Org.2766 Improves Functional and Electrophysiological Aspects of Regenerating Sciatic Nerve in the Rat

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DE KONING, P. AND W. H. GISPEN. *Org.2766 improves functional and electrophysiological aspects of regenerating sciatic nerve in the rat.* PEPTIDES 8(3) 415-422, 1987.—The beneficial effect of short-term (8 days) melanocortin therapy on regenerating peripheral nerves is demonstrated using functional and electrophysiological tests. Following a crush lesion of the rat sciatic nerve, recovery of sensory function is monitored by assessing the responsiveness of the rat to a small electric current applied to the footsole. Recovery of motor function is assessed by means of an analysis of walking patterns. Normalization of the walking pattern reflects reinnervation of different muscle groups. The motor and H-reflex related sensory nerve conduction velocity of the regenerated nerves are longitudinally investigated in the same rats in which the recovery of motor and sensory function had been assessed previously. Functional tests show an enhanced recovery under melanocortin therapy, but in the end both saline- and melanocortin-treated rats show 100% recovery. However, when compared to the contralateral sciatic nerve, in the peptide-treated animals motor nerve conduction in the regenerated nerves has fully recovered after about 90 days following the crush lesion and the sensory conduction after about 120 days, whereas in the saline-treated rats a deficit of 20-40% in both motor and sensory conduction remains. This difference is observed even 214 days following crush.

Sciatic nerve Regeneration Sensory function Motor function Electrophysiology Melanocortins

MELANOCORTINS (peptides derived from ACTH/MSH) exert trophic influences on the central and peripheral nervous system in the rat [8,29]. The trophic influence is apparent during development and regeneration of nervous tissue, i.e. the two situations in which neural plasticity is most expressed [1-3, 9, 15, 26, 30]. Administered during the early postnatal period, melanocortins enhance the maturation of the central nervous system, delay the onset of muscle fatigue upon repetitive nerve stimulation and increase distal nerve sprouting [1, 26, 30, 33]. Several endocrine inert fragments and analogs of ACTH/MSH have been shown to reduce the recovery period of sensory function after sciatic nerve crush or cut in the rat and to improve motor unit reorganization [2, 3, 9, 23, 28]. In order to exert their beneficial effects melanocortins must be administered during the early phase of regeneration [12]. Furthermore, it has been repeatedly shown that this facilitation of the recovery process is highly dose dependent. The inverted U-shaped dose response curve necessitates testing of a wide range of doses [2,9].

The assessment of functional recovery after peripheral nerve crush or cut in the rat by means of tests that rely upon sensory parameters indicate an enhanced recovery as a result of melanocortin treatment [2, 9, 28]. At the end of such experiments, the peptide-treated group and their saline-treated controls are indistinguishable by the test procedures applied, pointing to a 100% recovery in both groups. On the

other hand, histological studies show an increased number of regenerated nerve fibers in melanocortin-treated rats even 96 days following crush [4]. Saint-Côme and Strand [23] show that as a result of ACTH4-10 treatment more and smaller motor units are formed after a crush lesion of the rat sciatic nerve. These observations suggest that melanocortin treatment results in lasting beneficial differences in the regenerated nerves that cannot be picked up by the sensory function tests. We therefore decided to investigate whether Org.2766 (a trisubstituted ACTH4-9 analog) could influence the performance of rats with a crush lesion in the sciatic nerve in a motor function test. In this test the walking pattern of a rat is analyzed at fixed time points following the crush lesion [10]. Since the walking pattern reflects the coordinated use of different muscle groups, we felt that this function test might give insight into the qualitative function of the regenerated nerve.

A different qualitative aspect of nerve function is the nerve conduction velocity, which is one of the most thoroughly studied functions of regenerating nerves. The results of these studies are contradictory in that some authors state that nerve conduction velocity fully recovers about 150 days following nerve crush [11,24], while others find only 76% [7] or 60% [14] recovery. The differences can partly be explained by the fact that control measurements have not been made on the healthy contralateral side in the same animals,

$$SFI = \left(\frac{ETO - NTO}{NTO} + \frac{NPL - EPL}{EPL} + \frac{ETS - NTS}{NTS} + \frac{EIT - NIT}{NIT} \right) 46$$

$$FP = \left(\frac{NPL - EPL}{EPL} \right) 169$$

$$TS = \left(\frac{ETS - NTS}{NTS} + \frac{EIT - NIT}{NIT} \right) 100$$

FIG. 1. The upper part of the figure shows the walking pattern of a normal rat. Indicated are the different parameters that can be measured from this walking pattern. NTOF: orthogonal distance between the tip of the normal foot and the tip of the following contralateral toes (normal to opposite foot); ETOF: orthogonal distance between the tip of the foot on the experimental side (crushed side) and the tip of the following contralateral foot (experimental to opposite foot); NPL, EPL: length of the footprint on the normal (normal print length) and experimental side (experimental print length); NTS, ETS: linear distance between the first and fifth digit on the normal (normal toe spreading) and experimental side (experimental toe spreading); NIT, EIT: linear distance between the second and fourth digit on the normal (normal inter toe spreading) and experimental side (experimental inter toe spreading). The lower part of the figure shows the empirically derived formulas based on these parameters. SFI denotes sciatic functional index and gives an overall index of the motor function of the experimental (crushed) leg as a percentage of that of the contralateral (normal) leg. The factor 46 sets the SFI at 100% immediately following crush. FP denotes footprint and expresses the length of the footprint on the crushed side as a percentage of that on the contralateral side. Likewise TS denotes toe spreading which expresses the toe spreading between first and fifth digit, and second and fourth digit as a percentage of that on the contralateral side.

but in a group of younger animals and the fact that different experimental procedures were used (*in vivo*, *in vitro*) as well as different animals (rats, cats, rabbits). In normal nerves, conduction velocity correlates with fiber diameter [20]; this holds also for regenerated nerves [11]. By using needle stimulation electrodes and recording the muscle action potential from the small foot muscles we have studied the recovery of motor and H-reflex related sensory nerve conduction velocity of regenerating sciatic nerves in rats that were treated with Org.2766 or their saline-treated controls. This method offers the possibility of repeated measurements in the same animals and comparing the conduction velocity of the regenerating sciatic nerve to that of the healthy contralateral nerve in the same animal [27].

Short-term melanocortin treatment following nerve crush resulted in an improved performance of rats in the motor function test and an enhanced sensory recovery. In some experiments both functional and electrophysiological examinations were performed successively in the same group of animals. This was done to underscore the observation that short-term melanocortin therapy after a crush lesion gives rise to a long-lasting difference in the conduction velocity of regenerated sciatic nerves of melanocortin-treated rats as compared to saline-treated rats even after functional tests show full recovery in both groups.

METHOD

Animals

Female Wistar rats of an inbred strain (TNO, Zeist, NL) weighing approximately 120–140 g at the onset of the experiments were used. Rats were housed in groups of 5 per Makrolon cage (RUCO, NL) on sawdust and had access to com-

mercial rat chow and water ad lib. They were maintained on a 12 hr dark-light cycle with the lights on beginning at 7:30 a.m.

Peptides

Org.2766, a degradation-resistant ACTH4–9 analog (H-Met(O₂)-Glu-His-Phe-D-Lys-Phe-OH) is dissolved in 0.1% BSA/0.05 N HCl and diluted with a 0.9% NaCl solution to a final concentration of either 1 or 10 µg/0.5 ml solution. (The peptide was a gift from Organon Int. BV, Oss, NL).

Anaesthetics

All operations and electrophysiological examinations were carried out under general anaesthesia using Hypnorm® (Philips-Duphar, NL) containing fluanisone 10 mg/ml and phentanyl citrate 0.2 mg/ml, dose 0.8 ml/kg body weight, administered subcutaneously.

Surgery

In all experiments a crush lesion was placed in the right sciatic nerve in the upper leg. The surgical procedure and reproducibility of the crush lesion have recently been described [9].

Functional Tests

Motor function. An index of sciatic nerve function (SFI) was calculated from the walking pattern of rats. This test was originally described by De Medinacelli [10]. In short, after dipping the hindfeet in photographic developer (Eukobrom, Tetenal, FRG) rats are made to walk over photographic paper (Ilford, 2.24 M, semi-matt), which lies on the bottom

of a 50 cm long confined walkway at the end of which a dark box is placed. The corridor has a slight inclination of about 10° with the horizontal plane. Footprints show up immediately after the rat has passed the corridor. From these walking patterns different parameters can be measured (see Fig. 1). (1) Distance between opposite feet. This value is measured orthogonally from the tip of the normal foot to the tip of the following contralateral toes (NTOF: normal to opposite foot). The value between the tip of the footprint at the crushed side to the tip of the following foot on the normal side is called ETOF (experimental to opposite foot). (2) Print length. The length of the longest footprint on both the normal (NPL) and the crushed side (EPL) was measured. (3) Toe spreading. The linear distance between the first and fifth toe (NTS and ETS) and between second and fourth toe (NIT, EIT) was measured on the normal and crushed side.

Entering of these four variables from each side into an empirically derived formula yields an index of motor function: the sciatic functional index (SFI). For a description of this formula and arbitrary values in case no measures can be made on the experimental side see De Medinacelli [10]. Besides using the total formula, we have also measured the normalization of the individual parameters which together make up the SFI. Normalization of the print length (PL) is considered to correlate with reinnervation of the calf muscles, whereas normalization of the toe spreading (TS) reflects reinnervation of the small muscles of the foot.

Sensory function. Sensitivity to local noxious stimulation of the footsole was assessed using a slightly modified version of the test procedure described recently [9]. In short, in this test a small electric current is applied to the footsole through two stimulation poles. A normal rat invariably retracts his paw instantaneously as soon as the skin of the footsole closes the electric circuit. In the original description of the test method we used only one strength of current for testing (0.1 or 0.2 mA). We now test a range of strengths of current: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mA and assess the lowest strength of current at which a rat retracts his paw from the stimulus on a certain day. A rat is considered fully recovered (100% recovery) when it retracts his paw on a stimulus of 0.1 mA whereas absent retraction on a stimulation strength of 0.6 mA indicates complete absence of sensitivity to a noxious stimulus (0% recovery). Withdrawal upon one of the intermediate strengths of current is scored as incomplete recovery e.g., when the lowest strength of current upon which the rat retracts his paw is 0.2 mA, this rat is considered to have a 84% recovery of sensory function. Likewise, reaction to 0.3 mA indicates 67% recovery, 0.4 mA 50%, 0.5 mA 33% and 0.6 mA 17%, respectively. Using this modification of the originally described test, the recovery of sensory function can now be graded which also allows more sensitive statistical analysis.

Electrophysiology

Upon stimulation of the mixed sciatic or tibial nerve, two

FIG. 2. Indicated is the recovery of motor function following a crush lesion of the sciatic nerve based on the analysis of walking patterns. A: the recovery of the overall index of sciatic nerve function (SFI) of Org.2766 treated rats (○) and saline-treated rats (●). Indicated are the mean percentages of each groups $\pm$ SEM, both groups $n=10$. B: the recovery of the footprint parameter (FP) in the same groups of rats as in A. C: the recovery of the toe spreading index (TS) again in the same groups of rats as in A.

responses can be recorded from the small muscles of the foot by means of surface electrodes. At first, the M-response which is a direct motor response with short latency due to stimulation of the α -motor fibers. The H-reflex is an indirect response with long latency due to stimulation of the afferent proprioceptive I^a-fibers, which in the spinal cord monosynaptically excite α -motor neurons. The sciatic and tibial nerve were stimulated at the sciatic notch and ankle, respectively, by means of monopolar needle electrodes. The stimulus was a 0.1 msec square wave pulse of supramaximal strength (14 V) delivered by a Grass S8 stimulator through a Grass stimulus isolation unit. After amplification the signals were recorded on a VUCO VKS 22-16 digital storage oscilloscope. The latencies of the M- and H-response were measured as the time between stimulus and the beginning of the deflection of the M- or H-response. The motor and H-reflex related sensory nerve conduction velocity were calculated from the following formulas:

(1) Motor conduction velocity = distance (sciatic notch – ankle)/latency (M-response sciatic notch – M-response ankle).

(2) H-reflex related sensory nerve conduction velocity = distance (sciatic notch – ankle)/latency (H-reflex ankle – H-reflex sciatic notch).

Using this method, groups of rats were longitudinally screened for recovery of nerve conduction across the crush and in the regenerated nerve. The contralateral sciatic nerve of the same animal served as control value and the condition velocity in the crushed nerve was expressed as a percentage of the conduction velocity at the healthy contralateral side. In normal rats the conduction velocity on one side as determined with this method is within 80–120% of the contralateral side. In the same way the distal motor latency on the crushed side was expressed as a percentage of that on the control side. The distal motor latency reflects the conduction in the distal axon branches and neuromuscular synapse [27,31].

Experimental Design

For reasons of clarity the results of one experiment in which the functional (sensory and motor) and electrophysiological tests were successively carried out in the same groups of animals will be more extensively discussed. When necessary, results of separate functional or electrophysiological experiments will be given. In twenty rats a crush lesion was placed in the right sciatic nerve. Immediately after the operation 10 rats received an injection of 10 μ g Org.2766 SC, whereas the other 10 received 0.5 ml saline SC. At 48 hr interval (post-operative day 2, 4, 6 and 8) the peptide-treated animals received an injection of 1 μ g Org.2766 SC and the control rats received 0.5 ml saline SC following the same schedule. Throughout the experiments, the experimenter was unaware of the particular treatment the rats had received, the treatment code was broken after the data had been statistically analyzed.

Statistics

All data were analyzed using an analysis of variance with repeated measures, followed by a supplemental *t*-test.

RESULTS

Motor Recovery

The overall index of sciatic nerve function (SFI) calcu-

FIG. 3. Recovery of sensitivity to noxious stimulation of the foot-sole following a crush lesion of the sciatic nerve as measured by the local electric footsole stimulation test. Indicated are the mean percentages recovery of the Org.2766-treated rats ($\pm$ SEM) and of the saline-treated controls ($\pm$ SEM). Both groups $n=10$.

lated from the walking patterns shows an enhanced recovery in the Org.2766-treated animals ($F=5.3$, $p<0.05$) (Fig. 2A). On day 24 following crush, the SFI in both groups was within normal limits. The first parameter to show recovery was the print length (PL), indicating reinnervation of the calf muscles. The Org.2766-treated animals show an enhanced normalisation of this parameter over their saline-treated controls (Fig. 2B). An enhanced normalisation is also observed for the toe spreading parameter (TS), which indicates reinnervation of the small foot muscles (Fig. 2C).

Sensory Recovery

Measurements on the third day after operation indicated that in *all* rats sensitivity to noxious stimulation of the foot-sole on the crushed side was absent. From day 14 through 21 the rats were tested each day for recovery of sensibility. The lowest strength of current to which they reacted by withdrawing their paw was assessed and in this way the percentage recovery was determined. The results of these measurements are shown in Fig. 3, in which the mean percentage recovery for each group on a certain test day is indicated ($\pm$ SEM). As can be seen recovery in the peptide-treated animals is enhanced ($F=12.4$, $p<0.005$). As determined by this test, both groups are fully recovered by day 20 after crush. In another experiment, it was observed that prolonged treatment with Org.2766, that is, continued administration every other day till full recovery, did not further enhance recovery as compared to treatment during the first 8 days after crush. In one experiment, the effect of only one injection of 10 μ g Org.2766 on the day of operation was studied, but no beneficial effects on the recovery were observed.

Electrophysiology

From day 32 following sciatic nerve crush, M-responses could be reliably detected. The amplitudes were less than 10% of those on the contralateral side and the responses showed a large dispersion. In the group of rats that had been treated with Org.2766 for 8 days following the crush the

FIG. 4. A: Recovery of motor conduction velocity in the crushed sciatic nerve of rats that were treated with either Org.2766 (○) or saline (●). The conduction velocity in the crushed nerve is expressed as a percentage of that in the contralateral nerve of the same rat. Indicated are mean percentages recovery of the groups $\pm$ SEM. Both groups $n=10$. CL denotes control level that is the limit within which the conduction velocity in a normal sciatic nerve is, when expressed as a percentage of that on the contralateral side. B: recovery of the H-reflex related sensory nerve conduction velocity in the same groups of rats as in A. C: recovery of the distal motor latency that is the latency of the M-response after stimulation of the tibial nerve at the ankle. The latency is expressed as a percentage of that on the contralateral side. Same groups as in A.

mean motor conduction velocity was 62% of the contralateral side, whereas in the saline-treated group it was only 45% ($p < 0.01$) (Fig. 4A). At post-operation day 94 the motor nerve conduction velocity (MNCV) in the Org.2766-treated animals was within normal limits (80–120% of contralateral side). In saline-treated animals the motor conduction velocity did not reach normal values even 214 days following crush. Based on the levelling-off of the curves further improvement seemed unlikely. Throughout the observation period the two groups differed significantly ($F = 72$, $p < 0.001$) and also at the last test point the difference in motor conduction velocity was very significant ($p < 0.001$).

The distal motor latency (latency of the M-response after stimulation at the ankle), represents the conduction in the most distal part of the motor nerve fibers and the neuromuscular synapse. This value did not differ significantly between the two groups and was within normal limits 214 days following crush (Fig. 4C).

H-responses could be reliably detected from day 62 following crush onwards. At this time in the Org.2766-treated rats the mean H-reflex related sensory nerve conduction velocity (H-SNCV) was 64% of the contralateral side, whereas it was only 51% in the saline-treated rats ($p < 0.02$). At day 158 following crush the H-SNCV in the Org.2766-treated rats was within normal limits, whereas a large deficit remained in the saline-treated animals even 214 days following crush. The groups differed significantly throughout the observation period ($F = 54$, $p < 0.001$) and also at the last test point ($p < 0.001$) (Fig. 4B). The large dispersion of the M- and H-response at the first test day following the crush gradually disappeared during the follow-up period although the responses appeared to remain somewhat broader as compared to the contralateral side. We have not quantitated this observation.

DISCUSSION

This is the first time that Org.2766 is reported to enhance normalization of the walking pattern of rats following a crush lesion of the sciatic nerve. Since the walking pattern reflects the coordinated use of muscle groups, this once more stresses the fact that the enhanced recovery as a result of melanocortin therapy is of functional significance. In their studies on the effects of ACTH1–39 on recovery of neuromuscular function following crushing of peripheral nerve, Strand and Kung [28] report an enhanced return of normal movement by means of an unexplained method and an enhanced recovery of toe spreading as determined by the method originally described by Gutmann [17] and Hasegawa [18].

The presently reported experiments measuring sensory recovery by means of the local electric footsole stimulation test demonstrate an enhanced recovery under melanocortin therapy. They underscore earlier observations that melanocortin therapy is most effective when installed early after nerve damage during a period up to 8 days after the damage [12]. Prolonged administration of Org.2766, until functional recovery is completed, does not further shorten the recovery period, whereas only one injection of 10 μ g Org.2766 is ineffective in promoting recovery. Earlier reports by our group have shown lower and higher dosages than 1 or 10 μ g Org.2766 to be not effective in enhancing sensory recovery [2,9]. Strand and Kung [28] conclude from their studies in ACTH-treated adrenalectomized rats that more rapid outgrowth of the regenerating axons is in part

responsible for the enhanced return of function. However, our earlier studies using functional tests and histology indicated an influence of melanocortins on the initial sprouting response rather than on the rate of outgrowth of the newly formed sprouts [9,34]. These different observations might be explained partly by the fact that Strand and Kung used the whole ACTH1–39 molecule whereas we used the ACTH4–9 analog Org.2766. It might be possible that the whole ACTH molecule is needed for the elongation of the nerve sprouts. Analysis of the walking pattern of rats following sciatic nerve crush also demonstrates the influence of Org.2766 therapy on the initial regeneration process. The first parameter to show recovery is the print length, due to reinnervation of the calf muscles. The toe spreading parameter shows recovery as a result of functional reinnervation of the small muscles of the foot, which occurs later in time due to the longer distance these reinnervating sprouts have to travel. If melanocortins would have influenced the rate of outgrowth of the sprouts, then the enhancement of recovery as determined by the toe spreading parameter should have been larger than the enhancement of recovery of the print length parameter. As can be seen in Fig. 2B and 2C, this is not true.

Edwards *et al.* [13] hypothesized that early melanocortin therapy is effective in enhancing recovery from nerve damage, because the melanocortins would mimic the sprout-initiating actions of a MSH-like peptide formed in the degenerating distal nerve stump by specific proteolysis of the 150 kDa neurofilament protein. This may explain why enhancement of recovery is limited to a couple of days, as that is the time needed by untreated rats to form the endogenous MSH-like peptide. Immediate exogenous peptide treatment possibly overcomes this delay in the sprouting process.

The question as to which factors cause the observed difference between nerve conduction velocities of rats with short-term Org.2766 therapy following nerve crush and conduction velocities of saline-treated controls must remain largely unanswered at the moment. The factors that influence nerve conduction velocity are: fiber diameter, myelin thickness, internode distance, 'nodal, internodal and axoplasm parameters,' and temperature [35]. In normal nerves fiber diameter correlates positively with nerve conduction velocity [20]. Devor and Govrin-Lippmann concluded from their studies that this correlation also holds for regenerated nerve fibers [11]. Light and electron microscopical observations indicate that as a result of melanocortin therapy following a crush lesion, more nerve fibers regenerate which on the mean are thinner than in saline-treated rats, although the thickest nerve fibers are also found in the melanocortin-treated rats [4]. Cragg and Thomas [7] observed a lasting smaller fiber diameter (–11%) in peroneal nerves of rabbits following nerve crush. Nerve conduction velocity measurements rely on the beginning of the deflection of the M- or H-response for the determination of latencies. This initial deflection represents the contribution of the fastest conducting nerve fibers and since these also in regenerated nerves are the thickest, this might partly explain the higher nerve conduction velocity in melanocortin-treated animals. When the difference in diameter between the thickest nerve fibers in regenerated nerves of melanocortin- or saline-treated rats is transferred with the formula of Hursh to a difference in conduction velocity it can be seen that the difference in fiber diameter accounts for only 20% of the observed difference [20]. So the difference in fiber diameter cannot be held solely responsible for the observed difference in conduction veloc-

ity. Following nerve regeneration the ratio between myelin thickness and total fiber diameter is decreased due to the thinner myelin sheath [25]. Electron microscopical studies have shown that melanocortins do not influence myelin thickness of regenerated fibers following nerve crush [4].

The distance between nodes of Ranvier is decreased in regenerated nerves. It is not known whether melanocortins influence this parameter but it seems unlikely that it is of much importance in explaining the observed difference in conduction velocity since in the range of internodal distances of normal and remyelinated axons this distance has little influence on the conduction velocity [5,24]. A last possible explanation for the observed difference in conduction velocity is a melanocortin-induced change in internodal, nodal and axoplasm parameters. The influence of these parameters upon nerve conduction velocity has not been studied extensively, but there are studies indicating that especially myelin capacitance and axoplasm conductance are of more significance than nodal disturbances per se [22]. It is, however, unknown whether short-term melanocortin therapy brings about long-term structural changes in regenerated nervous membranes, axoplasm or myelin which then influence nerve conduction. Nerve conduction in the non-crushed contralateral sciatic nerves was the same in the Org.2766 and saline-treated rats indicating that Org.2766 does not influence nerve conduction in healthy nerves. The observation that the distal motor latency did not differ significantly between Org.2766 and saline-treated rats makes it unlikely that Org.2766 has much influence on the neuromuscular synapse. The different extent to which the conduction velocity recovers in motor and sensory nerves in saline-treated animals (73 and 62%, respectively) is difficult to explain. Following nerve section with prevention of reinnervation, Hoffer *et al.* [19] observed a more marked atrophy in sensory fibers than in motor fibers. However, their conditions differ markedly from those in our experiments and the difference was only observed

when reinnervation had been prevented for more than 45 days.

In conclusion, the results of the primarily motor sciatic functional index, the primarily sensory local electric footsole stimulation test and of the nerve conduction measurements indicate that Org.2766 influences regeneration of both sensory and motor nerve fibers. Histological and electrophysiological studies indicate that melanocortins not only influence the time course of regeneration, but also induce changes that persist after reinnervation has been completed. At day 96 following nerve crush there was still a significantly greater number of myelinated nerve fibers in the tibial nerve due to melanocortin therapy [3]. Nerve conduction measurements show a long-lasting difference between nerve conduction velocities in regenerated nerves of Org.2766 or saline-treated animals. These differences are observed when functional tests no longer show any differences between melanocortin- or saline-treated animals. Also the formation of more and smaller motor units as a result of ACTH4-10 therapy following nerve crush indicates a lasting difference as compared to saline-treated controls [23]. As to the functional consequence of the observed lasting histological and electrophysiological differences following melanocortin therapy we can only speculate. During aging motor neurons are lost, which is compensated for by collateral sprouting from residual nerve fibers [6]. This compensatory mechanism will be exhausted sooner when fewer nerve fibers have regenerated after nerve damage. Furthermore, full restoration of nerve conduction velocity might indicate more complete recovery on the basis of which regeneration following repeated nerve damage might be more successful.

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