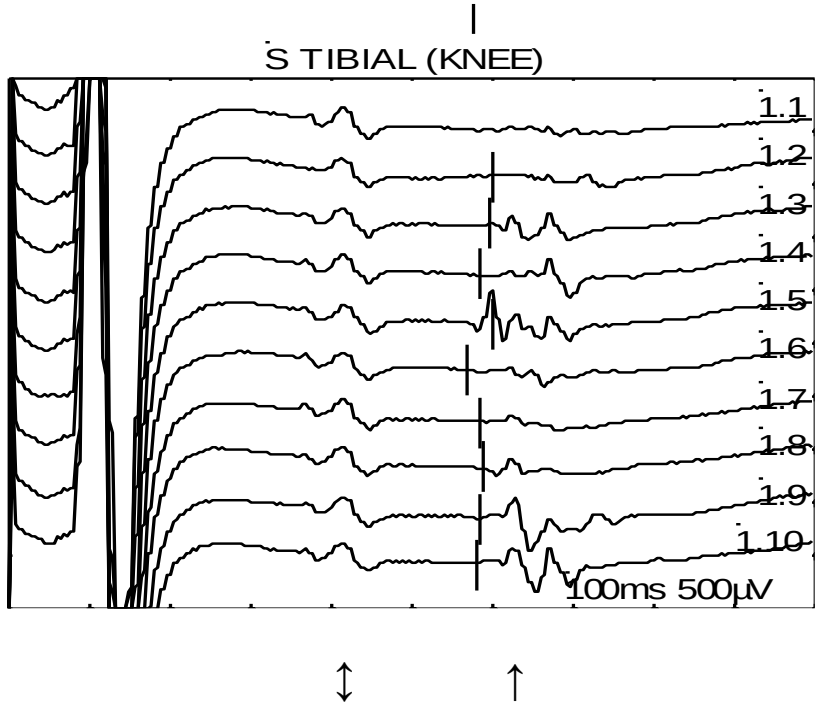


Fig.28 A waves (↕) pre-F (↑), with 100% persistence, with constant latency and identical morphology; the F waves present with significant temporal dispersion, consistent with the pathophysiological significance of the A waves (demyelinating disease of the nerve to which both belong).

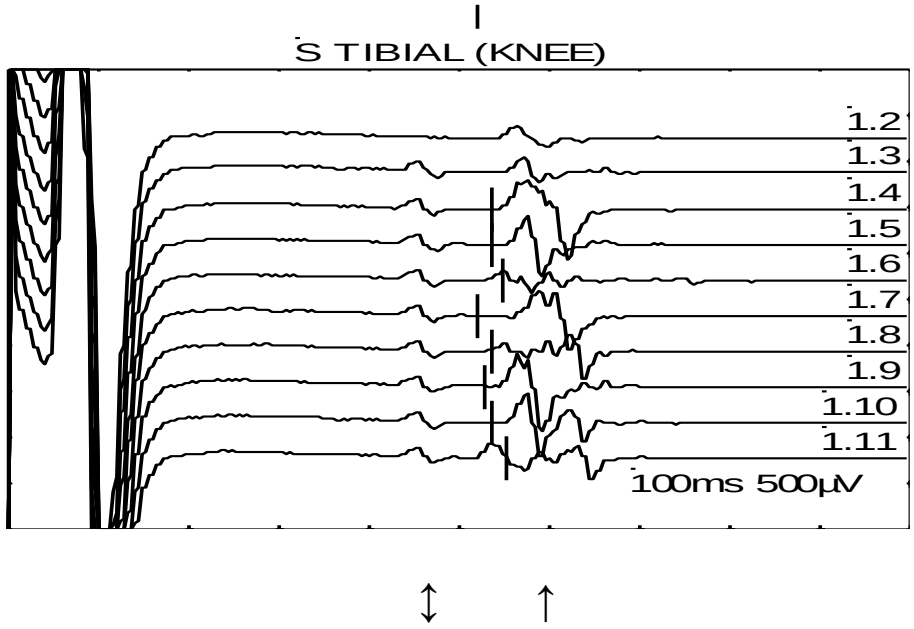


Fig. 29 A waves (↕) pre-F (↑), with 90% persistence, with constant latency and identical morphology; the F waves present with significant temporal dispersion, typical of demyelination.

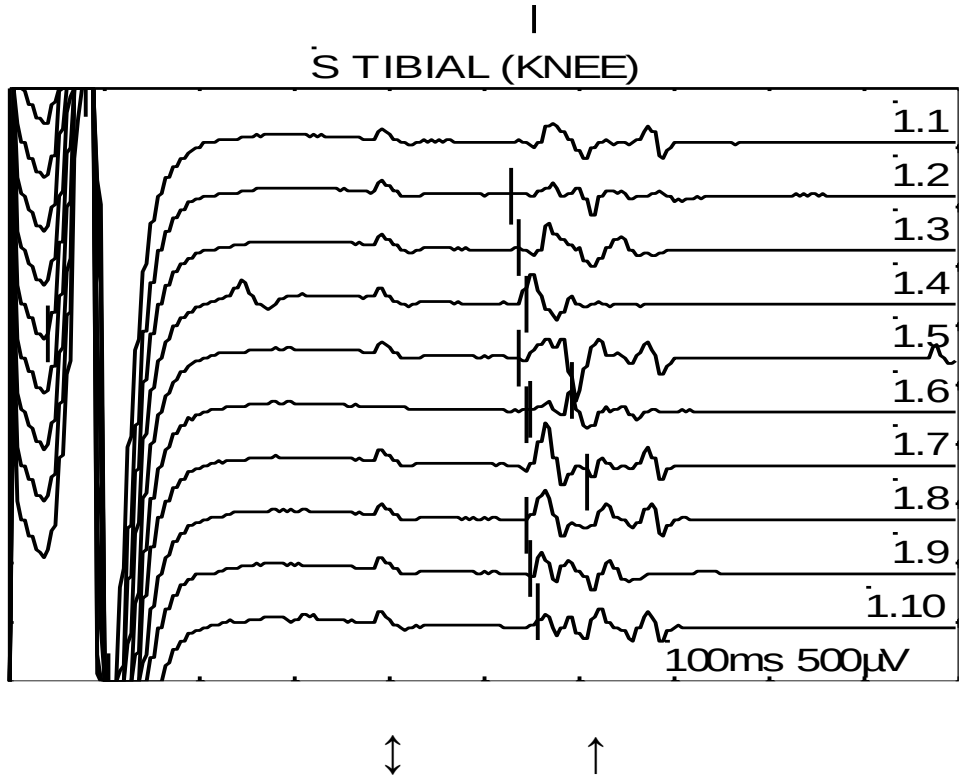


Fig. 30 A waves (↕) pre-F (↑), with 90% persistence, with constant latency and identical morphology; F waves with 100% persistence present with significant temporal dispersion, indicating demyelinating disease.

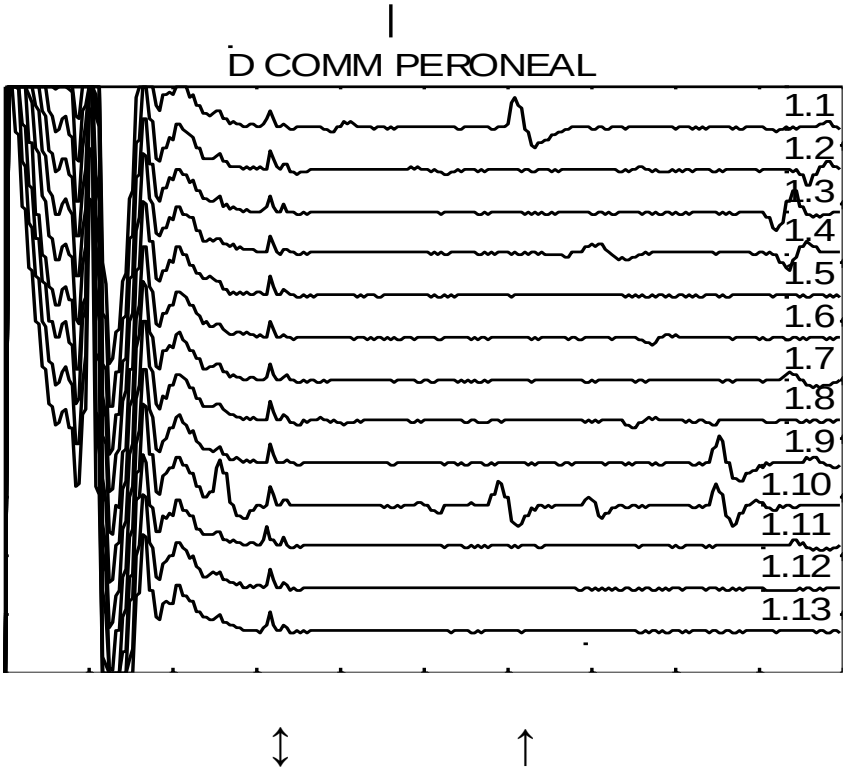


Fig.31 A waves (↕) pre-F (↑), with 100% persistence, with constant latency and identical morphology; F-waves with low persistence and dispersed (demyelination)

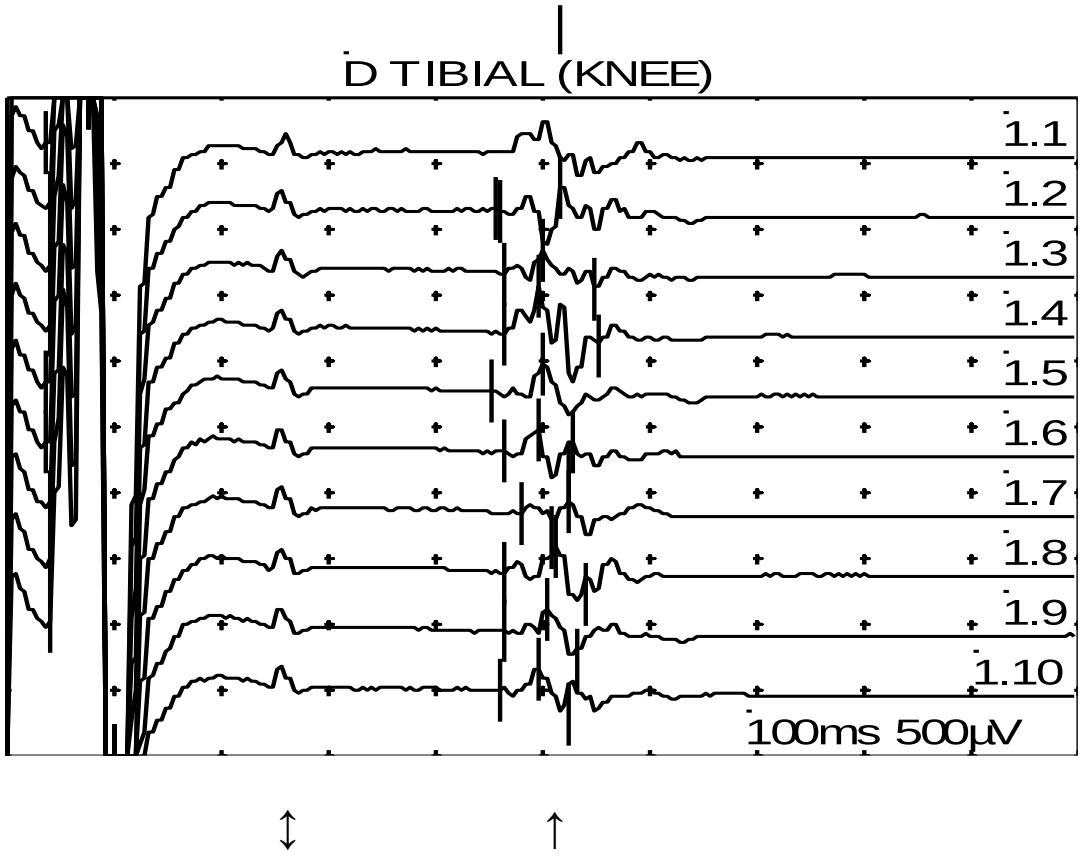


Fig. 32 A waves (↕) pre-F (↑) of tibial n. with 100% persistence, with constant latency and identical morphology; also the F waves with high persistence (100%).

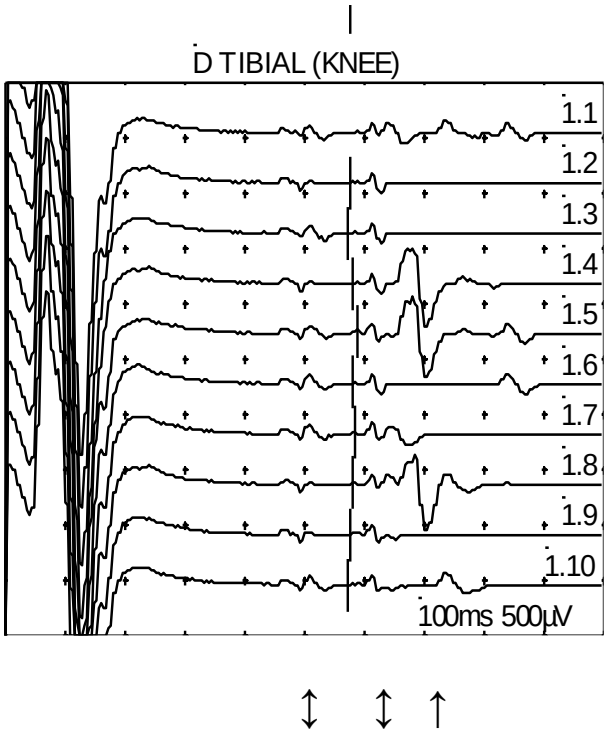


Fig. 33 Double series of A (↕) pre-F (↑) waves, both with identical morphology and latency of each series and persistence (100%).

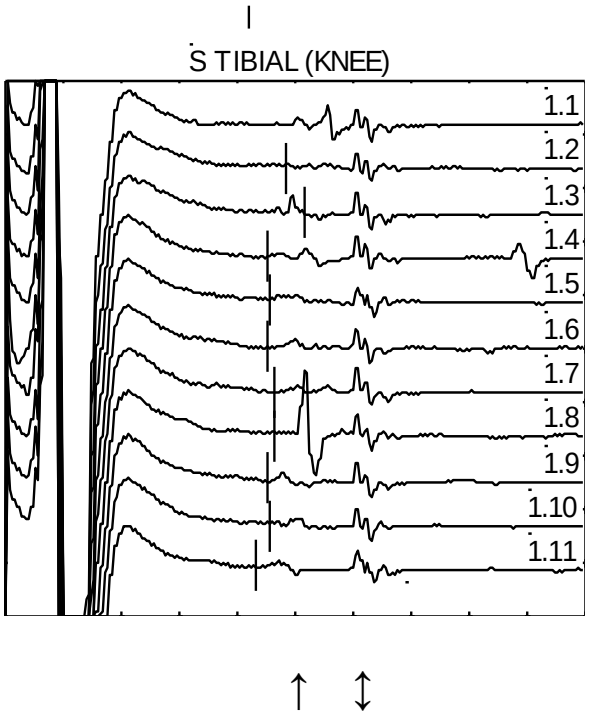


Fig. 34 Low persistence F (↑) waves with presence of post-F (↕) A waves, with 100% persistence

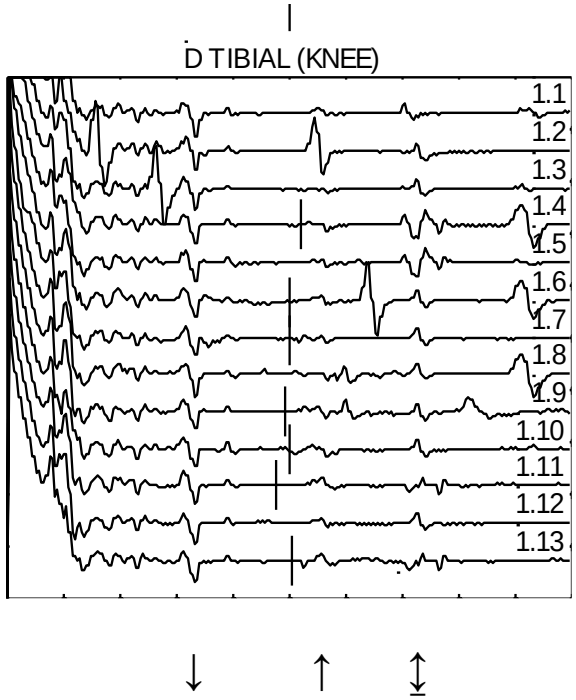


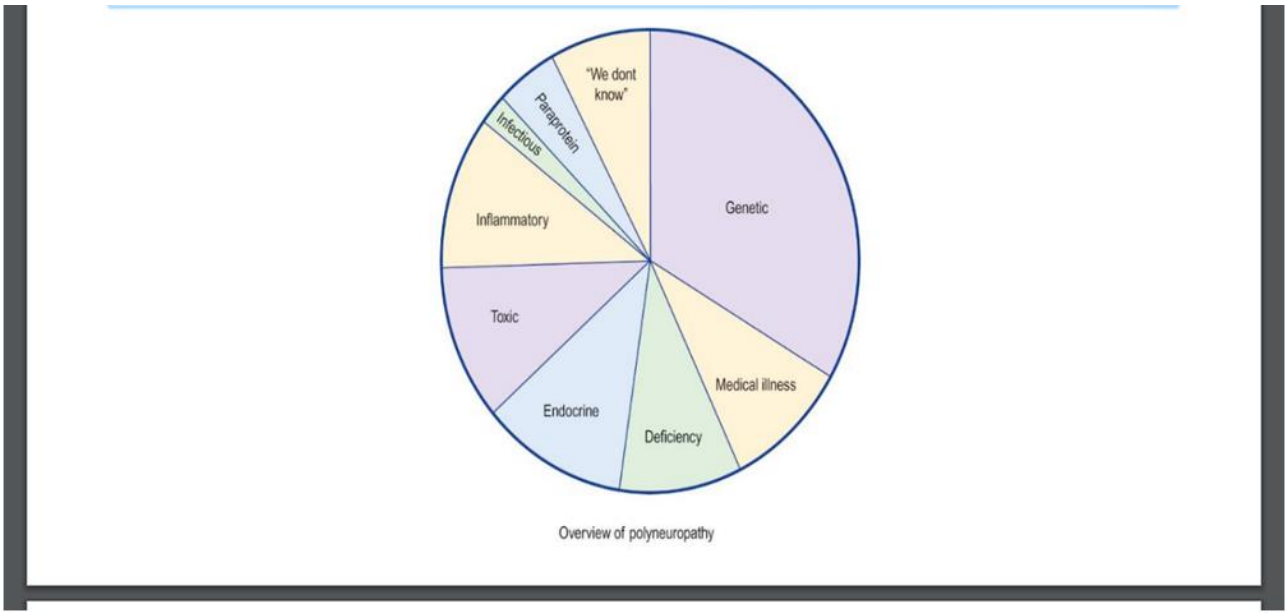
Fig. 35 A waves (↓) pre-F (↑) and post-F (↕), with 100% persistence, with constant latency and identical morphology; the F waves present with significant temporal dispersion and poor persistence, consistent with the pathophysiological significance of the A waves (demyelinating disease of the nerve to which they belong).

DISCUSSION

Previous experimental observations have reported a loss of myelinated and unmyelinated nerve fibers in old age and several abnormalities involving myelinated fibers, such as demyelination, remyelination and the presence of myelinated swellings. These anomalies was morphologically already documented through the finding of deterioration of the myelin sheaths, during aging, due to a decrease in the expression of the main myelin proteins (P0, PMP22, MBP) (29).

The percentage of alterations observed in our series didn't have an equal frequency of clinical symptoms reported by patients, who in many cases were asymptomatic; it is also specified that the significant percentage of neuropathies found in our case series is due to the inclusion not only of polyneuropathies, for which the international literature reports an incidence from 7% (30,31) up to 13% (32), but also of multineuropathies and mononeuropathies.

Our neurophysiopathological results could be considered as a contribution to the interpretation of those clinical pictures reported in authoritative scientific treatises (Adams and Victor's Principles of neurology 2019 McGraw-Hill Education) and in the series of numerous clinical trials, which report forms of "chronic benign polyneuropathy of the elderly", of “neuropathies of uncertain etiology”, of “neuropathies of unknown cause”, “cryptogenic” or “primitive”, frequently reported in clinical case histories (table 6), such as the absence of the SNAP of the sural nerve, currently considered a "normal" finding in the elderly in electromyography laboratories when it's alone.



Tab. 6 Percentage distribution of neuropathies

In aging, the peripheral nerves show a progressive slowdown in the conduction speed of the impulses, and consequent intensity and sensory reduction; reflexes become slower and often lead to the appearance of sensory symptoms, negative (hypo-anesthesia) or positive (paraesthesia), and / or the appearance of symptoms and motor signs, such as weakness and development of unnatural attitudes and behaviors.

Nerve conduction can slow down because myelin sheath around the nerves show progressive degeneration with consequent qualitative / quantitative sensory stimuli alterations, which appear to be slowed down.

The morpho-functional deterioration of the peripheral nerves can be interpreted assuming their degeneration connected with various multiple factors. In aging, the vasa nervorum show progressive obstruction with repercussions on the nutrition of the stromal and parenchymal structures of the nerves. This occurrence is overwhelmingly accentuated and amplified by pathological events such as traumatic ones, but specially metabolic

ones, such as in the onset of glycidic metabolism alteration, when appears obliteration of the microcirculation of epi-meso-endoneurium. The plasticity of the peripheral nervous system response to traumatic injuries is also reduced. When the axon of a peripheral nerve is damaged in a young man, the nerve can repair itself as long as its pyrenophore, situated in the spinal cord or spinal ganglia, is not damaged. This self-healing process in older people occurs more slowly and incompletely, making them more vulnerable to any type of injury.

As opposed to young subjects, in elderly man, even without pathogenic contexts, we found:

• a reduction in sensory and motor conduction speeds

• a reduction in the amplitude of motor unit potentials (CMAP) and sensory potentials (SAP)

• a slowing down of late responses

• an increase in the percentage of A waves

• occasional finding of denervation activity (fibrillation and / or fasciculation)

In the evaluation of elderly patients, our study consisted of the analysis of the changes in the latencies of the responses, the amplitudes of the cMAPs and the conduction speeds, the study of late waves, in particular of A waves, which, if present, represent valuable complement to neurophysiological evaluation, considering all the evaluable elements: morphology, presence / absence, persistence.

Previous studies focused attention on the regulation of the expression of the three main myelin proteins: P0, MBP, PMP22, whose alteration seems to be the basis of the predominant structural changes in the elderly myelin nerve fibers.

P0 (30 KDa) is the most abundant myelin protein (33).

It consist of an extracellular domain similar to an immunoglobulin (Ig), a single transmembrane helix and a cytoplasmic extension (P0ct); it has an important role in ensuring the integrity of compact myelin in the extracellular compartment. In vitro studies suggested a fundamental function in myelin compaction as a result of its homophilic adhesive properties when expressed transgenically in various cell types. For P0, several mutations have been identified that causes peripheral neuropathies, demonstrating its physiological importance, P0 point mutations represent 10-12% of all cases of demyelinating Charcot Marie Tooth (CMT) type 1 (29, 34).

MBP (21KDa), the second major protein in central nervous system myelin, is responsible for adhesion of the cytosolic surfaces of multilayer compact myelin and include several isoforms that differ in molecular weight and amino acid composition, resulting from an alternative splicing process of a common precursor mRNA. First discovered in the mouse and then in man, they are encoded by a located on chromosome 18q22-qter gene formed by seven exons, which form the protein expressed at the CNS with respect to the forms present in the immune system or within them. The different isoforms derive from alternative transcripts and the most represented have molecular masses of 21.5, 18.5, 17 and 14 kDa. The isoforms of 21.5 and

20.2 kDa, which contain the transcript of the second exon, are more expressed during myelination and reappear in chronic lesions from multiple sclerosis during remyelination. The amino terminal subunit shows post-transcriptional changes (acetylation, phosphorylation and methylation). There is indirect evidence of how its methylation can play an important role in giving a compact shape to membranes during maturation. MBP is located on the cytoplasmic side of myelin membranes and it is believed that it's responsible for the electron line visible on electron microscopy. The protein has an important role in the stabilization of myelin spiral structure by interacting with the negative charges of the membrane phospholipids. It can act as a membrane actin-binding protein, which could allow it to participate in the transmission of extracellular signals at the cytoskeleton in oligodendrocytes and at tight junctions in myelin (35,36).

PMP22 (18 KDa) represents approximately 2% to 5% of myelin proteins and, in common with P0, mutations or duplications in the gene often lead to peripheral neuropathy (29).

It's a very hydrophobic glycoprotein and is highly expressed in the compact myelin of the PNS. Comparison of marker genes on mouse chromosome 11 and human chromosome 17 revealed that PMP22 is thought to be involved in a very common autosomal dominant demyelinating peripheral neuropathy in humans, Charcot-Marie-Tooth disease type 1A (CMT1A). In accordance with these statements, PMP22 was found to be overexpressed in CMT1A patients who have the characteristic duplication. The crucial role of PMP22 in the etiology of

CMT1A was confirmed by the generation of transgenic mice and rats with an increase in the expression of the PMP22 gene, which caused severe peripheral myelin deficiency (2).

These proteins are already synthesized during embryogenesis and show a peak of expression in the post-natal period.

Two other less important proteins are myelin-associated glycoprotein (MAG, 100 KDa), formed by a single long strand, organized in 5 globular domains similar to those of antibodies, which plays a role in glio-axonal interactions (37-38 -39), and connexin 32 (Cx32, 32 KDa), whose function in the myelin of the peripheral nervous system is not yet fully understood, but is believed to be responsible for the formation of channels in the myelin that allow communication between the cell body of the Schwann cell and the axon, whose gene is located on chromosome Xq13 (40).

Mutations found in the two major glycosylated peripheral myelin transmembrane proteins, peripheral myelin zero protein (P0) and peripheral myelin protein 22 (PMP22), have been independently associated with the most common described above hereditary peripheral demyelinating neuropathies (41,1) .

It can be also assumed an alteration of the expression of the main myelin proteins (P0 and PMP22) as co-responsible for the reduction of the biochemical functions of the nerve with aging (42-43).

About the effect produced by aging, recent studies show that the speed of nerve conduction remains unchanged during adulthood until the last third of life, when it tends to decline: there is a marked loss of fibers, which

mostly involves non-myelinated axons rather than myelinated fibers, resulting in a decrease in the density of nerve fibers in the endoneurium. Myelinated nerve fibers were also altered, with a decrease in the size, circularity and thickness of myelin in elderly subjects over 70 years of age. In aging, the proportion of myelinated fibers of all sizes showing irregular shapes in cross nerve sections increased by 50%. Changes in the shape and structure of axons in neurons of all sizes have been correlated with a decrease in neurofilament mRNA expression with aging (44,45).

Already in the early 1980s it had been shown that the endoneurial levels of ATP and creatine phosphate progressively decrease with age; furthermore, the aging of the peripheral nerve also seems to reduce the energy requirement and at the same time also the energy reserves (46).

From several studies conducted on rats, many authors concluded that nerve blood flow decreases with aging due to a reduction of microvascular caliber, and it also been observed a reduction in the disposal of oxygen free radicals with increasing age; reduced axonal transport has also been demonstrated in older nerves compared to younger nerves (47).

Numerous studies conducted on elderly animals have seen a progressive reduction in myelin thickness associated with structural changes (48).

This impairment can be countered by the use of neuroactive steroids, such as progesterone (PROG), dihydroprogesterone (DHP), and tetrahydroprogesterone (THP), which are able to increase the expression of these proteins in the sciatic nerve of old age rats improving at the same time morphological alterations of the myelin sheath (49).

Similarly, treatment with pregnenolone (PREG), PROG and DHP is able to improve morphological alterations of the peripheral nerve caused by trauma from crushing, resection or cryolesion by increasing the levels of gene expression of P0 and counteracting the myelin sheath reduction.. This statement can explain the significant improvements observed during steroid therapy in cases of various nerve injuries (e.g. entrapments, diabetic neuropathy, etc.) but also in various peripheral nervous system pathologies that lead to a reduction in functional parameters of the nerve, including aging. In the elderly subject, in fact, there is an improvement in motor performance and electrophysiological parameters, mainly affecting the limbs, with steroids treatments (50).

The electrophysiological findings we found were a slowdown in motor and sensory conduction speeds with an increase of distal latencies and the F response latency increase. The reduction in both motor and sensory conduction speeds, an indication of myelin sheath damage / remodeling, has been observed with greater frequency in over 75 subjects. These changes were already reported in the second half of the last century, in many studies conducted on mice nerves in relation to their developmental age as shown in Table 7 and Fig. 36, (51) while from 12 to 20 months of age there were only slight changes, such as a slight decrease in the number and density of myelinated and unmyelinated nerve fibers, starting from 20 months the nerves showed a marked loss of fibers involving more non-myelinated axons than myelinated fibers, and consequent decrease in the nerve fibers density of the endoneurium.

Species	Nerve tested	Age	NCV (m/s)	*Reference*
Human	Median (sensory)	16–36 yr	46.1 _ 6.8	*Drechler 1975*
		67–91 yr	39.2 _ 7.4	
Human	Ulnar (sensory)	16–36 yr	45.7 _ 5.9	*Drechler 1975*
		67–91 yr	37.3 _ 6.8	
Human	Median (motor)	18–45 yr	59.4 _ 2.6	*Dorfman and Bosley 1979*
		60–86 yr	52.6 _ 4.0	
Human	Median (sensory)	18–45 yr	64.3 _ 3.2	*Dorfman and Bosley 1979*
		60–86 yr	56.9 _ 5.0	
Human	Median (motor)	21–29 yr	61.0 _ 2.9	*Bouche et al 1993*
		60–80 yr	54.7 _ 4.0	
		81–103 yr	53.0 _ 5.6	
Human	Median (sensory)	21–29 yr	47.8 _ 4.3	*Bouche et al 1993*
		60–80 yr	42.7 _ 6.0	
		81–103 yr	42.0 _ 7.8	
Human	Sural (sensory)	21–29 yr	43.0 _ 4.3	*Bouche et al 1993*
		60–80 yr	39.7 _ 6.4	
		81–103 yr	37.6 _ 5.2	
Cat	Masseter	1–3 yr	81.1 _ 9.7	*Chase et al 1992*
		_15 yr	59.2 _ 8.6	
Rat	Caudal	3 mo	45.2 _ 3.5	*Schmelzer and Low 1987*
		13 mo	70.1 _ 5.3	
		20 mo	50.5 _ 6.9	

Species	Nerve tested	Age	Latency (ms)	*Reference*
Human	Median (sensory)	20–29 yr	3.15 _ 0.16	*LaFratta and Canestrari 1966*
		30–39 yr	3.19 _ 0.21	
		40–49 yr	3.43 _ 0.30	
		50–59 yr	3.67 _ 0.30	
		60–69 yr	3.82 _ 0.54	
		70–79 yr	3.96 _ 0.60	
		_80 yr	4.08 _ 0.50	
Mouse	Sciatic (motor)	2 mo	2.14 _ 0.29	*Verdú et al 1996*
		6 mo	1.80 _ 0.24	
		9 mo	1.84 _ 0.18	
		12 mo	1.75 _ 0.22	
		18 mo	2.16 _ 0.28	
		24 mo	2.07 _ 0.48	
Mouse	Sciatic (sensory)	2 mo	1.89 _ 0.37	*Verdú et al 1996*
		6 mo	1.64 _ 0.30	
		9 mo	1.69 _ 0.37	
		12 mo	1.81 _ 0.52	
		18 mo	1.84 _ 0.40	
		24 mo	1.97 _ 0.74	

Table 7. Age-related changes in nerve conduction velocity and in latency time of peripheral nerves. Values are expressed as mean _ SD.

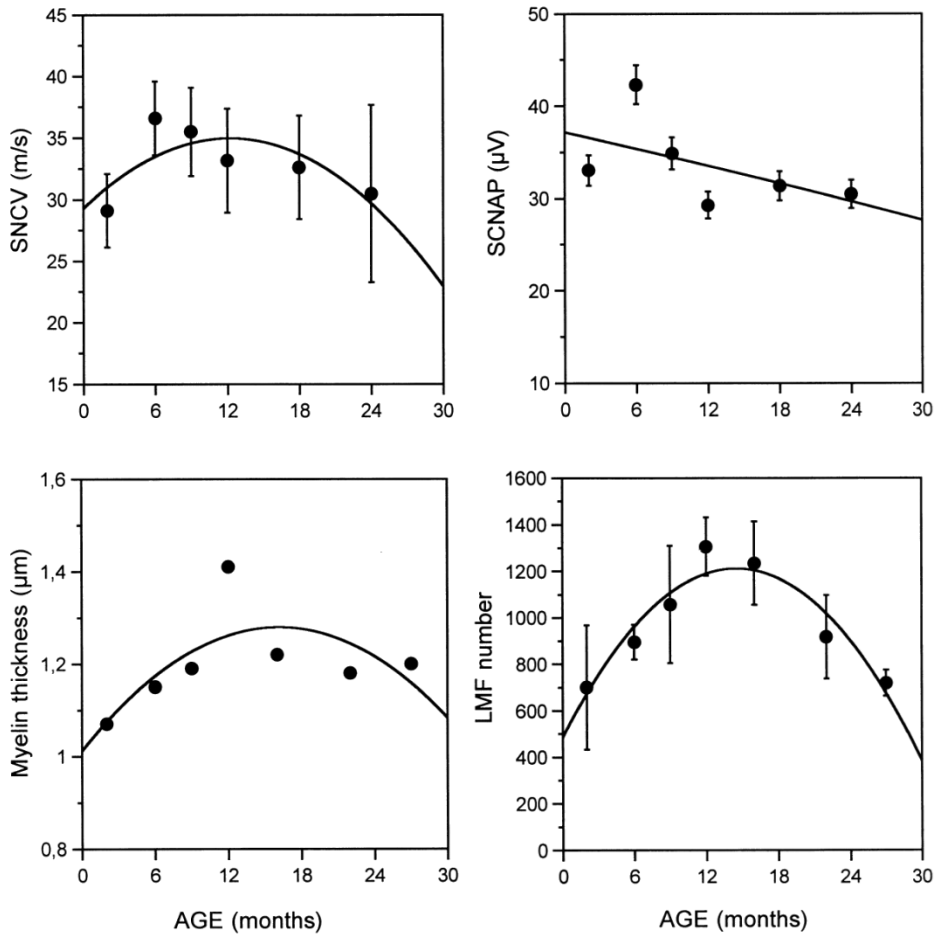


Figure 36. Changes in sensory nerve conduction velocity (SNCV), amplitude of compound nerve action potentials (SCNAP), myelin thickness and number of large myelinated fibers (LMF, .7 mm) with aging in the mouse. (51).

In our cluster we also examined the late responses alterations, mentioned above, about the F wave and the A wave.

The first interpretations on the generation of late responses go back to Roth et al. Some of these, were considered additional discharges resulting from an afferent impulse; others have been attributed to an axonal or myoaxonal ephapsis mechanism (19,24,25).

The physiological F waves with well-coded characteristics of persistence, latency, and morphology have certainly been better known for some time, the pathological ones (ie axonic response and partly A waves) have received less attention in international literature, even with discordant interpretative evaluations on their pathophysiological meaning.

The alterations found in the F waves in our series consisted of a reduction in their conduction speed and an accentuation of the chronodispersion. This phenomenon can be correlated with a progressive decrease in the number of motor units that occurs with aging and it can also be hypothesized that this reduction is progressive and continuous throughout life interesting initially larger and faster motor units and may also explain age-related changes in motor nerve conduction velocity (41,44).

Conversely, we have not found any correlation with persistence. Despite the rather varied and routine use of F waves, their studies can technically challenge even the most experienced electromyographers because they vary in latency, amplitude and configuration in the same train of stimuli, while A waves show no changes in latency or morphology.

Regarding, instead, A waves detection in the study group of elderly patients, these, as explained above, were observed, in our series, with a variable frequency in healthy subjects of advanced age, mostly in the lower limbs and specifically in the tibial nerves (table 4,5). These late responses, as well known in neurophysiology, when present, represent a precious complement to the neurophysiological evaluation. They are detected with higher frequency in subjects with diabetic neuropathy, are evoked by intense stimuli (supramaximal), are associated with proximal nerve lesions and may be the expression of acute lesions (days or even

hours, such as in the aforementioned case of onset of demyelinating G.-B.-S.); the effects of demyelinating neuropathic processes, acute or chronic, rarely observed in axonal lesions (motor neuron diseases, poliomyelitis, etc.) are unanimously observed. only in the limbs, specially in the lower ones, and in particular in the tibial nerves. The cases observed more frequently were localized in the typical site, that is before the F wave, but also in the subsequent site or, very rarely, both before and after.

Obviously the varable position of A wave in relation to F wave is not certainly a neurophysiological artifact nor a random event; we believe that a pathophysiological rationale certainly underlies the reason for such variability, which we try to explain.

It is believed that the pre-F A-wave, with typical intermediate latency between wave M and F, is due to possible reflection of the stimulus prematurely (before reaching the nerve root) in the site of demyelination, sometimes multiple, while part of the stimulus continues normally in the other antidromically stimulated axons, vectors of the normal F wave, which follows it chronologically (see fig. 37) (3).

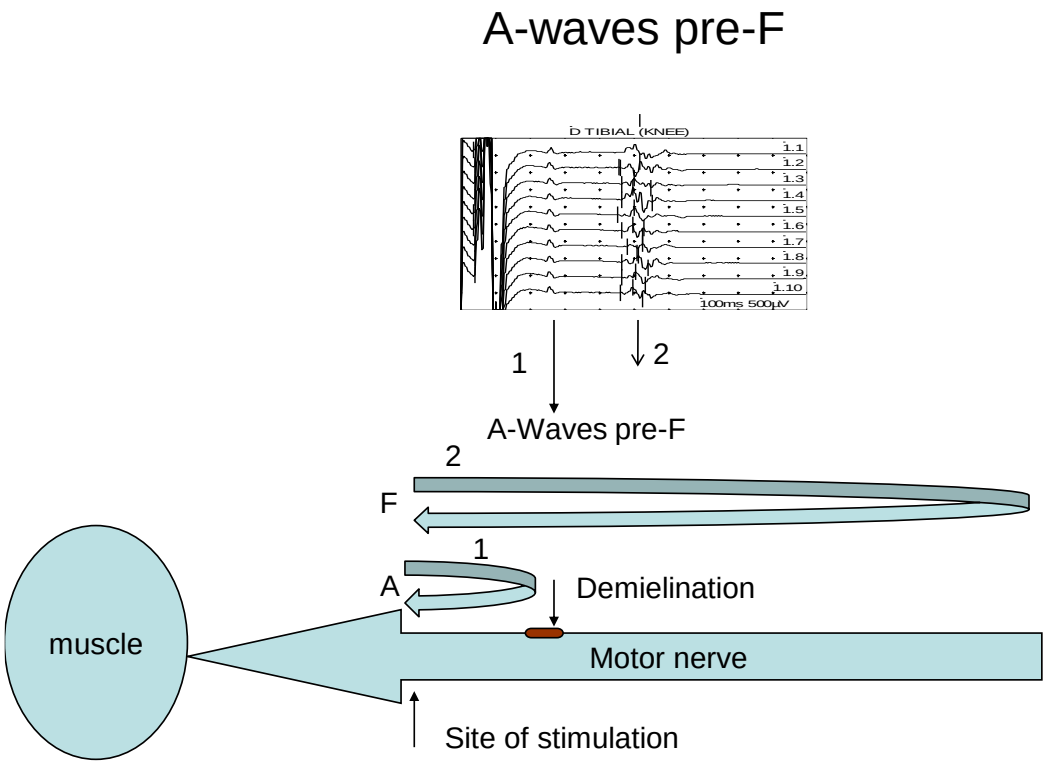


Fig. 37 Pre-F A waves

In the case of the infrequent post-F A-wave, a normal antidromic path is presumed, from the stimulation site to the nerve root, but with a differentiation of the return speed of the stimulus, from the nerve root to the muscle, between free, vector fibers. of the F wave, earlier, and demyelinated fibers, slower, carriers of the A wave, later than the F-wave (see fig. 38) (3).

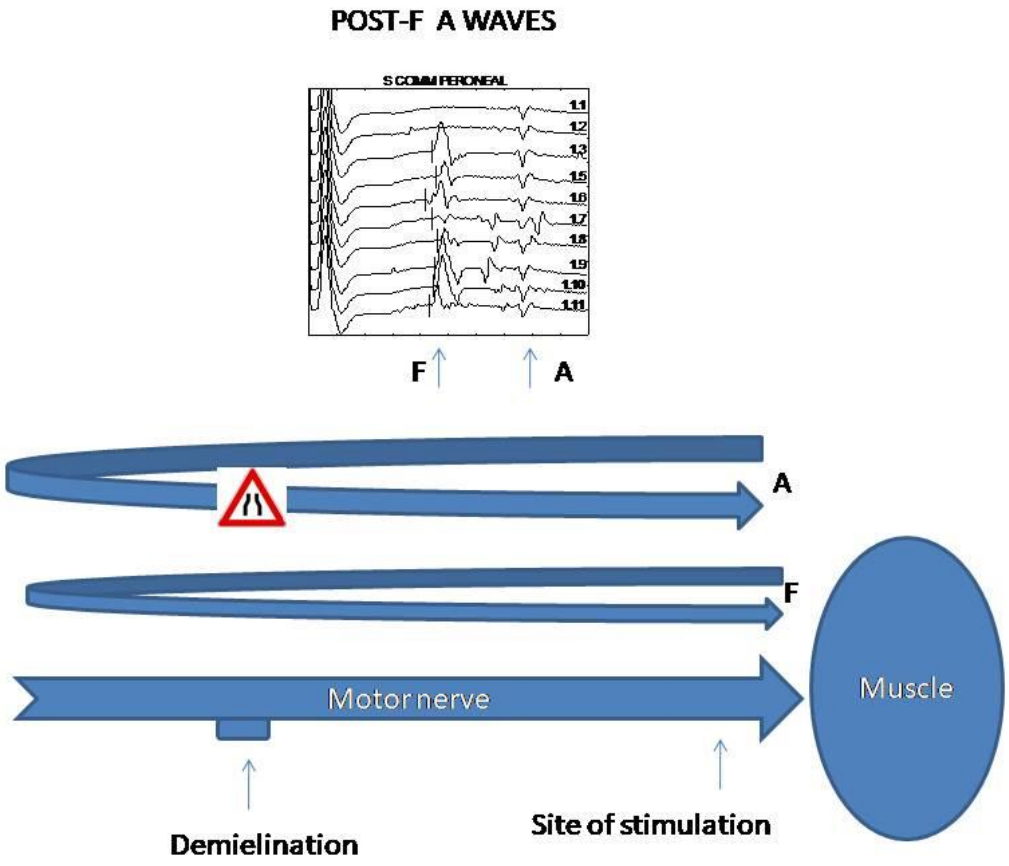


Fig. 38 Post-F A waves

It is possible to hypothesize sporadic lesions, probably demyelinating type, in people over seventy, not necessarily correlated to the presence of polyneuropathy, because the remaining electrophysiological examination showed a prevalent normal phenotype. These lesional sites, of little clinical relevance beacuse they are not correlated to evident sensory-motor deficits, could represent damage to the myelin sheath correlated to the physiological aging process and to the aforementioned reduction in the expression of myelin proteins; proof of this is that the conduction velocity of unmyelinated fibers is relatively unaffected by the aging process (52).

For some years it has been known that fragments of myelin are gradually released from the aged myelin sheaths and subsequently eliminated by the microglia. This myelin fragmentation process increases with age and leads to the formation of insoluble, lipofuscino-like lysosomal inclusions in the microglia; due to this, there is an increased lysosomal load which contributes to the senescence of microglia (53).

A common feature of regenerative processes is that the efficiency of remyelination decreases with aging, which explains why the chronic demyelinating diseases, such as multiple sclerosis (MS), typical CNS demyelinating disease, are characterized by ineffective remyelination. Numerous studies have also confirmed a reduced axonal terminal sprouting capacity with aging, also supported by histological confirmations of aging-related reduction in the number of regenerating axons after crushing injuries in the sciatic nerve of rats, and also of axonal diameter reduction and myelin sheath thickness in regenerated nerves of older mice compared to those of young mice, demonstrating the reduced amount of cytoskeletal elements in elderly axons regeneration and myelin proteins lower levels in normal aged nerves (54- 56). A very important role in this process of repair of axonal lesions is also played by the vascular endothelial growth factor (VEGF), of which three isoforms have recently been identified: they are angiogenic cytokines involved in the angiogenic reparative response after an injury during nerve regeneration, which appear under-expressed in the peripheral nerves of elderly mice (57).

Some metabolic changes, affecting the axonal transport of cytoskeletal components and possibly trophic factors, have been reported to occur in

neurons during aging (43,58). Another involved protein in axonal growth and repair processes, laminin (glycoprotein belonging to the group of adhesion molecules of the extracellular matrix, together with fibronectin, tenascin and perlecan) which promotes axonal growth of sensory neurons in vitro and regeneration of the rat sciatic nerve in vivo, is reduced by up to half in elderly rats peroneal nerves rather than in those of young rats (54,59). The alteration of the amplitude of the evoked responses, observed mainly in the peroneal and median nerves in the group of patients under examination, is due to the axonal loss which in part can also explain the slowing of conduction speeds, beacuse, when axonal degeneration occurs in a nerve, this can cause a slowdown in the conduction of the impulse if axons faster are involved, that are greater caliber, myelinated and high conduction velocity axons, a phenomenon that is it characteristically observed in the elderly, more frequently than in the young (41,44). A variable reduction in the number and density of myelinated and unmyelinated axons has been reported with aging in different types of peripheral nerves of different animal species (41,49,60,61,62).

This process of axonal loss is a phenomenon that can explain, even intuitively, the loss of muscle strength that occurs physiologically with aging, although it is more believed that this is due to muscle fibers lost and muscle mass reduction (63); at the same time it is also known that in aging there is a deterioration of the thermal, tactile, vibratory sensitivity (64) and an autonomic dysfunction (41) as for the taste fibers. Marked axonal atrophy has been demonstrated in elderly animals nerves which has been correlated with a decrease in neurofilament expression in aging (65).

Consequently, the axonal diameter in regenerated nerves is smaller in older mice than in young adult mice, and this may partly explain the regenerated nerves reduced conduction velocity (55).

In addition, numerous studies show that the presence of an unaltered number of myelinated axons in elderly subjects does not necessarily indicate axonal compromise absence because changes in size, alterations in composition, presence of regeneration clusters have been observed in the present axons (61); so much so that an aged nerve has a reduction in ATP-related axonal transport which causes a reduced energy requirement and an energy reserves progressive reduction. Therefore, in aging, we see functional as well as structural nerve modifications (43,58,66,67).

CONCLUSIONS

To conclude we can state that aging represents an important modification factor also for the peripheral nervous system, as well as for all other tissues of the organism and that the data collected in our study, together with the support of the scientific literature acquired to date, are a clear demonstration of this. Much remains to be clarified, so further clinical and experimental efforts will be required to elucidate what today does not allow us an exhaustive explanation of peripheral neurodegeneration and the consequent functional limitation that characterizes advanced age. This appears all the more compelling if we keep in mind that longevity increases more and more as generations follow one another and therefore that we will have more elderly people.

Therefore it is appropriate to recall the invitation, globally shared in various clinical areas (cardiological, neurological, neuropsychological, physiatric, sports, rehabilitative), to try to contrast as much as possible all the risk factors, shared by all international scientific societies, such as smoking, alcohol, obesity, sedentary lifestyle, hypertension, diabetes, substances of abuse, hyperlipidemias, globally recognized as harbingers of neurological, cardiovascular and neoplastic diseases, but which we do not hesitate to borrow here also for the prevention of related performance decay with the process of structural degradation and reduction of the repair capacity of the peripheral nervous system.

Therefore we firmly insert aging among the certain beneficiaries of the constant contrast to aforementioned risk factors because we are convinced that, between the unknowns of genetics and the pitfalls of epigenetics, *health is not a gift but a continuous conquest.*

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