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Assessment of Human Motoneuron Afterhyperpolarization Duration in Health and Disease

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The results of the investigation of afterhyperpolarization (AHP) duration in normal aging and selected neuromuscular disorders are presented. This investigation yielded unexpected results: the AHP shortening in myogenic disease (DMD) and no significant difference from control values in neurogenic disease (ALS). However, introduction of age factor revealed novel aspects of the human ALS, which can be interpreted on the basis of the results obtained in a SOD1 mice, thus confirming usefulness of this animal model of ALS. In spastic patients the AHP was prolonged and the difference from the control AHP duration decreased with age and disease duration. Our results suggest that the match between temporal characteristics of the AHP of MN and of the twitch of its muscle unit is preserved during normal aging and in spasticity, but not in the DMD.

K e y w o r d s: human motoneurone, afterhyperpolarization, aging, duchenne muscular dystrophy, amyotrophic lateral sclerosis, post-stroke spasticity

1. Introduction

In spinal motoneurons (MNs) each spike is followed by the long-lasting afterhyperpolarization (AHP), which is an important factor in control of repetitive firing. In cat MNs firing repetitively in response to injected steady current, maximum possible interspike interval (ISI) is approximately equal AHP duration [1].

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Human MNs are investigated in much more physiological conditions. Here, characteristics of the MN discharge also depend on its AHP duration. This influence, however, is not as straightforward as in animal experiments. Human MNs often fire with the ISIs exceeding the AHP duration. This is the consequence of the presence of synaptic noise, which results in the interspike interval (ISI) variability. Below, we will show how the analysis of ISI variability of MN may allow to compare the MNs with respect of their AHP duration. This analysis was applied to study the AHP duration in the healthy and diseased MNs.

2. Methods

Every subject (or his/her parents) gave informed consent for the experimental procedures, which conformed to the standards set by the latest revision of the *Declaration of Helsinki* and had the approval of the appropriate ethical committee, whenever possible.

In the majority of the experiments, the subject was comfortably seated in an armchair with the left forearm placed on a support assuring isometric muscle contractions. The arm was positioned horizontally and the angle in the elbow was 90º. The wrist was supported by a cuff suspended from the ceiling. During the experiment the subject exerted a force by pressing a lever attached to a strain gauge. The signal of the force transducer and the required force level were displayed on the screen together with the electromyogram. The subject was provided with an auditory feedback of the MU discharges and was instructed to perform a series of 10–100 seconds constant force isometric contractions, keeping the MUs firing steadily. Between the consecutive recordings the subject was allowed to rest for 3–4 minutes. At the beginning of each experiment, the force of maximum voluntary contraction, MVC, was measured and used as a reference.

Motor unit potentials (MUPs) were picked up from the brachial biceps by intramuscular disposable bipolar electrodes made from a Teflon-coated tungsten wire of 90 μm diameter according to the procedure described by Basmajian and Stecko [2]. Two wires were introduced into a hypodermic needle and their ends were hooked. Before use the electrodes were sterilized in an autoclave (130ºC). The needle was inserted into the muscle and then withdrawn, whereupon the hooked ends fixed the wires in the muscle. Their external ends, from which the insulation was removed, were connected to input of an amplifier. The electrodes were very selective, which enabled us to distinguish single MUPs sometimes up to 100% MVC. The MUPs were amplified and transferred for an off-line analysis to a PC computer by an A/D converter and recorded on a magnetic tape. The sampling was performed with rates from 10 to 20 kHz, depending on the frequency content of the signal, so that there was no aliasing.

2.1. Data Analysis

Usually, potentials of several simultaneously active MUs were recorded in each experiment (Fig. 1). The MU recordings were decomposed into the constituent single MU potential trains by an operator-computer interactive method described in detail elsewhere [3]. The results of the preliminary computer identification were verified by an experienced human operator who corrected the misclassifications. Only the sections of records with 100% proper identification were accepted for further analysis. The MU firing rates were calculated from 10–50 consecutive potentials. The longer fragments with proper MUP identification were also subjected to the variability analysis, as described below.

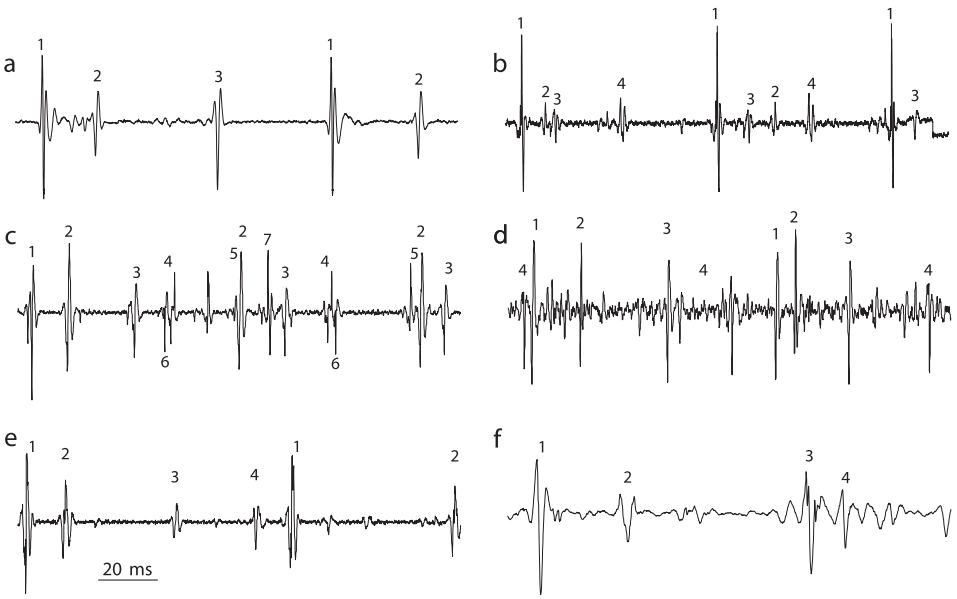

Fig. 1. Examples of MUP recordings made during moderate contractions (potentials of single MUs marked with distinctive numerals): a) adult control subject (age 58); b) young control subject (age 7); c) ALS patient with mild muscle involvement; d) dystrophic patient; e) ALS patient with severe muscle involvement; f) spastic post-stroke patient

The method of estimation of the AHP duration is based on the fact that there is a one-to-one relationship between the MUP and the MN discharge [1], and on the hypothesis of Person and Kudina [4, 5], that the plot of the standard deviation, *SD*, of MN interspike intervals (ISIs) vs. their mean value, *MISI*, may contain information about the motoneuronal AHP duration. On such a plot, two ranges of the ISI can be distinguished [6]. In the short-interval range the MN discharges regularly with a low ISI variability, whereas in the long-interval range the MN discharge is irregular, and the variability increases rapidly with the mean ISI. This is due to the

fact that the short-interval range corresponds to the *rhythmic firing mode*, whereas the long-interval range corresponds to the *occasional spike mode* [7]. In this last case, a large fraction of the ISIs is longer than the AHP of MN, and consecutive spikes are generated by random fluctuations of the membrane potential around threshold, after all post-spike ionic phenomena have been completed [8]. Obviously, the occasional spike mode produces substantially more of the ISI variability than does the rhythmic spike mode.

The long-interval range can hardly be observed in animal MNs, which are brought to firing threshold by a virtually noiseless electrical device. However, this range (subprimary range [9]) is commonly observed in human MNs, which are driven by a synaptic inflow always containing a variable component.

Using computer simulations [10] we have previously shown that the ISI corresponding to the transition between both ranges (transition interval, TI) is correlated with the AHP duration. This result was also confirmed by recording directly from cat MNs [11] and also in human experiments [12]. Figure 2 presents a *SD(MISI)* relationship compared with the AHP (simulation data). Although the TI is not equal to the AHP duration, the *SD(MISI)* plots with the shorter TIs correspond to the MNs with the shorter AHPs. Thus, this method can be used to compare single MNs with regard to their AHP durations. In this manner, we can obtain information on the physiology of human MNs comparable to that from acute experiments in animal models.

In most of the plots, linear sections in short- and long-interval range could be distinguished by visual inspection. For each of these plots, the transition ISI value delimiting the short- and long-interval sections was estimated in order to quantify the position of the plot with respect to the ISI axis. If the transition between ranges was curvilinear, the limits of the transition ISI range were determined by fitting straight

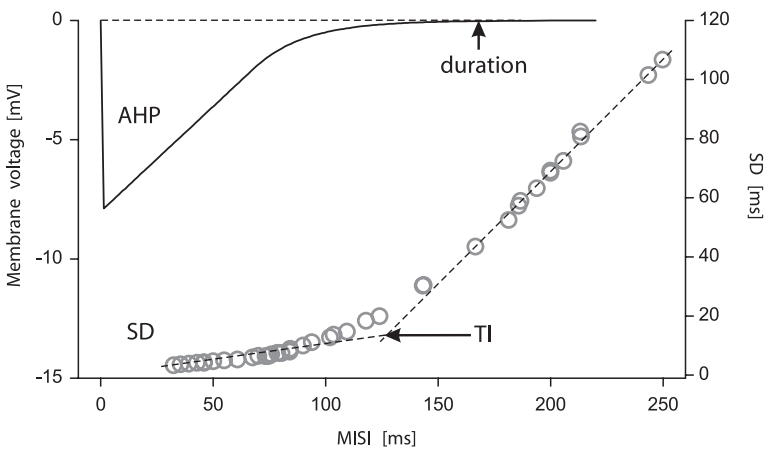

Fig. 2. Comparison of AHP time course and *SD*(*MISI*) plot. AHP duration and estimated transition interval indicated by arrows

lines to the initial and final parts of the plot (indicated by an operator) and the TI was set at the intersection of these lines. For the plots without a visible curvilinear section, the data were divided into two subsets with the division point close to the shortest mean interval. Straight lines were then fitted by means of the least-squares method to each subset; the division point was automatically varied and this value, which corresponded to the total least squares sum, was taken as the TI. More details on the latter procedure are given in [13]. In the muscular dystrophy and aging study this procedure was applied to the pooled data of each subject, for whom the global TI was determined.

Since SD strongly depends on the mean ISI, this had to be taken into account when comparing the ISI variability between different subject's groups. The result of comparison will depend on the ISI range over which the comparison is performed. The most reasonable way to assess the variability is to make the comparison at the same mode of spike generation. The short-interval range, corresponding to the rhythmic firing mode, seems to be more appropriate since the set of variability values here would be less dependent on the firing rate range, at which the data was collected. Accordingly, all the comparisons of SDs, reported below, were performed in the short-interval range, meaning that for each subject only these SD values were selected, which corresponded to the mean ISIs shorter than his/her transition interval. The statistical analysis of data was performed using Student's *t*-test (built-in Microsoft Excel 2003).

3. AHP Duration in Duchenne Muscular Dystrophy

3.1. Method

The data were collected from 8 controls aged 5.5–19 years and 25 patients aged 5.5–17 years. The patients were children suffering from Duchenne muscular dystrophy (DMD) of different severity. The advancement of this disease causes gradual muscle degeneration that results in deterioration of patient's mobility, beginning from gait disturbances and ending with the dysfunction of the respiratory musculature. This was evaluated by a physician who assigned a clinical score to each patient on the modified Vignos-Archibald 10-point scale [14].

3.2. Results

In total, 159 MUs from the normal muscles and 285 MUs from the dystrophic muscles were taken for the analysis. *SD(MISI)* plots for the dystrophic patients were shifted towards shorter intervals (Fig. 3a). Consequently, the transition intervals were shorter as compared with the control data. This shortening was significant and correlated with the disease severity (Fig. 3b). Note that in Fig. 3a the ISI range of 54–81 ms (between vertical arrows, indicating TIs) corresponds to the short-interval range for

the control subject, and to the long-interval range for the dystrophic patient. Therefore, the variability calculated over the range of 54–81 ms was significantly higher for the patient ($p < 0.002$). However, the patients' SDs were significantly lower ($p < 0.001$), when compared with the control values in the short-interval ranges. Both tendencies, the shorter TIs and the lower SDs, were typical for the AHP shortening [10, 11].

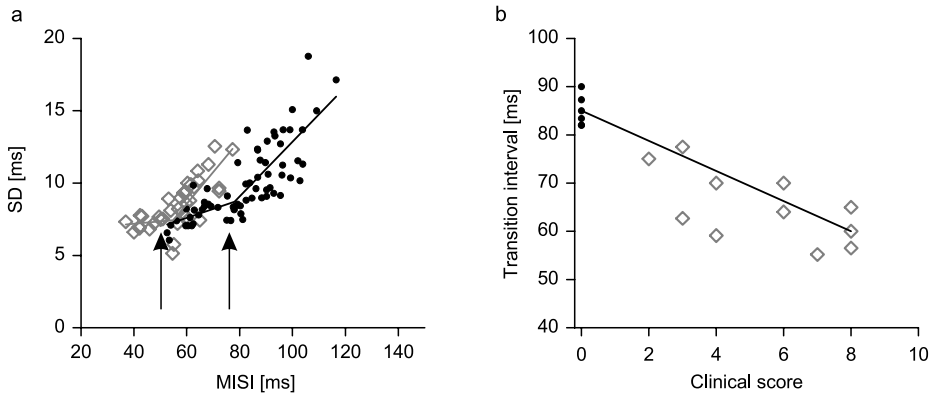

Fig. 3. a) examples of *SD*(*MISI*) plots for the control subject (dots) and the dystrophic patient (diamonds); b) TI vs. clinical score of the subject. See text for the explanation of the arrows

Firing rates at the lowest force levels did not differ significantly between the dystrophic and control MUs. However, at the higher force levels majority of the dystrophic MUs fired with rates significantly exceeding those of the control subjects ($p < 10^{-5}$) and their increase in firing rate with increasing force was more rapid. Also the threshold firing rates for the dystrophic MUs were higher than those for the control ones (more details on firing rates in the DMD in [15]). All these results are also in concert with the AHP shortening.

3.3. Discussion

Summing up, an alteration of motoneuron properties in the DMD is well documented. This result, at the first glance, seems unexpected since there is no doubt that the primary pathogenic process in the DMD begins in muscle fibers due to the lack of dystrophin and it is a common belief that only the muscle fibers are affected. However, the trophic interaction between nerve and muscle cell is also a commonly appreciated fact. It would be rather surprising if the advanced changes in the muscular part of MU would not affect the MN as well. The loss of muscle fibers, which are targets for axonal branches, may be compared to a partial axotomy. Huizar et al. [16] provided experimental evidence that partial denervation of a muscle may decrease duration of the AHP not only in the axotomized but also in the intact MNs. This decrease depends on the degree of the partial denervation, being greater in the severely denervated preparations.

An alternative explanation could be that the change in MN properties is a result of the disease itself. Certain impairment of spinal root axons was found in dystrophic mice (e.g. [17]). Moreover, according to our current knowledge, the lack of dystrophin affects not only muscles but also the nervous system [18–20]. Thus, there is a possibility that dystrophin deficiency may affect MNs as well, either directly or by alteration of supraspinal influences.

It should also be taken into account that the properties of dystrophic muscle change towards those of slow muscle [21, 22], possibly by a selective degeneration of fast muscle fibers [23, 24]. For slow muscle, the range of optimal rates is lower [25, 26]. Therefore, increased firing rates may not be appropriate for the diseased muscle fibers, which may enhance the degeneration process [27].

A more detailed report on the AHP duration changes in Duchenne muscular dystrophy has been presented elsewhere [15].

4. Age-Dependence of AHP Duration

4.1. Methods

The experimental material is based on data collected from 16 healthy subjects in three other series of experiments: 1) a study of the MN involvement in the DMD (8 control subjects, aged 5.5–19, [15]); 2) a study of the discharge properties of the normal human MNs (4 subjects, aged 51–58, [12]); 3) a study of the MN properties in ALS (4 control subjects aged 27–79 years). The subjects from the first series of experiments will be identified as the young group and the others as the adult group. None of the subjects had any record of a neuromuscular disease. Some of the subjects were investigated more than once.

4.2. Results

Altogether, 261 motor units (MUs) were identified in this study, 159 from the young group and 102 from the adult group. The MU firing rates were in the ranges of 8.3–21.7/s (mean 12.87/s) and 5.9–18.7/s (mean 11.08/s), respectively. The difference was statistically significant ($p < 0.001$).

Typical dependencies *SD*(*MISI*) are presented in Fig. 4a for a child (7 years, diamonds) and an adult subject (58 years, asterisks). Both short- and long-interval fragments of the plot for the adult subject are shifted towards the longer *MISI*. Figure 4b presents the relationship between transition intervals and the subject's age. The transition interval gradually increased with age. The regression line fitted to the pooled data was described by the equation $y = 0.441x + 78.083$ (correlation coefficient $R = 0.766$). This correlation was statistically significant ($p < 0.001$). When only the adult subjects' data was analyzed, the correlation was much weaker (coefficient $R = 0.387$) and insig-

nificant ($p > 0.22$), although the regression equation was virtually the same: $y = 0.455x + 77.265$.

ISI variability was compared between the young and adult subjects in the short-interval range. SD values ranged 4.84–11.57 (mean 8.39) ms for the young group and 4.26–12.23 (mean 7.76) ms for the adult group. The difference was statistically significant ($p < 0.001$), although small (0.63 ms, i.e. around 8% of mean).

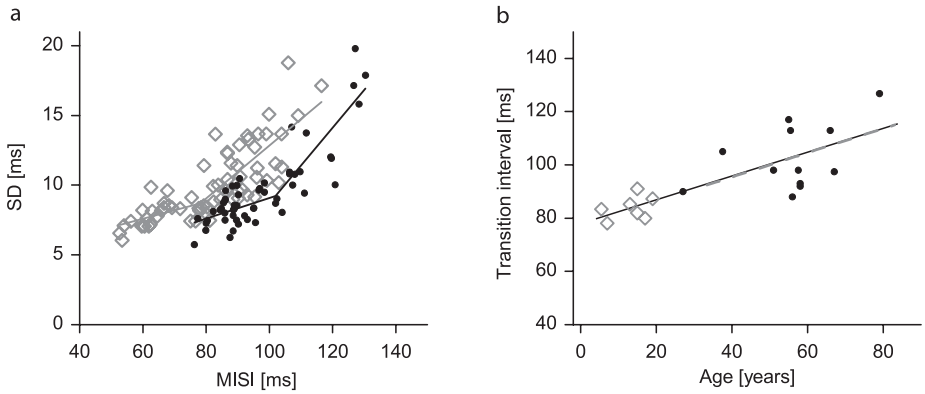

Fig. 4. a) examples of *SD*(*MISI*) plots for 7- (diamonds) and 58- (circles) year-old subjects; b) transition intervals, determined from pooled data, plotted vs. age of the subject. Diamonds, data for the young group; circles, broken line, data for the adult group; solid line, pooled data. See text for regression equations

4.3. Discussion

The variability in the short-interval range was significantly higher for the young group, although the difference was small. This result is closer to those studies reporting no difference (Barry et al., 2007; Vaillancourt et al., 2003) than to those in which higher variability was encountered in aged subjects (Laidlaw et al., 2000). The discrepancy is undoubtedly due to the differences in data collection and analysis.

Correlation of the AHP duration with age was statistically insignificant, when analyzed in the adult group separately. It was evidently due to the limited age range and the scatter of experimental points, considerably greater than in the young group. The latter can be explained by the differences between the individual subjects in the type and amount of preferred physical activity, which are known to influence the "fastness" of MNs and muscle fibres. These differences could be expected to be more pronounced in the adult group, compared to the young one. The differences in the age range may also explain the discrepancy between the results of two studies investigating electrophysiological properties of cat MNs. Morales et al. [29] compared the AHP duration between young adult (1–3-yr.-old) and old (14–15-yr.-old) cats and did not find statistically significant differences in their AHP durations. In contrast, Cameron et al. [30] found an age-related increase in the AHP duration in kittens vs. adult cats.

The increase in the transition interval, found in the brachial biceps, means that there is a systematic increase in the AHP duration of the low-threshold MNs with age, which covers virtually the entire lifespan of a human subject. This is in line with gradual age-related slowing of muscle phenotype [31–33], which suggests that the good match of temporal characteristics of MN and its muscle unit [34] is preserved in healthy aging. The slowing of the neuromuscular system, in contrast to the muscle force decrease, seems to be a continuous transformation, which begins in childhood and continues until senescence. A more detailed report on the age-related AHP duration changes can be found elsewhere [13].

5. AHP Duration in Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a late-onset, lethal neurodegenerative disease of unknown aetiology. Its multifactorial origin is nowadays widely accepted, but the initiating mechanism(s) underlying this process have not yet been defined.

5.1. Method

The data analyzed in this study were collected from 20 patients, aged 31–75 (mean 55.0 years), and 12 control subjects, aged 27–79 (mean 54.8 years), without known neurological disorders. All patients had been diagnosed as having the definite ALS according to El Escorial criteria [35]. The control group included 8 subjects who had also participated in earlier studies [12, 13]. Repeated experiments were run on a few of the control subjects.

For the patients, the severity of the muscle involvement was quantified by the *relative force deficit* (RFD), expressing the reduction in the patient's force as a percentage of the sex-respective mean control maximum voluntary contraction (MVC).

5.1.1. Variability Analysis

SD should be calculated from long stationary fragments of the recording (at least 50 consecutive potentials). Therefore, it is a sufficiently good measure of variability only for the regular spike trains, like those recorded in the normal muscle. However, in the neurogenic diseases such as ALS, the MN discharge is often irregular. In such cases getting a complete SD-mean plot for a given MN requires joining together shorter periods of stationary discharge with similar mean ISI and SD. This means imposing joining criteria, which are always disputable. Therefore, in the present study another method of variability analysis was applied, which we adopted from Holt et al. [36]. This method has been shown to be specifically useful for the variability analysis of irregular neuronal spike trains.

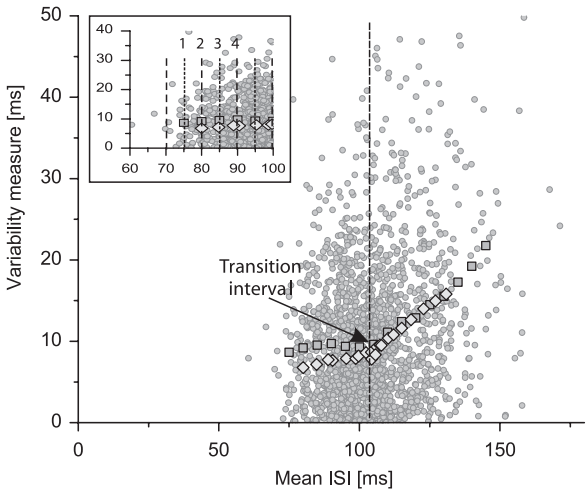

Fig. 5. Variability measures plotted against mean ISI. Dots, *CD2* vs. *MISI*2, both calculated for two adjacent intervals; squares, CD_m vs. *MISI*, both calculated over 10-ms bins; diamonds, *SD* vs. *MISI*, both calculated from a stationary fragment of at least 50 ISIs. Transition interval is indicated by an arrow. Insert explains the calculation of CD_m: point #1 for 75 ms is an average of the points falling into the MISI range delimited by broken lines at 70 and 80 ms; point #2 for 80 ms is an average of the points falling into the MISI range delimited by dotted lines at 75 and 85 ms and so on

Instead of SD, the variability was expressed as consecutive difference between two adjacent ISIs (Θ_i and Θ_{i+1}): $CD2 = |\Theta_{i+1} - \Theta_i|$. It was plotted against the mean ISI, calculated from the same two adjacent intervals: $MISI2 = (\Theta_{i+1} + \Theta_i)/2$. An example of the plot *CD2* vs. *MISI2* is shown in Fig. 5 (grey dots). The relationship $SD(T_m)$ (diamonds) is comparable with the relationship $CD_m(MISI)$ (squares). The CD_m values were calculated by averaging of the *CD2* values falling into the vertical bins of 10 ms width (moved every 5 ms), and *MISI* was the central value of the bin (cf. insert to Fig. 5). The computer simulations based on our threshold-crossing model [10] have shown that both measures of variability are equivalent with respect to the TI estimation. This conclusion was recently confirmed experimentally in the study on the MN discharge properties after spinal cord injury [37].

5.2. Results

The CD_m(MISI) relationships were obtained for 124 MUs from 16 ALS patients. The results were compared with 102 MUs from the control subjects. The patients' data overlapped control data, but were shifted towards higher variability (Fig. 6a). To assess the statistical significance of this shift, the variability was compared between the patients and the controls in the short-interval range. The mean values for the patients and the controls were 11.2 and 9.4 ms, respectively, and the difference was statistically significant ($p < 0.0001$).

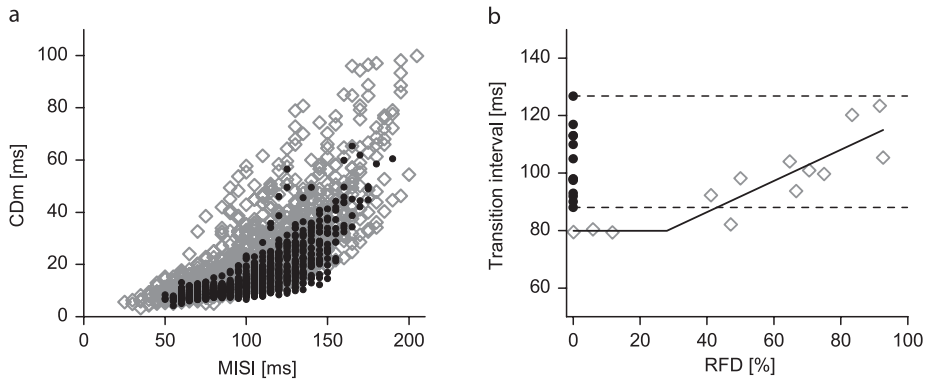

Fig. 6. a) pooled $CD_m(MISI)$ relationships; b) mean TIs plotted vs. RFD; dashed lines represent upper and lower limits of the control data. Diamonds, the ALS patients, dots, the control subjects

The average of the transition intervals of the ALS patients was not significantly different from that of the control subjects. However, the mean TI (calculated for each subject) was significantly correlated with the degree of muscle involvement, expressed as RFD (linear regression coefficient $R = 0.87$, $p < 0.0002$). For the patients with minor involvement, the TIs were shorter than control ones and were independent of the RFD for values up to 30% (Fig. 6b). Above this value, the TIs increased with the RFD (linear regression coefficient $R = 0.81$, $p < 0.01$) but did not exceed the limits of the control subjects' TIs. A comparison of single MN TIs for the patients with minor muscle involvement (RFD < 30%) with those of the age-matched control subjects revealed that the TIs of the patients were significantly shorter ($p < 0.001$) than those of the controls. For the remaining patients (RFD > 30%), the TIs did not differ from the control values.

ALS is known as an age-dependent disease [38, 39]. In our study the RFD was significantly correlated with the patient's age ($R = 0.71$, $p < 0.002$). The age factor influences also the AHP duration in the healthy subjects, as has been shown above. It seemed therefore reasonable to compare the ALS patients with the control subjects in this respect. The relationship between TI and age for the patients appeared to be qualitatively different from that of the control group. However, substantial scatter of the RFD values for the younger patients suggests that the factor of age has lesser impact on the AHP duration than the disease progress.

Firing rates were analyzed in 17 ALS patients. The range of firing rates in the control subjects was 8.4–20.4 spikes/s. The relationship between the MN firing rates and the contraction levels varied between the individual ALS patients. The firing rates of the patients with the RFD < 30% were higher than those of the controls (range: 10.9–29.5 spikes/s), whereas those of the patients with the RFD ≥ 75% (range: 4.7–18.0 spikes/s) were lower than those of the controls. These differences were statistically significant ($p < 0.01$).

It was already evident during MUP recognition that the ranges of firing rates of the simultaneously active MNs were much broader in the patients than in the controls. Extreme values of firing rates were inversely related to the AHP durations of the respective MNs. In all patients two distinct populations of MNs could usually be distinguished according to the AHP duration. In the patients with the RFD<30% fewer MNs exhibited longer AHPs. In the patients with the RFD≥75%, MNs with longer AHPs prevailed, but MNs with shorter AHPs were also present.

5.3. Discussion

The analysis performed in this study has confirmed the higher variability of ISIs in the MNs of ALS patients [40]. This finding is consistent with the increased excitability of the ALS MNs, documented both in the human ALS [41, 42] and in the animal ALS model [43, 44]. Enhanced excitability should result in an increase in the amplitude of the single EPSP in MN; this factor together with decreased number of excitatory inputs to the MN [45, 46] would increase the variability of the synaptic inflow, which is directly related to the ISI variability.

When the pooled data on TIs from the ALS and control MNs were compared, we found no significant differences. Since TI is correlated with the AHP duration [10, 11], this result is consistent with measurements from spinal cord slices from neonatal SOD1 mice [43, 47]. However, there was a clear dependence of the AHP duration on the degree of the muscle involvement in the patients (Fig. 6b). Compared with the MNs in the controls, the MNs supplying less affected muscles (patient group 1) had shorter AHPs, with the AHP duration gradually increasing for the MNs of the patients with the RFD values above 30%. This result indicates that the MN pools supplying muscles of the ALS patients initially exhibit an excess of the "faster" MNs; as the muscle degeneration progresses, the MNs change their activity patterns to become "slower."

Recently, researchers working with SOD1 mice found a marked decrease in firing thresholds of high input conductance (presumably "fast") MNs cultured from neonatal spinal cord [44]. If this was also the case in the human ALS, the levels of muscle contraction at which "fast" MNs are recruited would be lower than normal. Consequently, in the less affected MN pools, proportion of the "fast" MNs recruited at low force levels would increase as compared to the case of a healthy neuromuscular system. In fact, this is what we have observed. Motoneurones with short AHPs ("fast" MNs) prevailed in a sample of MNs collected from the patients with minor muscle lesions. This finding is also in concert with the result of a recent study on presymptomatic SOD1 mice (animal model of ALS), where the AHP was significantly shortened compared to wild type animals during early postnatal development [48].

To our knowledge, disease- and age-dependent changes in the AHP duration were not analyzed in MNs of the SOD1 mice, although it has been shown that the "fast" MNs are more vulnerable to degeneration [49–51]. Nevertheless, in our study we observed the MNs with the comparatively short AHPs (presumably the "fast"

MNs) even in the patients with severe muscle involvement. Since the AHP duration was estimated from data collected at weak or moderate muscle contractions, these results may indicate that the firing thresholds of the remaining "fast" MNs are lower than normal even in advanced stages of the disease. It should, however, be noted that the "fast" MNs of the patients with severe muscle involvement (RFD $\geq$ 75%) had longer AHPs as compared with those of the patients with mild muscle involvement (RFD < 30%).

Several factors may be responsible for the elongation of AHP duration as the disease progresses, including the following possibilities: (i) normal aging, (ii) the vulnerability of "fast" MNs to degeneration [50, 51], which should be enhanced by their excessive use, and (iii) the process of reinnervation [52]. However, the reinnervation should be a less important factor because it is impaired in the ALS [53]. The reliability of our method for estimation of the AHP duration has been well documented both theoretically and experimentally [10, 11, 54, 37]. Our results indicating changes in the AHP duration in the ALS patients are further strengthened by observations on firing rates, which were inversely related to the TI and thus to the AHP duration. Increased firing rates were characteristic of the MNs supplying strong muscles of the ALS patients. Similar results were recently reported from the presymptomatic SOD1 mutant mouse model of the ALS [55].

In summary, the results of this study are compatible with results of the studies conducted on the ALS animal models. These results may contribute to further integration of clinical and animal research, leading to the eventual unravelling of the ALS aetiology. More detailed report on the AHP duration changes in the ALS can be found in [56].

6. AHP Duration in Post-stroke Spasticity

6.1. Method

The study participants were 11 post-stroke patients, aged 30–77 years, all suffering from spasticity and seeking BOTOX treatment. The patients were recruited from the Department of Rehabilitation in Chi-mei Hospital, Tainan, Taiwan. The control group consisted of 8 Taiwanese subjects aged 22–64 years. None of the control subjects had any record of neuromuscular disease. The experiments were performed in agreement with the declaration of Helsinki. Each subject gave informed consent to the experimental procedures. The procedures were approved by the Ethical Committee at Chi-mei Hospital, Tainan.

During these experiments, the subject lay comfortably on a bed with one arm slightly abducted. The subject was provided with an auditory feedback of the MU discharges and was instructed to perform minimal isometric contraction for 3 minutes and then slowly increase the contraction force during the 4th and 5th minute until roughly 50% MVC was reached.

The EMG data were collected from the brachial biceps of the left arm (control subjects) or the affected arm (patients). Potentials of the single MUs were recorded by a bipolar concentric electrode mounted in a needle used for BOTOX injection. The EMG signals were amplified by a Medelec Premiere Plus electromyograph (Vickers Medicals, Woking, UK), sampled at 10 kHz by an analog-to-digital converter with 12-bit resolution, and sent to a computer for an off-line analysis. Programs for the data acquisition were constructed using the LabView environment. The variability analysis was performed as described in the ALS section.

6.2. Results

Altogether, 39 MUs were recorded from the control subjects and 64 from patients. The firing rates were lower in the patient MUs (4.6–14.3/s, mean 7.9/s) than in the control subject MUs (5.4–20/s, mean 10.6/s). The difference was highly statistically significant ($p < 10^{-6}$). The patient's MU firing was often irregular and single MUs could not always be followed throughout the recording of the entire experiment, presumably due to fatigue. Therefore some MUs were not accepted for the variability analysis because of an insufficient number of discharges. The slow fluctuations of the MU firing rate were also much more pronounced in the patients than in the control subjects.

The complete $CD_m(MISI)$ relationships were obtained for 25 control MUs and 37 MUs from 10 patients. One patient's MU firing was so irregular, that for each identified MU only 200–300 potentials could be distinguished, so his data were not taken for the further analysis. The patients' relationships were shifted towards longer ISIs (Fig. 7a). The patients' ISI variability, calculated over the entire range of MISI, was lower than the variability of the control MUs. This difference was statistically significant ($p < 0.001$). However, when the variability was compared between the patients and the control subjects in their short-interval ranges, the difference was not significant.

In Figure 7b, mean values of the TIs (calculated for each subject from all MUs analyzed) are plotted vs. age for the control subjects (circles) and the spastic patients (triangles). The patients' TIs were substantially longer than the control values. Moreover, their dependencies on age differed dramatically from each other. The regression equations were $y = 0.53x + 78.952$ (regression coefficient $R = 0.692$) for the control group and $y = –0.324x + 151.56$ ($R = 0.342$) for the patient group. This difference was statistically significant ($p < 0.005$). Note that the patients' regression line tends to converge with the control one. We correlated also the increase in the TI (calculated with respect to the regression line of the control data) with the duration of the disorder (Fig. 7c). The regression was insignificant, when all points were considered ($R = 0.377$). When, however, data of the 3 oldest patients (age > 55 years, filled diamonds) were removed, the significance substantially increased ($R = 0.856$, broken line, open diamonds).

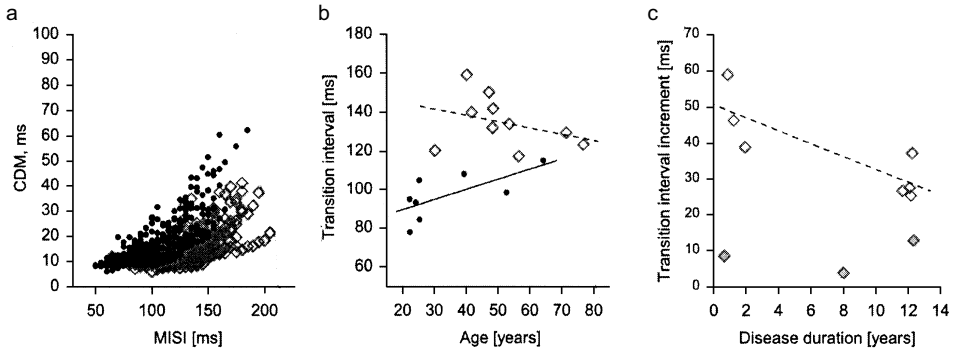

Fig. 7. a) pooled plot $CD_m(MISI)$; b) mean transition interval, calculated for each subject from single MU data, vs. age. In a) and b): dots, solid line, the control subjects, diamonds, broken line, the spastic patients; c) correlation between transition interval increase and disease duration: open diamonds, broken line, data of the patients younger than 55 years; filled diamonds, data of the older patients

6.3. Discussion

Our results concerning the MU discharge characteristics conform in general to the previous reports, showing lower MU firing rates [57–59] and lesser total ISI variability [60] in the patients compared with the control subjects. However, some of these findings need additional comments. The comparison of the short-interval variability yielded no difference between the control and patient groups. Similar results were obtained in an investigation by Sun et al. [61], in which short-term variability was studied separately from long-term variability. The short-term variability in the stroke patients was within control limits, whereas the long-term variability was significantly higher. It should be noted that the long-term variability in the cited paper represents another type of variability than that determined in our study. It is related rather to slow firing rate fluctuations as well as trends, occurring due to the less efficient control of the muscle force, commonly observed in the spastic patients.

Our results indicate that the MNs of the spastic patients become “slower” than those of the control subjects. This explains a commonly observed decrease in firing rates, since they are known to be controlled by the MN AHP. Since the contractile properties of muscles have been shown to change towards the slower phenotype after upper MN lesions [59], our results suggest that the match between MN and muscle properties is preserved in the post-stroke patients. The approximation lines for the control subjects and the patients tend to converge, which means that the susceptibility to changes due to the cerebral lesion decreases with age. This is without doubt a consequence of the age-dependent decrease in the MN plasticity. However, there is also another factor influencing the AHP duration. The increase in the MN AHP duration (expressed by TI) for the patients younger than 55 years was negatively correlated with the disease duration, which implies that there is a gradual recovery of the MN properties with time after the stroke accident. The youngest patient (30 years) who

had the accident 12 years before the investigation, presented the mean TI quite close to the control data (see Fig. 7b). When this point was removed, the regression coefficient of the line calculated from TI-age dependency increased from 0.342 to 0.699. The recovery is less pronounced in the older patients, which also may be attributed to the decreased MN plasticity. This issue, however, calls for further investigation on a larger patient sample with a wider range of the disease durations.

A more detailed report on the AHP duration changes in the post-stroke spasticity can be found in an earlier publication [62].

7. Summary

These investigations yielded unexpected results, revealing motoneuron (MN) involvement in the DMD, which is a myopathic disease, and no statistically significant difference in the AHP duration in the ALS, which is a neurodegenerative disease and where the MNs are no doubt involved. On one hand, these results suggest that the AHP is not a feature that can reliably differentiate "sick" from "healthy" MNs. On the other hand, our results reveal that the AHP duration is dependent on the subject's age and that this dependency for the ALS patients is different from that for the control subjects. These differences may be explained on the basis of the results obtained in the SOD1 mice, which is an argument for the usefulness of this animal model of ALS.

In the spastic patients the AHP was prolonged and the difference between the AHP duration in the control and diseased MNs decreased with age and the disease duration. Our results obtained in MNs of the spastic patients and during normal aging suggest that the match between the temporal characteristics of MNs and muscles is preserved when the muscles convert to the slower phenotypes, which is another manifestation of "muscle wisdom" [63], underlying MN spike frequency adaptation. This match, however, is not preserved in Duchenne muscular dystrophy, which might accelerate muscle devastation in this disease.

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References

1. Kernell D.: The limits of firing frequency in cat lumbosacral motoneurons possessing different time course of afterhyperpolarization. Acta Physiol. Scand. 1965, 65, 87–100.
2. Basmajian J., Stecko G.: A new bipolar electrode for electromyography. J. Appl. Physiol. 1962, 17, 849.

3. Mazurkiewicz Ł., Piotrkiewicz M.: Computer system for identification and analysis of motor unit potential trains. Biocyber. Biomed. Eng. 2004, 24, 15–23.
4. Person R.S., Kudina L. P.: Discharge frequency and discharge pattern of human motor units during voluntary contraction of muscle. Electroencephalogr. Clin. Neurophysiol. 1972, 32, 471–483.
5. Person R.S.: Spinal mechanisms of muscle contraction control. In: Soviet scientific review, T.M. Turpaev (Ed.), Harwood Academic Publishers. 1992, 1–83.
6. Tokizane T., Shimazu H.: Functional differentiation of human skeletal muscle. Springfield: Charles C. Thomas, 1964.
7. Calvin W.H.: Three modes of repetitive firing and the role of threshold time course between spikes. Brain Res. 1974, 69, 341–346.
8. Matthews P.B.C.: Relationship of firing intervals of human motor units to the trajectory of post-spike after-hyperpolarization and synaptic noise. J. Physiol. (Lond) 1996, 492, 597–628.
9. Kudina L.P.: Analysis of firing behaviour of human motoneurones within 'subprimary range'. J. Physiol. (Paris) 1999, 93, 115–123.
10. Piotrkiewicz M.: An influence of afterhyperpolarization on the pattern of motoneuronal rhythmic activity. J. Physiol. (Paris) 1999, 93, 125–133.
11. Powers R.K., Binder M.D.: Relationship between the time course of the afterhyperpolarization and discharge variability in cat spinal motoneurones. J. Physiol. (Lond) 2000, 528, 131–150.
12. Piotrkiewicz M., Kudina L., Hausmanowa-Petrusewicz I., Zhoukovskaya N., Mierzejewska J.: Discharge properties and afterhyperpolarization of human motoneurons. Biocyber. Biomed. Eng. 2001, 21, 53–75.
13. Piotrkiewicz M., Kudina L., Mierzejewska J., Jakubiec M., Hausmanowa-Petrusewicz I.: Age-related change in duration of afterhyperpolarization of human motoneurones. J. Physiol. (Lond) 2007, 585, 483–490.
14. Vignos P.J.Jr., Archibald K.C.: Maintenance of ambulation in childhood muscular dystrophy. J. Chronic Dis. 1960, 12, 273–290.
15. Piotrkiewicz M., Hausmanowa-Petrusewicz I., Mierzejewska J.: Are motoneurons involved in muscular dystrophy? Clin. Neurophysiol. 1999, 110, 1111–1122.
16. Huizar P., Kuno M, Kudo N.Y.M.: Reaction of intact spinal motoneurons to partial denervation of the muscle. J. Physiol. (Lond) 1977, 265, 175–191.
17. Rasminsky M.: Physiological properties of dystrophic mouse spinal root axons. Electroencephalogr. Clin. Neurophysiol. Suppl 1982, 36, 99–105.
18. Scott O.M., Hyde S.A., Vrbova G., Dubowitz V.: Therapeutic possibilities of chronic low frequency electrical stimulation in children with Duchenne muscular dystrophy. J. Neurol. Sc. 1990, 95, 171–182.
19. Kim T.W., Wu K., Black I.B.: Deficiency of brain synaptic dystrophin in human Duchenne muscular dystrophy. Ann. Neurol. 1995, 38, 446–449.
20. Licursi V., Caiello I., Lombardi L., De Stefano M.E., Negri R., Paggi P.: Lack of dystrophin in mdx mice modulates the expression of genes involved in neuron survival and differentiation. Eur. J. Neurosc. 2012, 35, 691–701.
21. Bateson D.S., Parry D.J.: Motor units in a fast-twitch muscle of normal and dystrophic mice. J. Physiol. (Lond) 1983, 345, 515–523.
22. Scott O.M., Vrbova G., Hyde S.A., Dubowitz V.: Responses of muscles of patients with Duchenne muscular dystrophy to chronic electrical stimulation. J. Neurol. Neurosurg. Psychiatry 1986, 49, 1427–1434.
23. Webster C., Silberstein L., Hays A.P., Blau H.M.: Fast muscle fibers are preferentially affected in Duchenne muscular dystrophy. Cell 1988, 52, 503–513.
24. Hayes A., Lynch G.S., Williams D.A.: The effects of endurance exercise on dystrophic mdx mice. I. Contractile and histochemical properties of intact muscles. Proc. Biol. Sci. 1993, 253, 19–25.
25. Kernell D., Eerbeek O., Verhey B.A.: Relation between isometric force and stimulus rate in cat's hindlimb motor units of different twitch contraction time. Exp. Brain Res. 1983, 50, 220–227.

26. Piotrkiewicz M., Celichowski J.: Tetanic potentiation in motor units of rat medial gastrocnemius. Acta Neurobiol. Exp. 2007, 67, 35–42.
27. Vrbova G.: Duchenne dystrophy viewed as a disturbance of nerve-muscle interactions. Muscle Nerve 1983, 6, 671–675.
28. Piotrkiewicz M., Hausmanowa-Petrusewicz I., Mierzejewska J., Jakubiec M.: Motoneuron "fastness" in Amyotrophic Lateral Sclerosis, in: Lecture Notes of 68th ICB Seminar "Motoneurons and motoneuron pools", G. Vrbova, M. Piotrkiewicz, W. Zmysłowski (Eds) 2005, ICB, Warsaw, 69–76.
29. Morales F.R., Boxer P.A., Fung S.J., Chase M.H.: Basic electrophysiological properties of spinal cord motoneurons during old age in the cat. J. Neurophysiol. 1987, 58, 180–194.
30. Cameron W.E., Jodkowski J.S., Fang H., Guthrie R.D.: Electrophysiological properties of developing phrenic motoneurons in the cat. J. Neurophysiol. 1991, 65, 671–679.
31. Vandervoort A.A., McComas A.J.: Contractile changes in opposing muscles of the human ankle joint with aging. J. Appl. Physiol. 1986, 61, 361–367.
32. Skorjanc D., Traub I., Pette,D.: Identical responses of fast muscle to sustained activity by low-frequency stimulation in young and aging rats. J. Appl. Physiol. 1998, 85, 437–441.
33. Hook P., Sriramoju V., Larsson L.: Effects of aging on actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. Am. J. Physiol. Cell Physiol. 2001, 280, C782–788.
34. Kernell D., Bakels R., Copray J.C.: Discharge properties of motoneurones: How are they matched to the properties and use of their muscle units? J. Physiol. (Paris) 1999, 93, 87–96.
35. Brooks B.R.: El Escorial world federation of neurology criteria for the diagnosis of amyotrophic lateral sclerosis. J. Neurol. Sci. 1994, 124 Suppl, 96–107.
36. Holt G.R., Softky W.R., Koch C., Douglas R.J.: Comparison of discharge variability in vitro and in vivo in cat visual cortex neurons. J. Neurophysiol. 1996, 75, 1806–1814.
37. Zijdewind I., Thomas C.K.: Firing patterns of spontaneously active motor units in spinal cord-injured subjects. J. Physiol. 2012, 590, 1683–1897.
38. Eisen A., Schulzer M., MacNeil M., Pant B., Mak E.: Duration of amyotrophic lateral sclerosis is age dependent. Muscle Nerve 1993, 16, 27–32.
39. Czaplinski A., Yen A.A., Appel S.H.: Amyotrophic lateral sclerosis: Early predictors of prolonged survival. J. Neurol. 2006, 253, 1428–1436.
40. Schmied A., Attarian S.: Enhancement of single motor unit inhibitory responses to transcranial magnetic stimulation in amyotrophic lateral sclerosis. Exp. Brain Res. 2008, 189, 229–242.
41. Kohara N., [Abnormal hyperexcitability in ALS]. Rinsho Shinkeigaku 1999, 39, 61–64.
42. Kostera-Pruszczyk A., Niebroj-Dobosz I., Emeryk-Szajewska B., Karwanska A., Rowinska-Marcinska K.: Motor unit hyperexcitability in amyotrophic lateral sclerosis vs amino acids acting as neurotransmitters. Acta Neurol. Scand. 2002, 106, 34–38.
43. Kuo J.J., Schonewille M., Siddique T., Schults A.N., Fu R., Bar P.R., Anelli R., Heckman C.J., Kroese A.B.: Hyperexcitability of cultured spinal motoneurons from presymptomatic ALS mice. J. Neurophysiol. 2004, 91, 571–575.
44. Kuo J.J., Siddique T., Fu R., Heckman C.J.: Increased persistent na(+) current and its effect on excitability in motoneurones cultured from mutant SOD1 mice. J. Physiol. 2005, 563, 843–854.
45. Schutz B.: Imbalanced excitatory to inhibitory synaptic input precedes motor neuron degeneration in an animal model of amyotrophic lateral sclerosis. Neurobiol. Dis. 2005, 20, 131–140.
46. Avossa D., Grandolfo M., Mazzarol F., Zatta M., Ballerini L.: Early signs of motoneuron vulnerability in a disease model system: Characterization of transverse slice cultures of spinal cord isolated from embryonic ALS mice. Neuroscience 2006, 138, 1179–1194.
47. Bories C., Amendola J., Lamotte d'Incamps B., Durand J.: Early electrophysiological abnormalities in lumbar motoneurons in a transgenic mouse model of amyotrophic lateral sclerosis. Eur. J. Neurosc. 2007, 25, 451–459.
48. Quinlan K.A., Schuster J.E., Fu R., Siddique T., Heckman C. J.: Altered postnatal maturation of electrical properties in spinal motoneurons in a mouse model of amyotrophic lateral sclerosis. J. Physiol. (Lond) 2011, 589, 2245–2260.

49. Mohajeri M.H., Figlewicz D.A., Bohn M.C.: Selective loss of alpha motoneurons innervating the medial gastrocnemius muscle in a mouse model of amyotrophic lateral sclerosis. Exp. Neurol. 1998, 150, 329–336.
50. Frey D., Schneider C., Xu L., Borg J., Spooren W., Caroni P.: Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. J. Neurosci. 2000, 20, 2534–2542.
51. Hegedus J., Putman C.T., Tyreman N., Gordon T.: Preferential motor unit loss in the SOD1 G93A transgenic mouse model of amyotrophic lateral sclerosis. J. Physiol. (Lond) 2008, 586, 3337–3351.
52. Totosy de Zepetnek J.E., Zung H.V., Erdebil S., Gordon T.: Motor-unit categorization based on contractile and histochemical properties: A glycogen depletion analysis of normal and reinnervated rat tibialis anterior muscle. J. Neurophysiol. 1992, 67, 1404–1415.
53. Gordon T., Hegedus J., Tam S.L.: Adaptive and maladaptive motor axonal sprouting in aging and motoneuron disease. Neurol. Res. 2004, 26, 174–185.
54. Piotrkiewicz M.: Modelling of motoneuronal rhythmic activity. Biocyber. Biomed. Eng. 2001, 21, 53–75.
55. Meehan C.F., Moldovan, M., Marklund S.L., Graffmo K.S., Nielsen J.B., Hultborn H.: Intrinsic properties of lumbar motor neurones in the adult G127insTGGG superoxide dismutase-1 mutant mouse in vivo: Evidence for increased persistent inward currents. Acta Physiol. 2010, 200, 361–376.
56. Piotrkiewicz M., Hausmanowa-Petrusewicz I.: Motoneuron afterhyperpolarisation duration in amyotrophic lateral sclerosis. J. Physiol. (Lond) 2011, 589, 2745–2754.
57. Young J.L., Mayer R.F.: Physiological alterations of motor units in hemiplegia. J. Neurol. Sci. 1982, 54, 401–412.
58. Gemperline J., Allen S., Walk D., Rymer W.: Characteristics of motor unit discharge in subjects with hemiparesis. Muscle Nerve 1995, 18, 1101–1114.
59. Frontera W.R., Grimby L., Larsson L.: Firing rate of the lower motoneuron and contractile properties of its muscle fibers after upper motoneuron lesion in man. Muscle Nerve 1997, 20, 938–947.
60. Andreassen S., Rosenfalck A.: Impaired regulation of the firing pattern of single motor units. Muscle Nerve 1978, 1, 416–418.
61. Sun T.Y., Chen J.J., Lin T.S.: Analysis of motor unit firing patterns in patients with central or peripheral lesions using singular-value decomposition. Muscle Nerve 2000, 23, 1057–1068.
62. Liang L.-Y., Chen J.-J.J., Wang Y.-L., Jakubiec M., Mierzejewska J., Piotrkiewicz M.: Changes in spinal motoneuron "fastness" in post-stroke spastic patients. J. Med. Biol. Eng. 2010, 30, 17–22.
63. Marsden C.D., Meadows J.C., Merton P.A.: "Muscular wisdom" that minimizes fatigue during prolonged effort in man: Peak rates of motoneuron discharge and slowing of discharge during fatigue. Adv. Neurol. 1983, 39, 169–211.