

NEUROPHYSIOLOGIC SIGNS OF LOWER MOTOR
NEURON IMPAIRMENT FOLLOWING LESIONS OF THE
UPPER ONES

Ph.D. Thesis

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LIST OF ABBREVIATIONS

ADM	abductor digiti minimi
ANOVA	analysis of variance
CMAP	compound muscle action potential
CSAP	compound sensory action potential
CT	computer tomography
EMG	electromyography
FD	fiber density
ISI	interstimulus interval
MUNE	motor unit number estimation
MUP	motor unit potential
NCS	nerve conduction study
SD	standard deviation
SE	standard error
SFEMG	single-fiber electromyography

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I. SUMMARY

BACKGROUND: Loss of lower motor neurons caused by upper motor neuron lesion following stroke has been recently suggested by neurophysiological data. Needle electromyographic studies have revealed pathological spontaneous activity in paretic muscles after a cerebrovascular insult and MUNE studies reported loss of functioning motor units on the hemiparetic side of stroke patients (3, 4, 25, 26, 31, 40, 46, 47, 56).

These changes might be due to trans-synaptic degeneration that occurs secondarily to the lesion of the upper motor neurons (25, 26). Alternatively the lower motor neurons could have been inactivated due to the lack of efferent impulses. However, these changes could have been caused by compression neuropathy in or inactivation of the paretic limb. Therefore, the concept about lower motor neuron injury with upper motor neuron lesion remained controversial.

The armamentarium of the clinical neurophysiologist enables him to assess specific aspects of the pathophysiologic changes in the nerves and muscles. Each lower motor neuron innervates many muscle fibers. The lower motor neuron and all the muscle fibers innervated by it constitute the motor unit. The number of muscle fibers innervated by large (high-threshold) motor neurons is higher than the number of muscle fibers innervated by smaller (low-threshold) motor neurons (28, 29). Judicious selection of appropriate neurophysiologic techniques can yield a significant amount of complementary information about the function (or dysfunction) of these structures. Motor nerve conduction studies are informative about the number of functioning lower motor neurons. Concentric needle EMG detects the spontaneous muscle electric activity following denervation, and also the reorganisation of the motor units in the subsequent, chronic stage after denervation (51). Using single-fiber EMG one can determine the FD, a sensitive measure of motor unit reorganisation already in the early, subacute stage (48). Macro EMG records from large territories in the muscle, making it possible to identify the different subpopulations of motor units (17, 49, 52). Recording F waves after conditioning stimuli yields valuable information about the changes in the excitability of the lower motor neurons (19, 20, 39). Thus various neurophysiologic methods are suitable to investigate the possible pathophysiologic changes in the lower motor neurons following the lesions of the upper ones.

OBJECTIVES: The objective of our studies was to assess specific aspects of the lower motor neuron affection following stroke:

- How do the different neurophysiological changes progress in time?
- Is there a selective loss of a fiber-subpopulation?
- Is there any change in the excitability of the remaining lower motor neurons?
- What is the clinical significance of these findings (i.e. correlation of the neurophysiological changes with the clinical severity of the symptoms)?

SUBJECTS AND METHODS:

Changes in the motor unit potentials and compound motor action potentials: The subjects in the study were 48 hospitalized patients with a unilateral ischaemic stroke in the territory of the middle cerebral artery as the first episode. The duration of hemiparesis ranged from one week to one year. Motor nerve conduction studies of the ulnar nerve and concentric needle EMG of the abductor digiti minimi muscle were performed on both sides, and hemiparetic side mean and extreme parameter values were compared with the unaffected side mean and extreme parameter values using non-parametric tests.

Changes in muscle fiber density: Fiber density was determined using single-fiber electromyography, in the abductor digiti minimi muscle. At first, we determined fiber density at low force output on the hemiparetic and the unaffected side of 59 patients with unilateral ischemic stroke in the territory of the middle cerebral artery, and 42 healthy controls. Duration of hemiparesis ranged from 2 weeks to 48 months. In order to survey a larger population of motor units, we measured the fiber density at high force output too. The subjects were 45 consecutively hospitalized patients (31 male, 14 female), aged 37-74 years (mean 59.0 years, median 62 years). To assess if the recruitment of larger motor units at higher force affects the fiber density we compared fiber density values recruited at low and high force output in 48 healthy subjects (32 male, 16 female) aged 29-76 years (mean 60 years).

Comparing the affection of high-threshold and low-threshold motor units: Forty-five stroke patients and 40 healthy controls participated in the study. The duration of the symptoms ranged from 8 months to 4 years. Macro EMG was recorded from the abductor digiti minimi muscle at two levels of force output (low and high). The median macro motor unit potential amplitudes on the paretic side were compared with those on the unaffected side and in the controls.

Changes in the excitability of motor neurons: F waves from 44 hospitalized stroke patients and 35 healthy controls were recorded from abductor pollicis brevis muscles in the course of

two experiments: (1) single stimuli following high-intensity ipsilateral cutaneous conditioning were used to stimulate the median nerve; (2) paired stimuli were given to the median nerve at gradually increasing interstimulus intervals to assess recovery curves. All patients had hemiparesis with the duration of the symptoms ranging from 2 weeks to 2 years. Mean F-wave amplitudes elicited by the conditioning stimuli were compared with mean F-wave amplitudes elicited by the test stimuli on both the hemiparetic and the unaffected side.

RESULTS:

Changes in motor unit potentials and compound motor action potential (M wave): The mean M wave amplitude was significantly lower, while the spontaneous activity and the mean number of motor unit potential phases and turns were significantly higher on the hemiparetic side. The outliers above maximum for motor unit potential duration and amplitude on the hemiparetic side were significantly higher than those on the unaffected side. Correlations were found between the hemiparetic side parameter values and time after stroke onset and hemiparetic severity.

Changes in muscle fiber density: In stroke patients the fiber density measured at low and at high force output was significantly increased on the hemiparetic side compared to the unaffected side and controls. This change was correlated with the severity of the clinical signs. The fiber density gradually increased during the first 10 months following the stroke and thereafter remained constant. The mean fiber density did not depend on the force output levels in healthy subjects either.

Selective injury of high-threshold motor units: In the control group and on the unaffected side, the macro motor unit potentials were significantly larger at the high force output than at the lower one. However, on the paretic side the macro motor unit potentials at the high force output had the same amplitude as those recorded at the low force output. These changes correlated with the severity of the paresis.

Changes in the excitability of motor neurons: The amplitude of the F waves was increased on the hemiparetic side. The recovery curve of mean F-wave amplitude after conditioning stimulation was prolonged on the hemiparetic side. A correlation was found between this delay of recovery and hemiparetic severity. There was no reduction of mean F-wave amplitudes elicited following high-intensity ipsilateral cutaneous stimulation on the hemiparetic side, as opposed to the controls.

CONCLUSIONS:

Shortly after ischaemic stroke trans-synaptic degeneration of the lower motor neurons occurs. The large, high-threshold lower motor neurons are selectively lost. Subsequently collateral reinnervation starts: the remaining lower motor neurons reinnervate the denervated muscle fibers, leading to changes in the structure of the motor units. The remaining lower motor units show pathophysiological changes: hyperexcitability, prolonged depolarisation, decreased inhibition from spinal interneurons. The abnormalities of the lower motor neurons following stroke are correlated with the clinical severity. Fiber density is a robust EMG parameter independent from the force output at which it is measured.

Our findings furnish further insight into a rarely investigated aspect of stroke: the trans-synaptic affection of the lower motor neurons.

I. INTRODUCTION

The circulatory disturbances of the central nervous system often involve motor structures, causing negative signs such as loss of muscle strength, incapacity for selective innervation, and poor contraction modulation.

The cerebrovascular lesion primarily affects the territory of the upper motor neurons, but the clinical signs manifest by means of the striated muscles controlled by the lower motor neurons. There is mounting evidence that the impairment of the lower motor neurons associated with lesions of the upper motor neurons is more than just a signal deprivation/decentralization. The concept of lower motor neuron injury following a stroke has been suggested recently by electrophysiological data.

Needle electromyographic studies have revealed pathological spontaneous activity in paretic muscles after a cerebrovascular insult (3, 4, 30, 46, 47, 56). Hara (25, 26) and McComas (40) reported loss of functioning motor units on the hemiparetic side of stroke patients. Datolla (11) has found that there is morphological muscle rearrangement after a stroke. These findings suggest drop-out of lower motor neurons together with an axonal lesion and may indicate both functional disturbances and changes in the microanatomy of the motor units. These changes could play a role in the pathomechanisms of the motor deficit of stroke patients.

However, few data have been published on changes in motor units in the case of upper motor neuron lesion, as in stroke. Moreover, it is unclear whether these changes would affect the motor units randomly or in some specific pattern.

Different types of motor units can be distinguished on the basis of various interrelated properties: recruitment threshold, size, and histochemical and biomechanical characteristics (44, 51). Clarifying this issue might furnish further insight into the pathophysiology of lower motor neuron injury following a stroke.

Alterations in the muscle fiber topography within the motor unit territory reflect events during degeneration and collateral sprouting. The study of the focal distribution of muscle fibers in the paretic muscles of stroke patients could provide additional information about lower motor neuron involvement in the case of upper motor neuron lesion (8).

Needle electromyography allows assessment of structural alterations of motor units.

Alterations in muscle membrane function, in motor unit organization, or in the number of fibers in a motor unit cause changes in the electrical signals. Several methods using different types of needle electrodes focus on different aspects of the changes in pathology of the motor unit. Concentric needle EMG detects the spontaneous muscle electric activity following

denervation, and also the reorganisation of the motor units in the subsequent, chronic stage after denervation (51). Using single-fiber EMG one can determine the FD, a sensitive measure of motor unit reorganisation already in the early, subacute stage (48). Macro EMG records from large territories in the muscle, making it possible to identify the different subpopulations of motor units (17, 49, 52). Individual electromyographic methods present different sensitivity and specificity for certain specific changes in disease.

Judicious selection of appropriate needle electromyographic techniques can yield a significant amount of complementary information about the state of motor units in the case of upper motor neuron lesion, and about the relationship between lower motor neuron injury and the severity of the hemiparesis.

In addition to muscle weakness, spasticity contributes to the motor disability following ischaemic lesions of the central nervous system. It was showed that spastic muscle tone has a significant neuronal component, resulting mostly from hyperexcitability of lower motor neurons. Electrophysiological studies in patients with spastic hemiparesis have found no specific patterns of abnormalities and no correlations with severity of spasticity. One possible explanation for this lack of correlation is that the studies employed the H reflex which involves an afferent loop of the Ia fibers and therefore may not be ideal for monitoring segmental spinal motor neuron excitability. Changes in F waves have been reported valuable for evaluating motor neuron inhibition (35). F waves are generated by backfiring of motor neurons and therefore could be used to explore altered lower motor neuron excitability in patients with spastic muscle tone.

II. OBJECTIVES

With these premises as starting-point, we investigated the controversial concept of lower motor neuron injury following stroke, using parameters and methods previously not reported. Our objectives were:

1. To assess the progression in time of the possible degeneration of the motor units following stroke, as reflected by the neurophysiologic changes.
2. To explore whether a subpopulation of motor units is selectively affected following stroke.
3. To investigate the pathophysiology of the remaining lower motor neurons.

4. To assess the clinical significance of these neurophysiological changes (i.e. correlation with the clinical severity).

III. SUBJECTS AND METHODS

1. Changes in the motor unit potentials and compound motor action potentials

Subjects in the study were 48 hospitalized patients with a unilateral ischaemic stroke in the territory of the middle cerebral artery as the first clinical episode. The exclusion criteria were age above 65 years, and any sign or data indicating peripheral nerve and muscle disease or predisposing pathological states such as diabetes mellitus, malignant tumors, immunological disease, critical state, or toxic damage.

The age range of the 34 male and 14 female patients was 36 - 64 years (mean 55 years, median 59 years). The ischaemic lesion developed in 29 patients in the right, and in 19 patients in the left hemisphere. The process proved to be atherothrombotic in 31 and thromboembolic in 17 patients. In every subject, the CT examination showed a unilateral hypodensity characteristic of ischaemic transformation in the territory of the middle cerebral artery. The maximal diameter of the lesion was – depending on the patient – between 2 and 6 cm and there were no radiological signs of intracranial hypertension.

The duration of hemiparesis ranged from one week to one year (mean value 5.3 months, median value 4.9 months).

A total of 37 healthy subjects aged 43-67 years (mean 50.5 years, median 54 years) were similarly investigated to establish the reference values of the study.

After a detailed explanation, all selected patients and controls gave their consent. The study was carried out with the approval of the authorized Ethical Committee of the Medical Chamber. The clinical status of the patient was evaluated using the long term items of the Scandinavian Stroke Scale ranging from 0 (no active movements) to 30 (full muscle strength) points (12). Our subjects had scores between 7 and 22 points.

Nerve conduction studies were carried out in our laboratory using Neuropack MEB 2200A equipment (Nihon Kohden Corporation, Tokyo, Japan).

The nerve conduction studies (NCSs) were performed using surface electrodes. In the case of motor NCSs, the frequency band of the filters were 2 Hz-3 kHz, and maximum M-potentials

were evoked with supramaximal stimulation using single square pulses of 0.2 ms duration. Averaged sensory potentials were elicited using sequences of supramaximal stimuli with duration of 0.2 ms and a frequency of 2 Hz at the band pass 20 Hz to 3 kHz.

Distal motor latency to the standardized distance of 80 mm and the amplitude of the motor (CMAP) and sensory (CSAP) potential were determined, and motor and sensory conduction velocity was calculated for the ulnar nerve on both sides. The active electrode was positioned over the belly of the abductor digiti minimi muscle (ADM). A ground reference electrode was positioned between the recording and stimulus sites, and the reference electrode was placed over the nearest metacarpal-phalangeal joint. The antidromic method was used for sensory neurography, the recording interelectrode distance being 23 mm. The values for conduction velocities were corrected to 34⁰C skin temperature.

Concentric needle electromyography Concentric needle (NM-131T/330T, Nihon Kohden Corporation, Tokyo, Japan) EMG was performed on both sides on the ADM muscle during inactivity and during voluntary contraction. Using 20 different needle positions, spontaneous electrical activity was examined. At least 20 MUPs were recorded from each investigated muscle and quantitatively analysed using multiMUP method (51). The signals were recorded at the band pass of 2 Hz to 5 kHz, using a 50 mikroV/cm gain for the spontaneous activity and 200 mikroV/cm gain for the multiMUP analysis. The sweep speed in the edit mode was 3 ms/div.

Different depths were studied at 3-4 skin insertions in the middle part of the muscle. The recorded MUAPs were checked visually. Those with a noisy baseline were rejected, and the duration markers were corrected. The following MUP parameters were measured: amplitude, duration, number of phases and turns.

For each subject the mean values and standard deviation of each MUP parameter of the first 20 MUPs were calculated.

In addition, for each subject of the control group, the third largest and third smallest value of the 20 MUPs for each parameter were selected. The highest and lowest of these values were chosen as the extreme outlier limits for the studied sample (50).

Statistical analysis The hemiparetic side mean parameter values in the stroke patients were compared with the unaffected side mean parameter values by the Wilcoxon signed rank test combined with the Bonferroni correction for multiple measurements. Differences were considered significant at $p < 0.01$. The hemiparetic side number of MUPs with parameter values

above maximum outlier limit was compared with the unaffected side number. Correlations were studied by Spearman rank correlation.

2. Changes in fiber density at low force output

Subjects enrolled in the study were 59 consecutively admitted patients (41 male, 18 female), aged 37-78 years (mean 61.0 years, median 55 years), with a unilateral ischemic stroke in the territory of the middle cerebral artery, and 42 healthy subjects (30 male, 12 female) aged 33-70 years (mean 55.4 years, median 50 years).

The exclusion criteria comprised any sign or data suggestive of peripheral neuropathy, muscle disease or predisposing pathological states such as diabetes mellitus, malignant tumors, immunological disease, a critical state or toxic damage. Ulnar nerve conduction studies performed in each subject excluded compression neuropathy.

The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki. All participants gave their informed consent.

The duration of the symptoms ranged from 2 weeks to 4 years (mean: 20.1 months, median: 14 months).

In all 59 patients, the CT examination revealed a unilateral hypodensity characteristic of an ischemic transformation in the territory of the middle cerebral artery. The maximum diameter of the lesion ranged from 1 to 8 cm. There were no radiological signs of intracranial hypertension. Twenty patients had subcortical lesions (confined to the white matter in 15 patients and involving the basal ganglia in 5 patients). Thirty-nine patients had cortical lesions. The stroke affected both the primary and secondary motor cortices in 30 patients, the premotor cortex in 3 patients, and the postcentral area in 6 patients. The ischemic lesion was situated in the right hemisphere in 37, and in the left hemisphere in 22 patients. All 59 patients were right-handed. The pathology was atherothrombotic in 44, and thromboembolic in 15 patients. None of the patients underwent thrombolysis.

The clinical status of the patients was evaluated by using the long-term items of the Scandinavian Stroke Scale, ranging from 0 (no active movements) to 30 (full muscle strength) points (12).

Single-fiber electromyography was carried out with Neuropack MEB 2200A equipment (Nihon Kohden Corporation, Tokyo, Japan).

The patient or the control subject was comfortably seated with their investigated arm pronated and placed on an elbow-rest. In order to activate the abductor digiti minimi muscle efficiently, the thumb, the second-fourth fingers, the wrist and the forearm were immobilized with straps. A single-fiber EMG needle electrode (NM-64OS, Nihon Kohden Corporation, Tokyo, Japan) was inserted into the abductor digiti minimi muscle and the subjects were asked to abduct the fifth finger. The single-fiber needle was slowly advanced into the contracting muscle with the recording surface of the electrode perpendicular to the longitudinal direction of the muscle fibers. After a single-fiber potential had been visually detected, the electrode was optimally positioned to maximize the amplitude of single-fiber waveform. Once the amplitude had been optimized, the number of associated single muscle fiber action potentials time locked to the triggering potential was determined (45). The signals were recorded at the bandpass of 0.5 kHz to 10 kHz, using a 50 μ V/cm gain. The sweep speed in the edit mode was 5 ms/div. The needle electrode was then advanced to a new position in the muscle and the process of counting potentials was repeated. Different depths were studied at 3-5 skin insertions in the middle part of the muscle until a minimum of 20 different recording sites had been examined. The summated value of potentials was divided by the number of recording sites to yield the fiber density (52).

Statistical analysis Differences among the fiber density values on the affected side, on the unaffected side and in the control group were assessed by using ANOVA. To compensate for inter-individual variability, we determined the ratio of the fiber density values on the hemiparetic and unaffected sides. This ratio was entered into the correlation analyses, by means of the Pearson correlation test.

3. Changes in fiber density at high force output

Subjects The previous fiber density study was performed at low force output. In order to survey a larger population of motor units, we measured the fiber density at high force output too. Forty healthy subjects (29 male, 11 female) aged 33-70 years (mean 55.2 years, median 58 years) and 45 consecutively hospitalized patients (31 male, 14 female) with a unilateral ischemic stroke in the territory of the middle cerebral artery, aged 37-74 years (mean 59.0 years, median 62 years), participated in the study.

The exclusion criteria were the same as in the concentric needle study. Ulnar nerve conduction examinations performed for each subject excluded compression neuropathy.

All participants gave their informed consent, and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

The duration of the symptoms ranged from 8 months to 4 years (mean: 26.2 months, median: 24 months).

In all 45 patients, the CT examination revealed a unilateral hypodensity characteristic of an ischemic transformation in the territory of the middle cerebral artery. The maximum diameter of the lesion ranged from 2 to 7 cm. None of the patients had lacunar stroke or watershed infarction. There were no radiological signs of intracranial hyper-tension. Fourteen patients had subcortical lesions (confined to the white matter in 10 patients and involving the basal ganglia in 4 patients). Thirty-one patients had cortical lesions. The stroke affected both the primary and secondary motor cortex in 27 patients, the premotor cortex in one patient, and the postcentral area in three patients. The ischemic lesion was situated in the right hemisphere in 27, and in the left hemisphere in 18 patients. All the patients were right-handed. The pathology was atherothrombotic in 34, and thromboembolic in 11 patients. None of the patients underwent thrombolysis.

Single-fiber electromyography (SFEMG) was recorded from the abductor digiti minimi muscle on both sides, at the level of 50% of the maximal force output. This was determined with a pulley set-up specifically designed for this purpose (Figure 1).

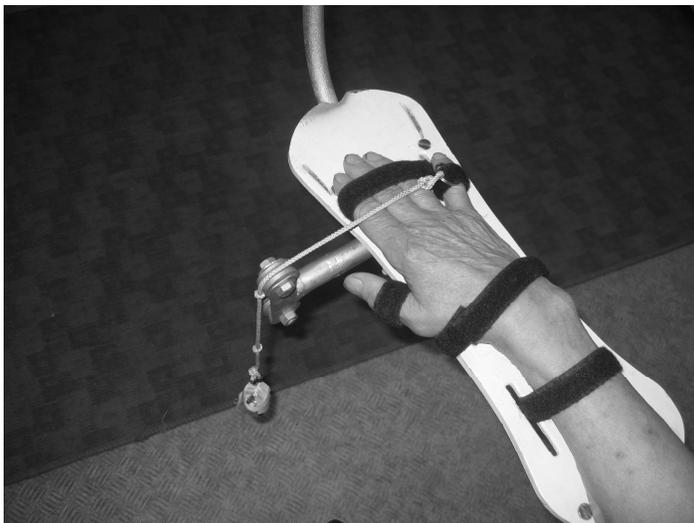


Figure 1.

A pulley set-up specifically designed was used for selective activation of the low and the high recruitment threshold motor units.

The subjects were seated comfortably with their investigated arm pronated and placed on an elbow-rest. In order to activate only the abductor digiti minimi muscle, we immobilized the thumb, the second-fourth fingers, the wrist and the forearm with straps. The end of a 30-cm-long cord was attached to a strap wrapped around the middle phalanx of the fifth digit. A metal hook was attached to the other end of the cord. In this set-up, the downward pulling of the hook caused adduction of the fifth finger. The subjects were asked to abduct the fifth finger as far as possible without any strain, and this position of the fingertip was marked with a visible line on the elbow-rest. Shots weighing 100 g were anchored successively on the hook, and the subjects were instructed to maintain the tip of the fifth finger in line with the mark for at least 5 s. They were aided by visual feed-back and verbal encouragement from the examiner. The placement of two successive shots was separated by a 1-min rest period. The load corresponding to the maximal force was taken to be the total weight of the shots anchored to the hook prior to the episode when the subject was unable for the first time to maintain the indicated position of the fifth finger for 5 s.

Shots with a total weight corresponding to 50% (high force output) of the maximal load were anchored to the hook and the subjects were asked to maintain the tip of the fifth finger in line with the mark, by adjusting their isometric contraction force.

A single-fiber needle electrode (NM-64OS, Nihon Kohden Corporation, Tokyo, Japan) was inserted into the abductor digiti minimi muscle and the fiber density was determined following the procedure used in the single-fiber EMG study at low force output.

Statistical analysis Differences among the fiber density values on the affected side, on the unaffected side and in the control group were assessed by using ANOVA. To compensate for inter-individual variability, we determined the ratio of the fiber density values on the hemiparetic and unaffected sides. This ratio was entered into the correlation analyses, by means of the Pearson correlation test.

4. Fiber density at low and at high force output in healthy subjects

As we described above, we measured the fiber density at low and at high force output in stroke patients. However, to assess if the recruitment of larger motor units at higher force affects the fiber density we compared fiber density values recruited at low and high force output in normal controls.

During a gradually increasing force output, the motor units are recruited according to the size principle of Henneman. The smallest motor units (with the lowest number of muscle fibers) are the first to be recruited, and larger motor units are progressively recruited in sequence of increasing size. Macro EMG studies in humans have confirmed that motor units with larger electrical size (amplitude and area) are recruited at higher force output. The amplitude of the macro motor unit potential is directly related to the number of muscle fibers within the motor unit. Thus, the large, high recruitment threshold macro motor unit potentials could be generated by motor units with either higher muscle fiber density or larger territory. In order to elucidate this, we determined fiber density by single-fiber EMG at low and at high force output.

Subjects Forty-eight healthy subjects (32 male, 16 female) aged 29-76 years (mean 60 years) participated in the study. All participants gave their informed consent, and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

Single-fiber electromyography Single-fiber and macro EMG was recorded from the abductor digiti minimi muscle, at two levels of force output: 10% and 50% of the maximal force. This was determined with the previously described pulley set-up designed for this purpose (Figure 1). The load corresponding to the maximal force was determined in the same way as in the previous single-fiber EMG study.

Shots with a total weight corresponding to 10% (low force output) or 50% (high force output) of the maximal load were anchored to the hook and the subjects were asked to maintain the tip of the fifth finger in line with the mark, by adjusting their isometric contraction force. Force levels were examined in a fixed manner: first lower force was applied until a minimum of 20 macro motor unit potentials and corresponding single fiber potentials were obtained, and then the procedure was repeated for the higher force level.

A single-fiber macro EMG needle electrode (NM-64OS, Nihon Kohden Corporation, Tokyo, Japan) was inserted into the abductor digiti minimi muscle. A conventional concentric needle electrode (NM-131T/330T, Nihon Kohden Corporation, Tokyo, Japan) was placed into the extensor digitorum communis muscle on the same side and secured with adhesive tape.

The conventional concentric needle electrode served as a reference electrode to the macro EMG cannula. Signals were fed into a two-channel electromyograph (Nihon Kohden Neuropack MEB-2200A). Single-fiber EMG signals were filtered in the range 500 Hz - 10

kHz, and were displayed at 200 μ V/cm gain in channel 1. Macro EMG signals were filtered in the range: 20 Hz - 3 kHz and were displayed at 50 μ V/cm gain in channel 2. The sweep speed in the edit mode was 5 ms/div.

At the positions where adequate single-fiber potentials were recorded, the macro EMG signal was averaged until a smooth baseline and constant macro motor unit potentials were obtained. Different depths were studied with 3-5 skin insertions. Successive contractions were separated by rest periods and were performed until a minimum of 20 macro motor unit potentials recorded at different needle positions were obtained at each force output level. During the isometric contractions, distinct single-fiber potentials were sought by slightly rotating and moving the needle backwards and forwards. The morphology of the macro motor potential remains relatively constant over a large portion of the motor unit's territory, therefore in the case of two macro motor unit potentials with the same shape, one of them was discarded. The peak-to-peak amplitudes, the area of the macro motor unit potentials, and the fiber density were measured. The mean value of the fiber density and the median value of the macro motor unit potential amplitude and area were determined for each force level. Because of the generally skewed distribution of the individual macro motor unit potential amplitude, the median value was preferred to the mean.

Statistical analysis The normality of the sample distribution was determined by means of the Shapiro-Wilk test. The amplitude and area of the macro motor unit potentials and the fiber density values recorded at low and at high force output were compared by using 2-tailed paired t-test. Correlation analysis was performed using Pearson test.

5. Macro EMG study at high and low force level

Subjects

The subjects were those 45 consecutively hospitalized patients (31 male, 14 female), aged 37-74 years (mean 59.0 years, median 62 years), who participated in the previously described single-fiber EMG study at high force output.

The clinical status of the patients was evaluated by using the long-term items of the Scandinavian Stroke Scale, ranging from 0 (no active movements) to 30 (full muscle strength) points (12).

Macro electromyography

The neurophysiologic investigations were carried out with Neuropack MEB 2200A equipment with the facility of dual-channel recording (Nihon Kohden Corporation, Tokyo, Japan).

Macro electromyography (macro EMG) was recorded from the abductor digiti minimi muscle on both sides, at two levels of force output: 10% and 50% of the maximal force. This was determined with a pulley set-up specifically designed for this purpose, as described before (Figure 1).

Shots with a total weight corresponding to 10% (low force output) or 50% (high force output) of the maximal load were anchored to the hook and the subjects were asked to maintain the tip of the fifth finger in line with the mark, by adjusting their isometric contraction force.

A single-fiber macro EMG needle electrode (NM-64OS, Nihon Kohden Corporation, Tokyo, Japan) was inserted into the abductor digiti minimi muscle. A conventional concentric needle electrode (NM-131T/330T, Nihon Kohden Corporation, Tokyo, Japan) was placed into the extensor digitorum communis muscle on the same side and secured with adhesive tape.

The conventional concentric needle electrode served as a reference electrode to the macro EMG cannula (filter settings: 20 Hz - 3 kHz; display: 50 μ V/cm gain). The sweep speed in the edit mode was 5 ms/div.

At the positions where adequate single-fiber potentials were seen, the macro EMG signal was averaged until a smooth baseline and a constant macro motor unit potential were obtained.

The averaging was preset to 128 discharges. Different depths were studied with 3-5 skin insertions. Successive contractions were separated by rest periods and were performed until a minimum of 20 macro motor unit potentials were obtained at each force output level. The peak-to-peak amplitudes of the macro motor unit potentials were measured, and the median value was determined. Because of the generally skewed distribution of the individual macro motor unit potential amplitude, the median value was preferred to the mean.

Statistical analysis Differences between the median macro motor unit potential amplitudes on the affected side, on the unaffected side and in the control group were assessed by using between- group ANOVA, with force output level and group (or side) as independent factors. When differences were present, we carried out a post-hoc analysis to pinpoint where the differences were, using the Dunnett T3 tests. Correlations were studied by means of the Pearson correlation test.

6. Changes in the excitability of motor neurons

Subjects in the study were 44 hospitalized patients with an initial unilateral ischaemic stroke in the territory of the middle cerebral artery. The age range of the 31 male and 13 female patients was 42 - 64 years (mean 57 years, median 55 years). The ischaemic lesion was on the right in 19 patients and on the left in 25. All patients had a hemiparesis with the duration of the symptoms ranging from 2 weeks to 2 years (mean 15 months, median 13 months). In every patient, the CT examination showed a unilateral hypodensity consistent with an ischaemic lesion in the territory of the middle cerebral artery. There were no radiological signs of intracranial hypertension.

The Scandinavian Stroke Scale was used to assess the clinical status of the patient. The muscle tone of the hemiparetic side forearm flexors was assessed by means of the modified Ashworth scale ranging from 1 (no increase in tone) to 5 (affected part rigid).

Normal control values were established by studying 35 healthy subjects aged 39-68 years (mean 58.1 years, median 59 years).

After a detailed explanation, all patients and controls gave their informed consent. The study was carried out with the approval of the hospital Ethics Committee.

F-wave measurements and statistical analysis The neurophysiological investigations were carried out in our laboratory using Neuropack MEB 2200A equipment (Nihon Kohden Corporation, Tokyo, Japan).

F waves were recorded from the abductor pollicis brevis muscles bilaterally, using stainless steel surface electrodes placed over the motor point of the muscle (active electrode) and the metacarpo-phalangeal joint of the thumb (reference electrode). A ground reference electrode was positioned between the recording and stimulus sites. The frequency bandwidth was 2 Hz to 3 kHz. The median nerve was stimulated with a bar electrode at the wrist, with the cathode positioned proximally. Maximum M potentials were evoked with supramaximal stimulation using single square constant current stimulus impulses of 0.1 ms duration. The F waves were elicited using stimulus intensity 25% higher than that required to elicit the maximal M potential.

The subsequent procedures were followed:

At first, sixty-four single stimuli were delivered to the median nerve at a rate of 0.2 Hz and mean peak-to-peak F-wave amplitude, mean F-wave latency and difference between maximal

and minimal F-latency values were determined. Differences between the affected side, unaffected side and controls were assessed using one-way ANOVA.

Next, two experiments using conditioning stimuli were performed:

- a. Paired ring electrodes were then used to stimulate the index finger with a 0.2 ms duration stimulus at intensity 15 times the perceptual threshold. The stimulus to the finger was delivered 40, 60 and 80 ms prior to an associated median nerve stimulation at the wrist. For each interstimulus interval a sequence of 64 consecutive associated stimuli was given at a rate of 0.2 Hz. To determine the effect of the ipsilateral sensory stimulation, an analysis of variance was performed, using a criterion of $p < 0.05$ for significance. Mean amplitude ratios of the sensory conditioned to single stimulated F waves were compared between the hemiparetic and unaffected sides as well as the controls. F-wave amplitudes were individually measured and the mean values were used for data analysis.
- b. Supramaximal paired stimuli at interstimulus interval ranging from 5 ms to 300 ms were delivered to the median nerve at a rate of 0.2 Hz. Sixty-four consecutive pairs of stimuli were given at each interstimulus interval with an interval of 1 min between each stimulus sequence. After the first step of a 15 ms interstimulus interval, steps of 20 ms were used between consecutive sequences. For each patient and each interstimulus interval, conditioning F-wave amplitudes were compared with test F-wave amplitudes using paired t test with Bonferroni correction for multiple measurements. We defined time of recovery of a patient as the shortest interstimulus interval at which there was no significant difference between the conditioning and test F-wave amplitudes. Recovery curves were constructed using mean peak-to-peak amplitude ratios of the test to conditioning F waves recorded at each interstimulus interval. The amplitude ratio was calculated using the F waves in each pair of stimuli. All 64 traces for each interstimulus interval were recorded. F-wave amplitudes were measured individually with the mean values used for graphing. Differences among the data from the recovery curves from the affected side, unaffected side and control group were assessed using between group-ANOVA with interstimulus intervals and group (or side) as independent factors. When differences were present, we carried out a post-hoc analysis to find out where the differences were, using the Dunnett T3 tests. Significance was defined as $p < 0.05$.

Correlations of clinical data with the results of the paired stimulation and sensory conditioning were studied.

V. RESULTS

1. Changes in the motor unit potentials and the compound motor action potentials

Nerve conduction studies

No statistically significant difference was found between the hemiparetic and unaffected sides for distal motor latency, motor and sensory conduction velocity, or CSAP.

The M wave amplitude was significantly smaller on the hemiparetic side than that on the unaffected side (Table 1), and showed good correlation with the time after stroke onset and severity of hemiparesis (Figure 2).

Concentric needle EMG

There was no significant difference between the mean values of the control subjects and the unaffected side of the stroke patients.

Pathological spontaneous activity (fibrillation potentials and positive sharp waves) was found on the hemiparetic side in 24 of 48 stroke patients, and it showed significant correlation with the time after stroke onset and severity of clinical signs (Figure 2). No pathological spontaneous activity was found beyond 6 months after stroke onset.

The mean number of MUP turns and phases on the hemiparetic side was significantly higher than that on the unaffected side, and significant correlation with time after stroke onset and severity of hemiparesis was found (Figure 2).

The mean MUP duration was longer, and the mean MUP amplitude was larger on the hemiparetic side, but this failed to reach the level of significance (Table 1).

Parameter	Number of cases	Hemiparetic side		Unaffected side		Significance of the difference
		mean	SD	mean	SD	
M wave amplitude (mV)	48	9,72	4,17	12,2	3,53	p<0,001
Number of MUP turns	46	4,9	1,05	3,2	0,48	p<0,001
Number of MUP phases	46	3,7	0,82	3,37	0,46	p=0,001
MUP amplitude (mikroV)	46	857,9	165,4	790,4	127,2	p=0,025
MUP duration (ms)	46	10,37	1,03	9,9	0,81	p=0,05

Table 1.

Summary of results

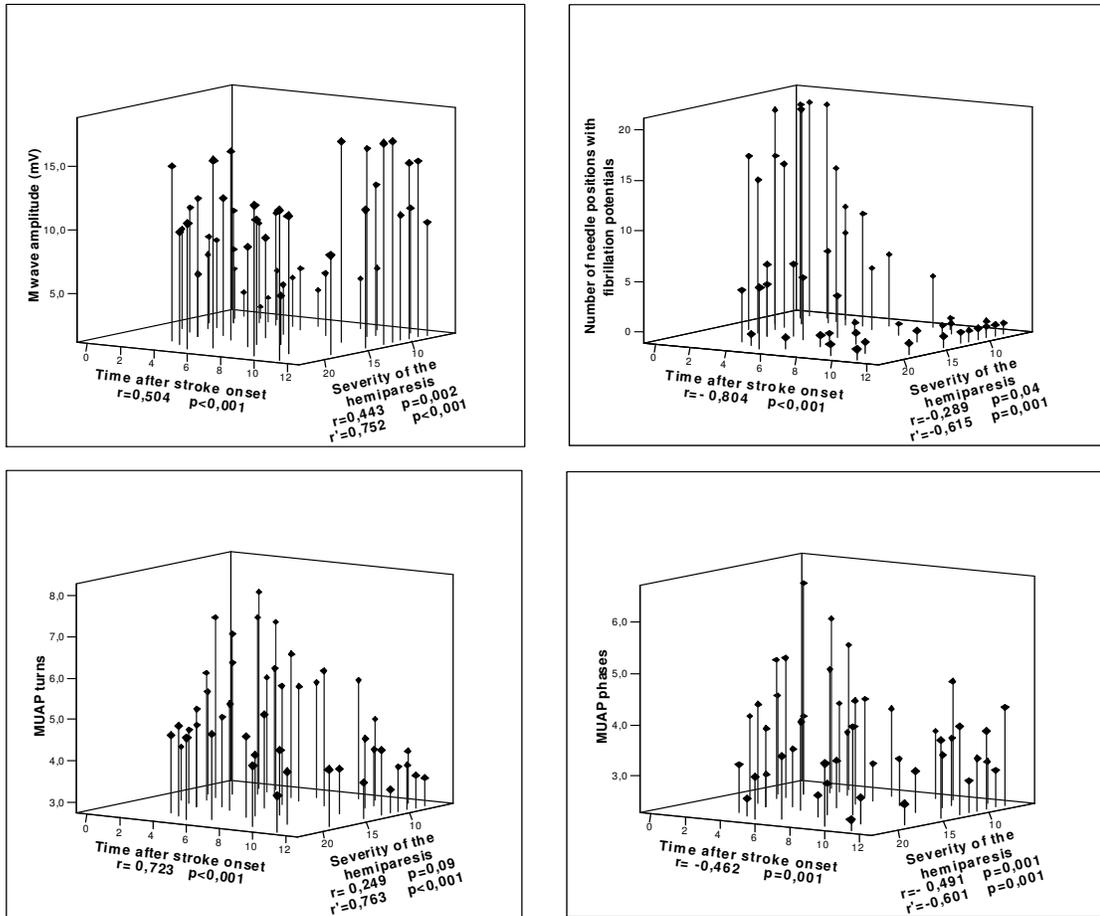


Figure 2.

Relationship between the significant EMG, nerve conduction findings and time after stroke onset (months) as well as severity of the hemiparesis (Scandinavian Stroke Scale) on hemiparetic side.

r indicates the correlation coefficient for all patients, r' for those less than 6 months after onset

More than two MUPs with duration above the maximum outlier limit were found in 22 patients on the hemiparetic side. The number of outliers above maximum for MUP amplitude was more than two in 14 patients.

The number of outliers above maximum for MUP duration and amplitude showed good correlation with the time after stroke onset and severity of hemiparesis (Figure3).

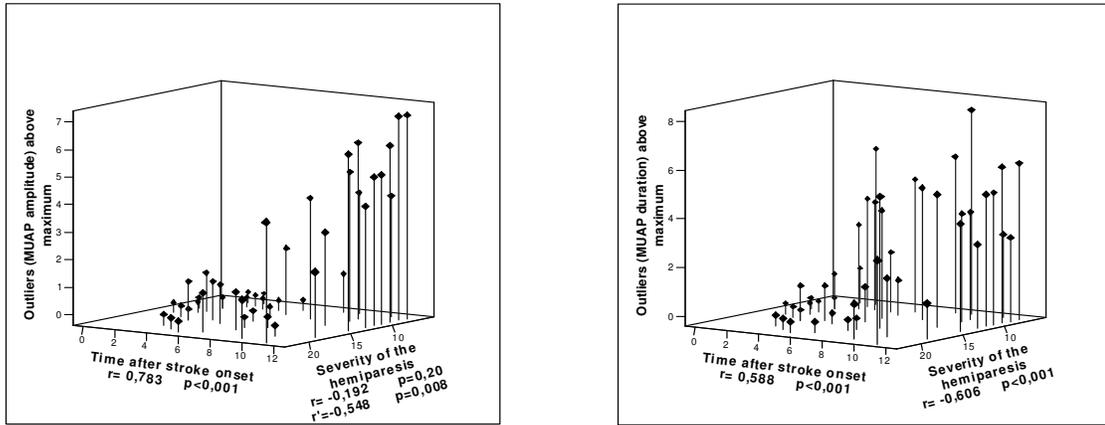


Figure 3.

Relationship between number of outliers above maximum and time after stroke onset (months) and severity of hemiparesis (Scandinavian Stroke Scale) on hemiparetic side. R indicates the correlation coefficient for all patients, r' for those with more than 6 months since onset

2. Changes in fiber density at low force output

Fiber density was significantly higher on the hemiparetic side as compared with the unaffected side ($p=0.016$) and the control group ($p=0.01$). The difference between the fiber density values determined on the unaffected side and in the control group was not significant ($p=0.956$) (Figure 4).

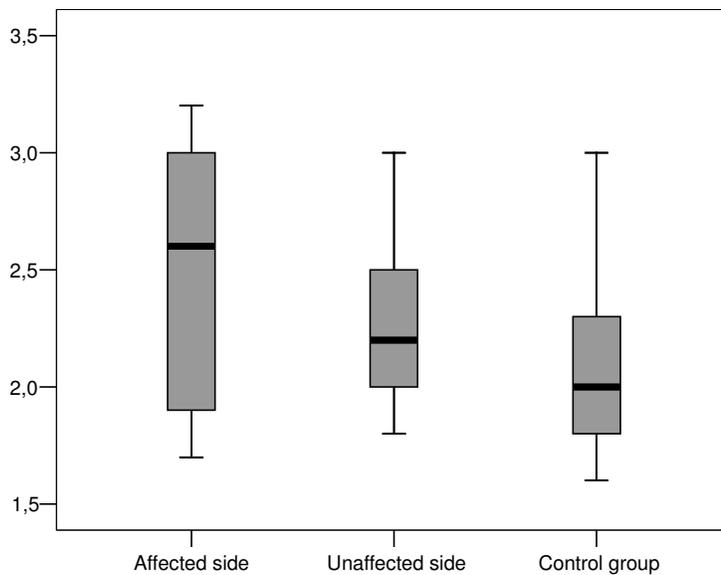


Figure 4.

Box and whisker plots of fiber density values at low force output

The ratio of the FD values on the hemiparetic and unaffected sides correlated significantly and inversely with the Scandinavian Stroke Scale (Pearson correlation coefficient = -0.67; $p < 0.001$), i.e. a patient with a higher FD had a more severe clinical score (Figure 5).

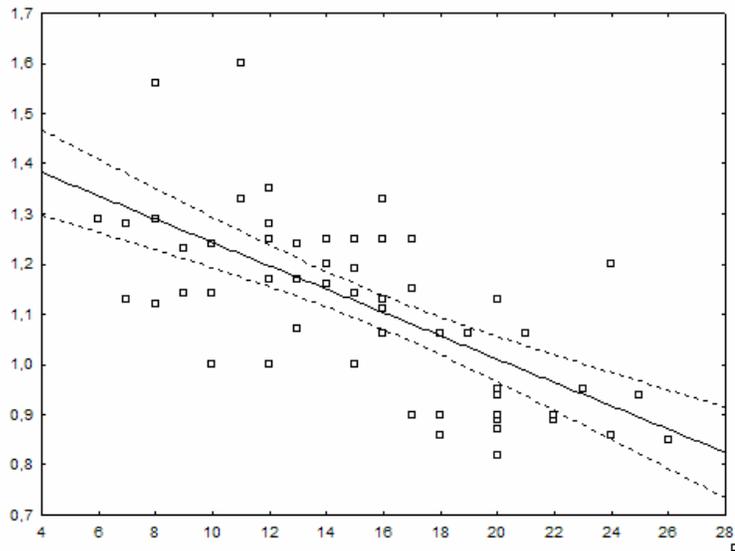


Figure 5.

Correlation with severity of hemiparesis

y axis: ratio between fiber density on the affected side and the unaffected side at low force output

x axis: clinical score (Scandinavian Stroke Scale: 0 = no active movement, 30 = full muscle strength).

Dotted line: 95% confidence

For the patients who had suffered the stroke within 10 months before the EMG, a significant correlation was found between the time elapsed since the onset of the stroke and the ratio of the FD values on the hemiparetic and unaffected sides (Pearson correlation coefficient = 0.59; $p = 0.02$) (Figure 6). The FD ratio did not increase further after the 10-month post-stroke period.

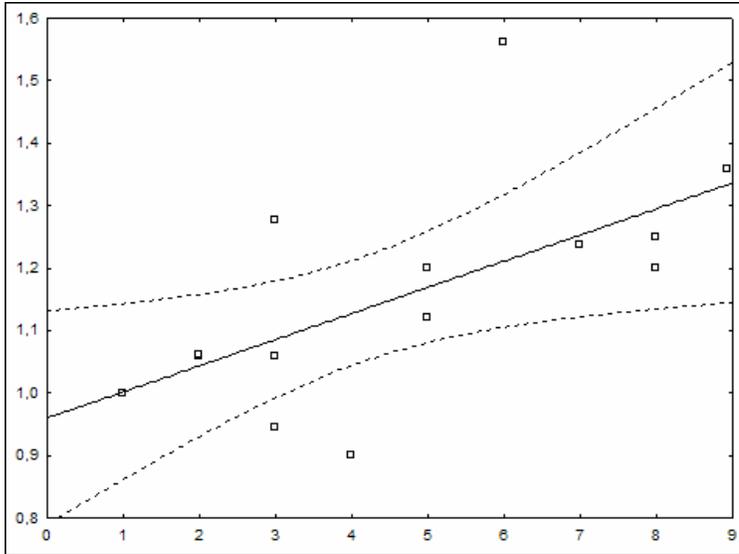


Figure 6.

Correlation with the time elapsed since the onset of the stroke (< 10 months)

y axis: ratio between fiber density on the affected side and the unaffected side at low force output

x axis: number of months elapsed since the onset of the stroke. Dotted line: 95% confidence

3. Changes in fiber density at high force output

Fiber density was higher on the hemiparetic side as compared with the unaffected side ($p=0.01$) and the control group ($p=0.001$). The difference between the FD values determined on the unaffected side and in the control group was not significant ($p=0.6$) (Figure 7).

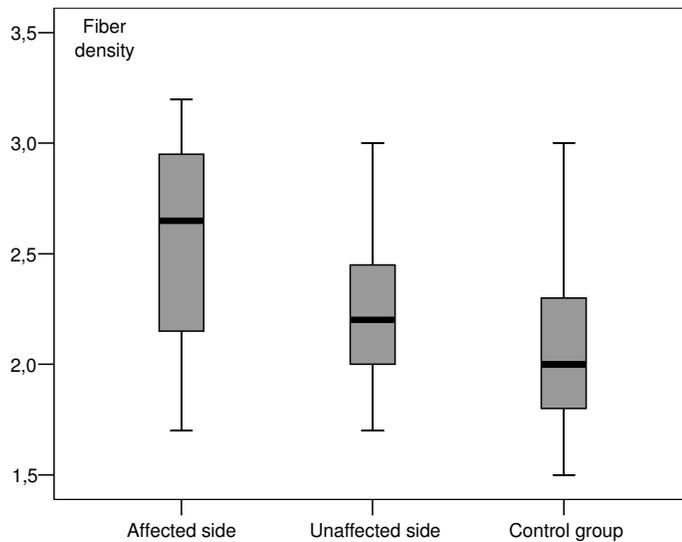


Figure 7.

Box and whisker plots of fiber density values at high force output

The ratio of the fiber density values on the hemiparetic and unaffected sides correlated significantly and inversely with the Scandinavian Stroke Scale (Pearson correlation coefficient = -0.724; $p < 0.001$), i.e. a patient with a higher fiber density had a more severe clinical score (Figure 8).

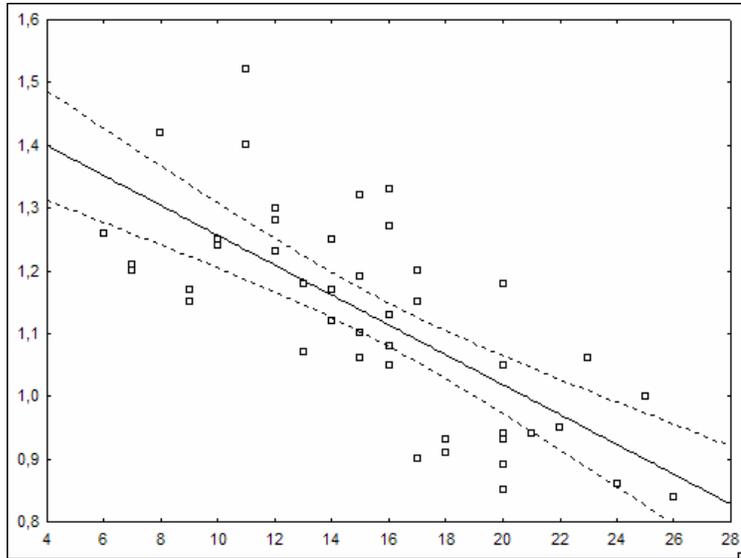


Figure 8.

Correlation with severity of hemiparesis

y axis: ratio between fiber density on the affected side and the unaffected side at high force output

x axis: clinical score (Scandinavian Stroke Scale: 0 = no active movement, 30 = full muscle strength).

Dotted line: 95% confidence

4. Fiber density at low and at high force output in healthy subjects

The fiber density and macro EMG data at low and at high force output are presented in Table 2 and Figure 9.

Force	FD: mean (SE)	MUP amplitude: mean (SE)	MUP area: mean (SE)
Low	2.22 (0.06)	286 (15)	0.49 (0.03)
High	2.23 (0.07)	591 (24)	0.9 (0.04)

Table 2.

Mean FD, macro MUP amplitude (μV) and area (mVms) values for the two force output levels (low=10% of maximum, high=50% of maximum).

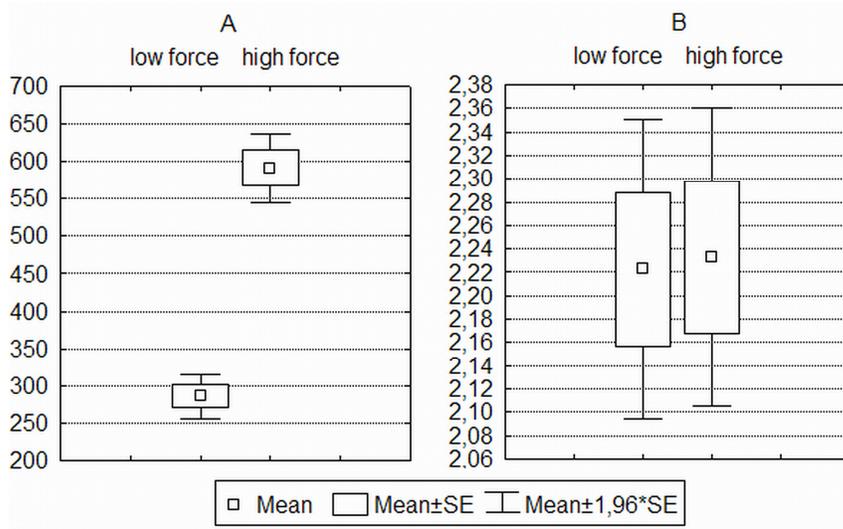


Figure 9.
Box-plots of macro-MUP amplitudes (A) in μV and of FD (B)

Macro MUP amplitude and area were significantly larger at the higher force output than at the lower one ($p < 0.001$). The mean fiber density did not differ at the two force output levels ($p = 0.72$). The subgroup analysis of the patients younger than 50 years ($n = 12$) yielded similar results: macro-MUP amplitude and area were larger at the high force output than at the lower one ($p < 0.001$), and fiber density did not differ at the two force output levels ($p = 0.52$). The ratio between fiber density at high and low force did not show correlation with the age of the subjects.

5. Macro electromyography at high and low force-output

The maximal loads differed on the affected side (mean 562 g, SD 290 g), on the unaffected side (mean 1277 g, SD 686 g) and in the control group (mean 1401 g, SD 625 g).

Macro motor unit potential amplitudes. The analysis of variance revealed that the macro motor unit potential amplitude was significantly influenced by the force output level and the side (group) on which the potentials were recorded. Multiple comparison tests provided details about this relationship (Table 3).

	F	Significance
Force level	215.33	p<0.001
Side/group	34.41	p<0.001

	p
<i>Multiple comparisons – Dunnett T3</i>	
Low force output on affected side / low force output on unaffected side	0.344
Low force output on affected side / low force output in control group	0.103
Low force output on affected side / high force output on affected side	0.912
Low force output on unaffected side / high force output on unaffected side	<0.001
Low force output in control group / high force output in control group	<0.001
High force output on affected side / high force output on unaffected side	<0.001
High force output on affected side / high force output in control group	0.002

Table 3.

Between-group ANOVA for macro motor unit potential median amplitude

The macro motor unit potentials recorded on the unaffected side and in the control group were significantly larger at the higher force output than at the lower one. On the affected side, the macro motor unit potential amplitudes recorded at the higher force output had the same amplitude as those recorded at the lower force output. Accordingly, at the higher force output the macro motor unit potentials on the affected side were significantly smaller than those on the unaffected side and in the controls. No significant differences were found between the macro motor unit potential amplitudes recorded at the lower force output on the unaffected side, on the affected side and in the control group (Figure 10).

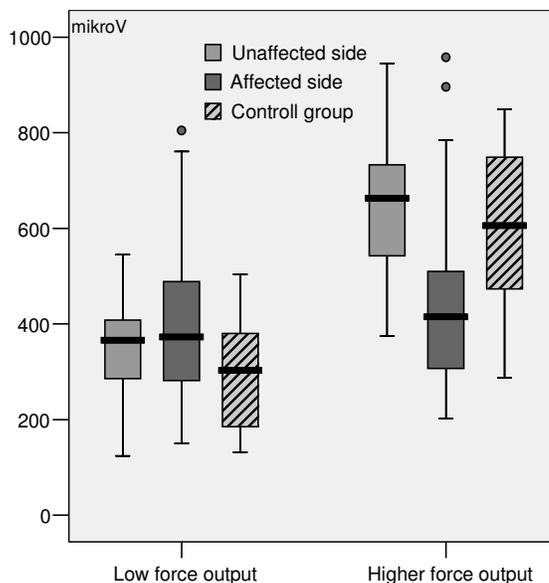


Figure 10. Box-plots of median values of macro motor unit potential amplitudes

Correlations with the clinical parameters. On the affected side, the ratio between the median macro motor unit potential amplitude at the high force output and the low force output demonstrated a significant correlation with the severity of the paresis (Figure 11), but not with the time since the onset of the stroke (Figure 12). Thus the loss of the high-threshold motor units was correlated with the clinical severity.

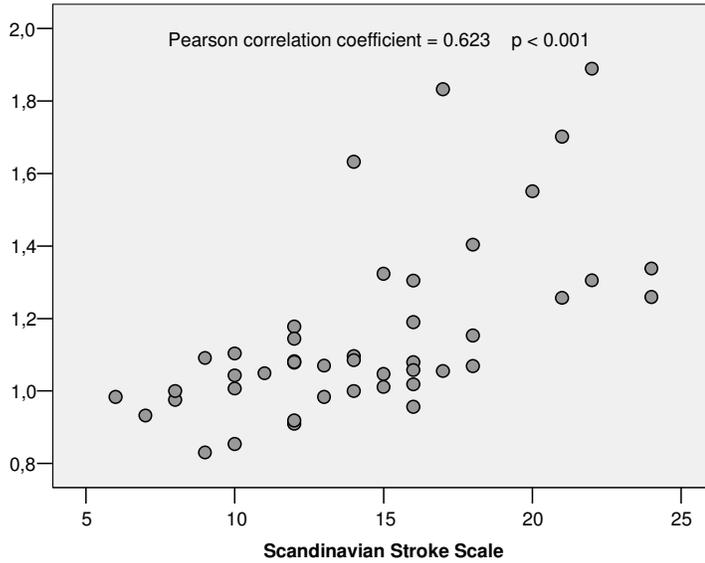


Figure 11. Correlation with severity of hemiparesis. y axis: ratio between median macro motor unit potential amplitudes at high force output and at low force output. x axis: clinical score (Scandinavian Stroke Scale: 0 = no active movements, 30 = full muscle strength)

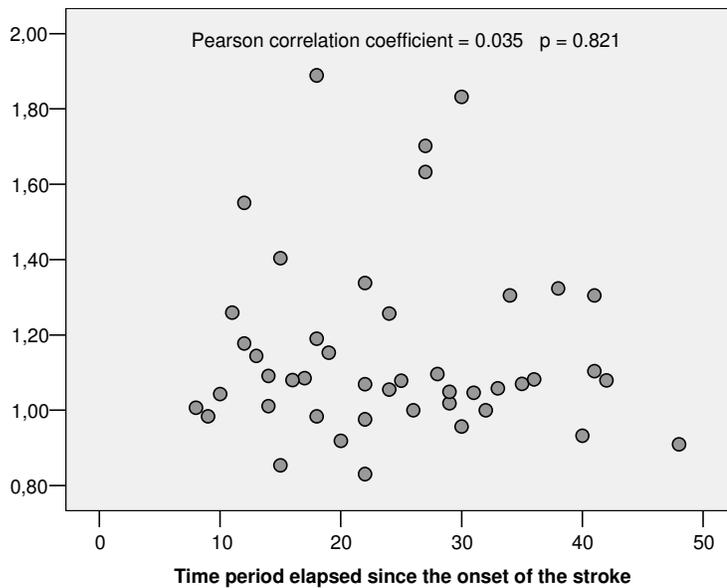


Figure 12. Correlation with the time elapsed since the onset of the stroke. y axis: ratio between median macro motor unit potential amplitudes at high force output and at low force output. x axis: number of months elapsed since the onset of the stroke

6. F-wave measurements

In comparison to unaffected side and controls, mean F-wave amplitude values were increased on the affected side (Table 4) and showed good correlation with the time after stroke onset (Pearson correlation coefficient = 0.721, $p < 0.001$).

	Values in mikroV	
	Mean	SD
Affected side	802.9	550.1
Unaffected side	166.8	67.1
Control group	174.5	54.3

ANOVA	
F ratio	p
49.69	<0.001

Table 4. Statistical analysis of single stimulated F-wave amplitudes

No significant differences were found among mean F latencies and chronodispersion on the affected side, unaffected side and control group (F ratio = 3.784, $p = 0.115$). Ipsilateral cutaneous stimulation inhibited F waves on the unaffected side and in controls, but not on the affected side (Figure 13).

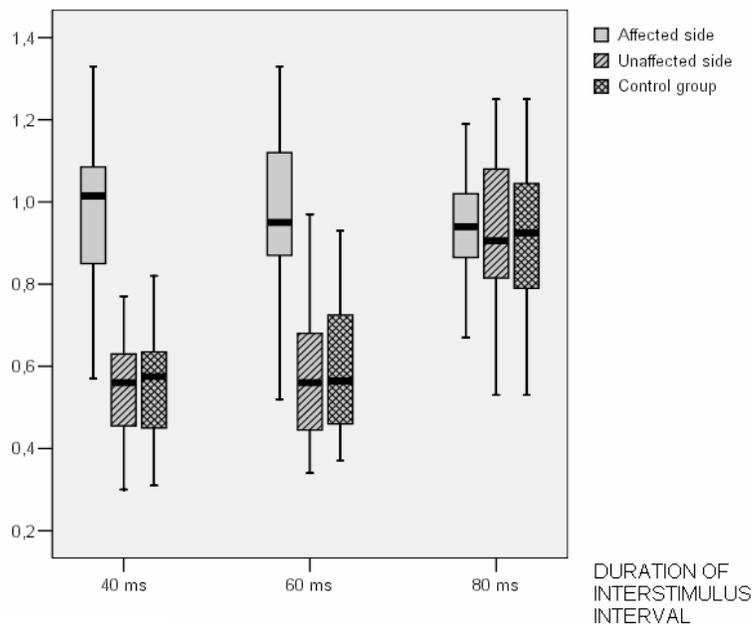


Figure 13. Box and whisker plots of sensory conditioned - single stimulus F-wave amplitude ratios

The ANOVA indicated a significant relation between cutaneous stimulation and the side on which F waves were measured (Table 5). Post-hoc testing showed that F-wave ratio measurements detected differences among sides and groups at 40 and 60 ms interstimulus intervals.

Between-group ANOVA

Dependent variable: sensory conditioned to single stimulated F-wave amplitude ratio

Independent variable	F	Significance
ISI	3.33	p=0.14
Side/group	4.43	p=0.09
ISI + side/group	25.29	p=0.001

Multiple comparisons – Dunnett T3

ISI	ANOVA		Affected side/unaffected side	Affected side/control group	Unaffected side/control group
	F	p	p	p	p
40	90.8	<0.001	<0.001	<0.001	0.99
60	65.3	<0.001	<0.001	<0.001	0.90
80	0.16	0.85	0.76	0.99	0.98

ISI: duration of interstimulus interval

Table 5. Statistical data of sensory conditioned to single stimulated F-wave amplitude ratio measurements

On the hemiparetic side, the ratio between the amplitude of the F responses conditioned by sensory stimulation and those elicited by single stimuli was poorly correlated to the time after stroke onset, F/M ratio, rate of spastic muscular tone and severity of hemiparesis (Table 6).

	Sensory conditioned to single stimulated F amplitude ratio (ISI = 60 ms)		Recovery time of test F wave amplitude	
	r	Significance (p)	r	Significance (p)
Time after stroke onset	0.372	0.012	0.711	<0.001
Rate of spastic muscle tone*	0.296	0.05	0.537	<0.001
F/M ratio	0.304	0.018	0.749	<0.001
Severity of hemiparesis**	0.325	0.024	-0.851	<0.001

r: Pearson correlation coefficient

*: Ashworth scale

** : Scandinavian Stroke Scale

Table 6. Statistical data of correlation analysis

In both patients and controls, the recovery curve showed an initial decline followed by a rise and a later horizontal part. The curves from the unaffected side showed points of inflexion similar to the curves from the controls, whereas the curve from the affected side became horizontal at different, later points (Figure 14).

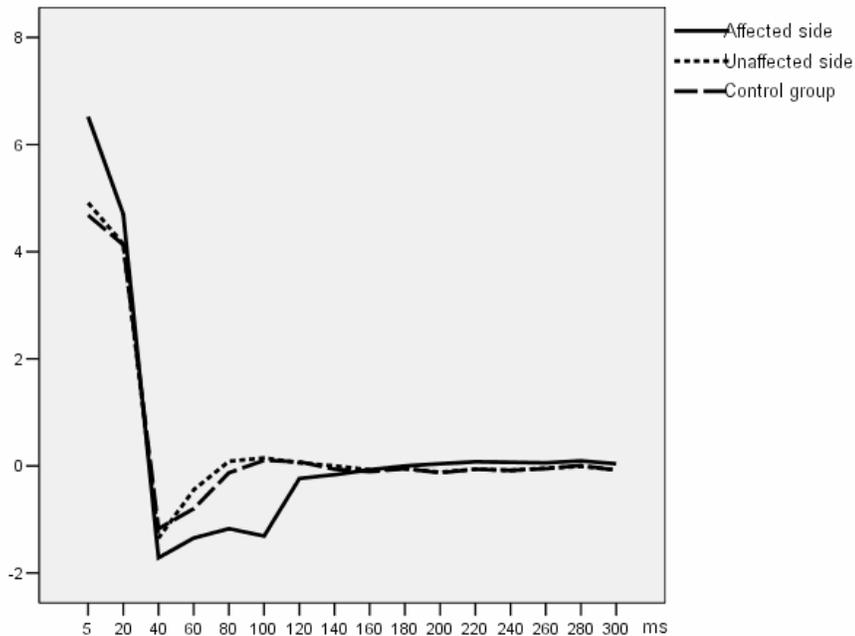


Figure 14.

Paired stimulation - recovery curves of F responses after a conditioning stimulus

y axis: natural logarithm of the test to conditioning mean F-wave amplitude ratio

x axis: duration of interstimulus interval

The analysis of variance revealed that the effect of the interstimulus interval on the F amplitude ratio was significantly influenced by the group and the side on which the F responses were registered. Multiple comparisons tests indicated that this influence was of importance between interstimulus intervals of 60 to 140 ms. In comparison to the unaffected side and the control group, the recovery curve from the hemiparetic side showed significant differences at interstimulus intervals of 60 to 140 ms, while no significant differences were found between the equivalent portions of the curves from the unaffected side and controls. At

longer interstimulus intervals the differences for specific points for all three recovery curves were similar. (Table 7).

The recovery time showed good correlations with the time after stroke onset, F/M ratio, severity of hemiparesis, and clinical grade of spasticity (Table 6).

Between-group ANOVA

Dependent variable: test to conditioning F-wave amplitude ratio

Independent variable	F	Significance
ISI	2.73	p=0.009
Side/group	1.17	p=0.322
ISI + side/group	32.17	p=<0.001

Multiple comparisons – Dunnett T3

ISI	ANOVA		Affected side/unaffected side	Affected side/control group	Unaffected side/control group
	F	p	p	p	p
5	34.69	<0.001	<0.001	<0.001	0.271
20	3.04	0.51	0.161	0.186	0.98
40	2.65	0.074	0.289	0.107	0.821
60	12.67	<0.001	<0.001	0.037	0.034
80	66.61	<0.001	<0.001	0.003	0.06
100	143.96	<0.001	<0.001	<0.001	0.99
120	9.77	<0.001	0.002	0.001	0.98
140	3.11	0.048	0.047	0.077	0.59
160	0.18	0.835	0.99	0.941	0.63

ISI: duration of interstimulus interval in ms

Table 7.

Statistical data of the F wave recovery cycle

VI. DISCUSSION

It was first suggested more than 30 years ago that lesions of the upper motor neurons cause degeneration of the lower ones. Concentric needle EMG revealed pathological spontaneous activity in paretic muscles of stroke patients (3, 4, 30, 46, 47, 56). Studies using MUNE demonstrated loss of the motor units as early as 4-30 h following stroke (1, 25, 26).

Morphologic studies revealed muscle fiber rearrangement after stroke (11). These changes might be due to trans-synaptic degeneration that occurs secondarily to the lesion of the upper motor neurons. Alternatively the lower motor neurons could have been inactivated due to the lack of efferent impulses. However, these changes could have been caused by compression neuropathy in or inactivation of the paretic limb (31). Therefore, the concept about lower motor neuron injury with upper motor neuron lesion remained controversial.

In order to elucidate the pathophysiology of the possible lower motor neuron affection caused by lesion of the upper one, we investigated patients with stroke, and we focused our studies on several, relevant aspects: How do the different neurophysiological changes progress in time? Is there a selective loss of a fiber-subpopulation? Is there any change in the excitability of the remaining lower motor neurons? What is the clinical significance of these findings (i.e. correlation of the neurophysiological changes with the clinical severity of the symptoms)?

Progression in time

During the first month after stroke onset, we found spontaneous EMG activity and decreased M wave amplitude on the hemiparetic side. This suggests that axonal or neuronal lesion occurs in the peripheral motor nerves.

There was an increased number of motor unit potential phases (polyphasic potentials) and turns (serrated potentials) on the hemiparetic side. These are caused by delays in the nascent neuromuscular junctions, and are hallmarks of the ongoing collateral reinnervation: the denervated muscle fibers are reinnervated by the remaining motor nerves (51). This leads to motor unit potentials that have longer duration and higher amplitude, as showed by the outlier-MUP analysis in our patients. Another neurophysiological parameter sensitive for the presence of the collateral reinnervation (as early as several weeks following denervation), the fiber density (FD) was significantly increased on the paretic side.

All these neurophysiological changes in the early post-stroke period showed significant correlation with the time period elapsed since the onset of the stroke, suggesting that the degeneration of the lower motor neurons occurs shortly after the lesion of the upper one.

In the chronic phase of the post-stroke period (after 10 months) the FD values did not increase any longer (they remained increased constantly in time). At this stage the collateral reinnervation became complete, and this explains why in the chronic patients the spontaneous EMG activity disappeared and the amplitude of the M-wave approached the normal values.

Thus our findings support that shortly after the stroke, degeneration (structural loss) of the lower motor neurons occur. Previously it was suggested that the abnormalities found in the peripheral nerves of stroke patients are the consequences of compression neuropathies due to inactivation of the limb (31). Our findings indicating that the process starts right after the stroke makes this assumption highly unlikely because it would imply that all these patients with abnormal neurophysiological parameters would have suffered a compression neuropathy simultaneously with the stroke. In addition, during the patient inclusion we carefully excluded those patients who were suspected of having compression neuropathy (on clinical and neurophysiologic grounds).

We hypothesize that the loss of lower motor neurons following stroke is the consequence of trans-synaptic degeneration. Supraspinal input provides trophic support to the motor neurons (42). Removal of the supraspinal input causes alterations in metabolism and axonal transport in the lower motor neurons (9, 10, 34).

The selectivity of the lower-motor neuron degeneration

It was unclear if the trans-synaptic degeneration affected the lower motor neurons randomly or in some specific way. In order to elucidate this, we recorded the macro EMG at two force output levels, corresponding to the activation of the smaller motor neurons (low recruitment threshold motor units) and large motor neurons (high recruitment threshold motor units) (27, 28). We found that, in contrast with the unaffected side and the controls, in the paretic muscle the amplitudes of the macro motor unit potentials at the high force output were not larger than those at the low force output. This means that the large, high-threshold motor units are lost following stroke.

Previous studies have revealed a direct relationship between the recruitment threshold and the size of the macro motor unit potentials: the later recruited units were larger than those recruited at low thresholds, reflecting the size principle (49). Thus, from a theoretical point of view three different pathomechanisms could explain our findings: degeneration of the large motor neurons (large motor units), selective failure of activation of the large motor units, and selective atrophy of the muscle fibers in the large (high threshold) motor units. Taken into account previous data concerning denervation and collateral reinnervation in the paretic

muscles after a stroke (11, 36) we hypothesize that our results are caused by a selective degeneration of the large (high threshold) motor units. Animal studies showed selective preferential vulnerability of the large motor units (innervated by large motor neurons) to toxic and metabolic injuries (6, 28, 54). Thus we speculate that the large motor neurons are more vulnerable to the trans-synaptic changes following the lesion of the upper motor neuron (5, 53).

Our findings cannot be attributed to disuse atrophy. More high-threshold motor units are recorded in disused muscles than in control muscles (14). In the disused muscles there is an increase in the expression of the type-II fibers (of the high-threshold motor units) (22). Thus changes in disused muscles are just opposite to the changes we recorded in the muscles of the stroke patients.

The lack of difference between the macro motor unit potential amplitudes at high and low force output on the paretic side cannot be explained in terms of an increase in the size of the low threshold motor units caused by the collateral reinnervation, because the amplitude at the low force output on the paretic side was the same as that at the low force output on the unaffected side and in the controls. Moreover, on the paretic side, the amplitude at the high force output was significantly smaller than that on the unaffected side and that in the controls at the high force output. Thus the changes observed in the macro EMG study (i.e. the failure of recruiting large motor units) are not biased by the structural changes (collateral reinnervation) in the motor units on the affected side. Thus following stroke fewer high threshold motor units are recruited on the paretic side, and the generation of the remaining muscle force falls to the lot of the low threshold motor units. This also explains the failure to increase the discharge rate of the motor units during the voluntary force increase in the paretic muscles following a lesion of the upper motor neurons (24).

Fiber density measured at high force-output was significantly increased on the affected side of the stroke patients. To exclude that this was only due to a possible influence of the force output level on the FD, we compared FD values recorded at high and low force output, in normal controls. We found that FD was a robust neurophysiological parameter that was independent from the force output-level at which this was recorded. To prove that the FD values were measured from different subpopulation of motor units at the two force levels we simultaneously recorded macro MUPs, which showed an increase from the lower to the higher force-output level, in normal controls. Our findings suggest that the larger electrical size of the high recruitment threshold motor units in normal controls is due not to a higher fiber density, but to a larger territory.

Taking this into consideration our findings in stroke patients suggest that some of the low-threshold (smaller diameter) motor neurons participated in the reinnervation of the muscle fibers belonging to the larger motor units, whose motor neurons selectively degenerated following stroke. Thus the remaining low-threshold motor units have to provide the muscle activity also at higher force output too.

Changes in the excitability of motor neurons

To shed light on the properties of the lower motor neurons that survived the trans-synaptic degeneration we investigated the excitability of these neurons by recording F-waves with and without conditioning stimuli.

In accordance with previous papers we found increased F-wave amplitude on the paretic side (19, 21). This has been interpreted as a sign of lower motor neuron hyperexcitability related to spasticity (13, 16, 19, 55).

To investigate the dynamics of the depolarization of the lower motor neurons in stroke patients we recorded F-waves after application of conditioning stimuli preceding the test stimulus at increasing time-intervals. The recovery curve in our subjects is in accordance with previously published data (39). There is an initial short refractory period (up to 5 ms) because the axons are still depolarized by the preceding conditioning stimulus. In the next phase collision occurs between the descending volley resulting from antidromic firing of motor neurons activated by the conditioning stimulus, and the ascending volley from the test stimulus. This causes the decrease in conditioning F responses at interstimulus intervals between 5-35 ms (high test-to-conditioning ratio). In the next phase the test F-responses are depressed. The bottom of the graph corresponds to the highest degree of test F-wave inhibition (the lowest value of the test to conditioning F amplitude ratio). Recovery of test F-waves is represented by the upward trace, which turns into the horizontal part when the test F-wave amplitude reaches the conditioning F-wave amplitude (Figure 14). On the hemiparetic side of the patients the recovery phase was significantly prolonged.

A possible explanation for the reduction of the test F-wave is that the lower motor neurons are still depolarized by the conditioning F-waves when the ascending test impulses reach the spinal cord. On the affected side of the stroke patients, the depolarization seems to be prolonged explaining why the changes in the recovery-curve. We hypothesized that this was due to lack of inhibitory inputs to the lower motor neurons.

To investigate the possible disturbance in the inhibitory influences on the lower motor neuron we investigated the influence of sensory conditioning stimuli on the F-waves. It was

previously reported that high-intensity ipsilateral sensory stimulation preceding the test-stimulus for the F-wave caused reduction of the F-wave amplitude in healthy subjects (20, 35). The cutaneous inhibition is thought to be produced by thinly myelinated, high-threshold afferents that provide input to spinal inhibitory interneurons (38). Although high intensity cutaneous stimulation activates low-threshold afferents too, these do not appear to be essential for eliciting the cutaneous inhibition (33). It seems likely that the cutaneous inhibition of F-waves results from the action of inhibitory spinal interneurons upon motor neurons. Therefore this approach seemed ideal for investigating a possible disturbance in the inhibitory inputs on the lower motor neurons.

On the hemiparetic side the ipsilateral high-intensity cutaneous stimulation failed to elicit the inhibitory influence seen in the controls. This suggests that the inhibitory input on the lower motor neuron is reduced in the case of upper motor neuron lesion, and this could be partly responsible for the increased excitability of motor neurons.

The results of our excitability studies indicate that following stroke, the remaining lower motor neurons have a higher excitability and have prolonged depolarization period. Decreased inhibitory input from the spinal interneurons is likely to contribute to this.

Clinical significance of the pathophysiologic changes in the lower motor neurons following stroke

To investigate whether the recorded abnormalities of the lower motor neurons following stroke are just coincidental findings or they are indeed related to the clinical picture we compared the degree of neurophysiologic abnormalities in the lower motor neurons with the severity of the clinical symptoms.

We found a significant correlation between the clinical signs (Scandinavian Stroke Scale) and the following neurophysiologic abnormalities: spontaneous muscle activity and decrease of M-wave amplitude (in the subacute phase), the mean number of MUP turns and phases, the number of outlier MUPs with prolonged duration and increased amplitude, the increase in the fiber density, the loss of high-threshold motor units (as showed by the macro-EMG study), and the changes in the F-wave recovery curve.

The severity of the stroke and the severity of the affection of the lower motor neurons were correlated.

VII. NEW RESULTS ESTABLISHED IN THE THESIS

1. Shortly after ischaemic stroke trans-synaptic degeneration of the lower motor neurons occurs
2. The large, high-threshold lower motor neurons are selectively affected
3. Subsequently collateral reinnervation starts: the remaining lower motor neurons reinnervate the denervated muscle fibers, leading to changes in the structure of the motor units
4. The remaining lower motor units show pathophysiological changes: hyperexcitability, prolonged depolarisation, decreased inhibition from spinal interneurons
5. The abnormalities of the lower motor neurons following stroke are correlated with the clinical severity
6. Fiber density is a robust EMG parameter independent from the force-output at which it is measured.

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10. ANNEXES