

Effect of exercise training on acute (crush lesion) and chronic (diabetes mellitus) peripheral neuropathy in the rat

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Abstract

The effect of moderate exercise training on acute and chronic neuropathy in two separate experiments was examined. Acute nerve dysfunction was induced by sciatic nerve crush lesion and chronic neuropathy by streptozotocin-induced diabetes mellitus (experimental diabetic neuropathy; EDN). Moderate exercise training was achieved by placing food and water, separately, at either end of a U-shaped tubular home cage (8 m). Recovery from the crush lesion and the development of EDN were monitored by evaluating the free walking pattern and nerve conduction velocity (NCV), respectively.

In the acute neuropathy model, 24 days of exercise after the crush lesion resulted in an enhanced return of motor function in the early phase of recovery ($P < 0.01$) and an increased sensory NCV after 250 days in the late phase ($P < 0.001$). Diabetic rats benefited from this exercise training by showing fewer signs of EDN, as evidenced by a superior motor function (toespreiding, calculated from the free walking pattern; $P < 0.05$) and an improvement in both motor and sensory NCV (both $P < 0.05$).

We conclude that moderate exercise training is effective in enhancing recovery from acute peripheral neuropathy and in ameliorating the consequences of experimental chronic neuropathy in diabetic rats.

Keywords: Crush lesion; Diabetes mellitus; Diabetic neuropathy; Electrophysiology; Exercise training; Nerve; Rat; Regeneration

1. Introduction

Peripheral nerve abnormalities usually lead to autonomic and sensorimotor impairments, and concomitant functional limitations. In the case of an acute lesion, such as a crush lesion, the axons are damaged but their endoneurial sheaths are preserved. The latter guide sprouted growth cones towards their target organs. Consequently axonal regeneration and functional recovery from this type of transient injury is fairly good

[30,39]. However, when the nerve is chronically compromised, as for instance in diabetes mellitus, by a combination of neurovascular insufficiency and neurochemical abnormalities [3,22,48], the negative consequences gradually increase. Symptoms like muscle atrophy and weakness (diabetic myopathy), paraesthesia, and pain are considered the direct consequences of chronic diabetic neuropathy. These impairments eventually lead to functional disorders that limit patients in their daily life [40].

Considerable pharmacotherapeutic research is focused on accelerating regeneration after acute neuronal lesions [11,29,37] and reducing the negative effects of chronic neuropathology, for instance diabetic neuropathy.

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thy [6,41]. Despite this effort, no decisive therapeutic breakthrough has been achieved until now.

Recent studies from our laboratory revealed that physical exercise training has beneficial effects on the rate and quality of recovery of nerve function in a model of acute neuropathology [45,47]. On the basis of these results, it seemed appropriate to study the effect of exercise training in a chronic neuropathy model. For this we chose the streptozotocin (STZ)-induced diabetic rat model. This well-documented model proved excellent for the longitudinal evaluation of functional locomotor behavior [12,13] and the effectiveness of therapeutic interventions in experimental diabetic neuropathy (EDN) [4,6,7,9,13,26–28,32,43,44].

Previously used training paradigms, such as running in a treadmill device or swimming, were considered inadequate because of their ineffectiveness in increasing functional recovery after sciatic nerve lesion in rats (Van Meeteren, unpublished observations), and because of the stress these physical exercise strategies may induce in the rats. The animals are motivated to make a physical effort in order to avoid negative reinforcement; however, stress is known to compromise functional recovery in acute neuropathy [46] and to elevate plasma glucocorticosteroid levels [2], which are known to cause muscle wasting [49]. Secondly, we considered our own drink-training model [45] as less suited for this experiment, as diabetic rats need to drink large volumes of water, which could result in overtraining. Consequently, we developed a new training device, a 'U'-shaped home cage with water and food 8 m apart. By doing so, we preserved our theoretical premise of inducing physical exertion by way of positive reinforcement.

It was the aim of the present study to evaluate the effect of exercise training in the 'U'-shaped home cage on the recovery of functional and electrophysiological variables in acute nerve injury (sciatic nerve crush) and in a chronic neuropathy model (STZ-induced diabetes mellitus).

2. Materials and methods

2.1. Experimental designs

2.1.1. Acute neuropathy

Twenty male rats (140–160 g) were obtained from our own bred Wistar RMI colony (originally obtained from CPB-TNO, Zeist, Netherlands). The rats were kept under a 12-h light/dark cycle. All rats were habituated to the U-shaped tubular home cage (8 m) and to the measurement of walking pattern, 4 days prior to injury of the sciatic nerve.

The sciatic nerve of the right paw was crushed. This standardized method yields a reproducible sensorimotor deficit in the involved paw and has been extensively

described by De Koning et al. [10]. In short, under Hypnorm® (Janssen Pharmaceutica, Tilburg, Netherlands) anaesthesia (0.8 ml kg^{-1}), an incision was made at the thigh. The sciatic nerve was carefully exposed and subsequently crushed with haemostatic forceps for 30 s at a point immediately distal from where it emerges from under the gluteus maximus muscle. The day of the crush was termed day 0.

The rats were randomly assigned to the training or the control group and were allowed to regain consciousness. The control rats (CONTR) were housed in standard Makrolon cages, three or four per cage. The training rats (TRAIN) were immediately housed in the U-shaped home cage (see training device).

2.1.2. Chronic neuropathy

Thirty-three male rats (220–240 g; age 11–12 weeks) were obtained from an inbred Wistar strain (RMI bred, originally obtained from TNO, Zeist, Netherlands) and were housed on sawdust in Makrolon cages (two rats per cage), with free access to food and water. The rats were subjected to a 12-h light/dark cycle.

Subsequently, experimental diabetes mellitus was induced in 24 animals by intravenous injection of streptozotocin (STZ; 40 mg/kg body weight; Zanosar, Upjohn, Kalamazoo, Michigan, USA). STZ obliterates β -cells in the islets of Langerhans [33], causing diabetes mellitus and concomitant chronic hyperglycaemia and severe neuropathy [26,27,31]. The week of STZ injection was termed week 0.

The diabetic rats were divided at random, 6 weeks after the STZ injection, into two groups: a training group (train; $n = 12$) and a diabetic control group (contr; $n = 12$). The twelve train rats were housed in the U-shaped tubular home cage (see training device), and their contr counterparts in standard Makrolon cages (two per cage).

As STZ causes a reduction of body weight, a group of nine age-matched weight control rats (DIET group) were used in this study. These animals had near-normal maturation of their nerves, as reflected by a regular gain of NCVs [8,38]. Rats were housed in Makrolon cages (three per cage). These weight-matched controls were kept on a strict diet (13–15 g chow per rat daily) in order to keep their mean body weights similar to those of the diabetic rats.

2.2. Training device

As other used training devices were considered inappropriate (see Section 1), we built a new device, in which physical exertion was rewarded with food and water. A U-shaped home cage was constructed out of four Makrolon cages, provided with sawdust and situated in a long rectangle and connected by a 12-cm-diameter tube. The total length of the home cage was 8

m. Rat chow pellets were placed at one end of the Makrolon cage, and bottles of water at the opposite end. Thus the rats were forced to walk in order to get food and water.

2.3. Measurements

2.3.1. Body weight measurements

The rats were weighed daily during the early phase of recovery, when training was started, and weekly after the training period (Experiment 1). In Experiment 2 body weight was measured weekly, in order to monitor weight loss, as STZ-induced diabetes mellitus is known to result in a striking impairment of the normal gain of body weight in maturing rats [26–28].

2.3.2. Motor function test

The return of motor function was monitored by analysis of the free walking pattern. This method was originally described by De Medinacelli et al. [14] and calculations were modified by De Koning and Gispen [11]. The test procedure is as follows. After both hind feet of the rats were dipped in photographic developer (Eukobrom, Tetenal, Germany), the animals were trained to walk over strips of photographic paper (Ilford, 2.24 m, semi matt) covering the bottom of a 50 cm long, 6.4 cm wide, inclining (10°) corridor ending in a dark box. The rat's footprints became visible within a few seconds. Analysis of spatial aspects of the walking patterns, using a computer program, enables the examiner to monitor intra- and inter-individual differences in the spontaneous motor function of sciatic nerve crush lesioned rats, as well as rats suffering from diabetic neuropathy [11–13].

2.3.3. Sensory function test

The return of sensory function was measured by the foot reflex withdrawal test (in Experiment 1 only). The rat was gently immobilized by hand and the sole of its foot was presented to the examiner. A small electric current was applied to the sole of the foot by using two stimulation electrodes. Six current strengths were tested, ranging from 0.1 to 0.6 mA. Rats with an intact innervation will instantaneously retract their paw upon skin contact with the electrodes. Rats subjected to sciatic nerve crush initially remain motionless. A lack of reaction to a 0.6 mA stimulus indicated no recovery. As reinnervation proceeds, the reflex is restored. Rats that reacted to a 0.1 mA current were considered completely recovered. The lowest current strength that provoked the reflex was registered daily, starting from postoperative day 16.

2.3.4. Electrophysiology

We used an electrophysiological test procedure as described in detail by De Koning and Gispen [11] to

monitor the recovery of nerve conduction velocities. In short, under general anaesthesia (Hypnorm®, Janssen Pharmaceutica, Tilburg, Netherlands: containing fluanisonem 10 mg/ml and fentanyl citrate 0.2 mg/ml; dose: 0.8 ml/kg body weight, injected subcutaneously) monopolar needle electrodes were inserted to stimulate the tibial nerve at the ankle and subsequently the sciatic nerve at the notch (stimulus: 500 μ s unipolar pulse, 42 V, 1 mA, generated by a Neurolog NL 300 pulse generator; the anode was inserted 5 mm proximal to the cathode). Responses to stimulation of these mixed peripheral nerves were recorded from the intrinsic foot muscles by surface electrodes (Nicolet, 1 mm diameter). The latency of the M-response and the H-response, caused by excitation of α -motor fibres directly and indirectly via monosynaptic I^A-afferents, respectively, enabled calculation of the motor nerve conduction velocity (MNCV) and the H-reflex related sensory nerve conduction velocity (SNCV). In order to minimize the effect of differences in body temperature on nerve conduction velocity, anaesthetized rats were placed in a cotton-wool bed before testing. Data were used only when the rectal temperature was within the range of 37–38°C.

2.3.5. Blood glucose levels

Blood samples from the tail vein were obtained 48 h after STZ injection and glucose levels were measured by use of a Minilab (Bayer GmbH, Munich, Germany). Rats with blood glucose levels exceeding 15 mmol/l were considered diabetic. Blood glucose levels were measured again at the end of the experiment.

2.4. Data analysis

All experiments and measurements were carried out in a blind fashion. The group code was broken at the end of the experiments to allow analysis of the data for all groups. The treatment code, indicating which group had received exercise training, was broken only after analysis of the data was completed.

In Experiment 1 data obtained from the free walking pattern are expressed as the mean (\pm SEM) overall index of sciatic nerve function (SF-index), footprint (FP-index), and toespreading (TS-index) per group. These data were used to evaluate the recovery of coordinated motor function of the injured hind paw.

In Experiment 2 for each individual rat the inner- and outer toespreading distance (mm) of both the left and right paw was summated and termed toespreading (mm). In a pilot experiment, STZ-induced diabetes significantly reduced toespreading after approximately 5–6 weeks (data not shown). In the experiment presented here, each rat was monitored one day before STZ-injection and during the entire experiment, starting at week 6. Changes in the toespreading of diabetic-

and weight-matched DIET control rats were calculated by subtraction of the week 6 value. Group mean changes were calculated and used for statistical analysis.

Motor and sensory nerve conduction velocity (MNCV and SNCV, respectively) data were calculated as a percentage of those of the contralateral intact nerve of the same rat in Experiment 1.

In Experiment 2 unprocessed conduction velocity data were used for statistical analyses.

Differences in body weight and walking patterns between groups were statistically analysed by analysis of variance for repeated measures (MANOVA). The data obtained in the foot reflex withdrawal test were analysed with a Mann-Whitney U-test on each consecutive day. Electrophysiological data were analysed by Student's *t*-test in Experiment 1. The nerve conduction velocity data of Experiment 2 were treated according to Fisher's procedure of planned comparison. A MANOVA was carried out over the three groups and subsequently a Student-Newman-Keuls test was used to indicate differences at time points chosen from the curve. Blood glucose measurements were analysed by an analysis of variance for repeated measurements (ANOVAR) followed by a supplemental *t*-test.

All data analysis¹ were processed using SPSS/PC + v2.0 (SPSS Inc., Chicago, IL, USA). Significance was defined at the 0.05 level.

3. Results

3.1. Experiment 1: acute neuropathy

3.1.1. Body weight

During the experiment the mean body weights of both groups were not significantly different (data not shown).

3.1.2. Early phase effect of training on recovery of sensorimotor function

Analysis of the free walking pattern before crushing of the right sciatic nerve showed that both paws functioned normally. Four days after the crush, the use of the injured paw of all animals was markedly limited, as indicated by the SF-index (< -100) and FP-index (< -60) values. SF-index and FP-index showed signs of recovery at day 12, the latter parameter indicating the return of function in proximal calf muscles. TS-index, which is a measure of the return of function in the distal calf and small foot muscles, started to increase at experimental day 16. Increased functional activity resulted in a more rapid normalization of SF-, TS-, and

FP- indexes when compared to those of the sedentary control group (Fig. 1).

3.1.3. Early phase effect of training on recovery of sensory function

Measurements on day 4 after the crush lesion showed that all rats were insensitive to noxious stimulation of the foot sole. Recovery of sensory function, indicating the establishment of functional free nerve endings of regenerating sprouts in the foot sole, became apparent at day 19. At day 28 all rats had recovered completely: they retracted their paws on 100 mA stimulation of the foot sole. Comparison of data from control rats and exercise-trained rats showed no effect of training on this parameter (data not shown).

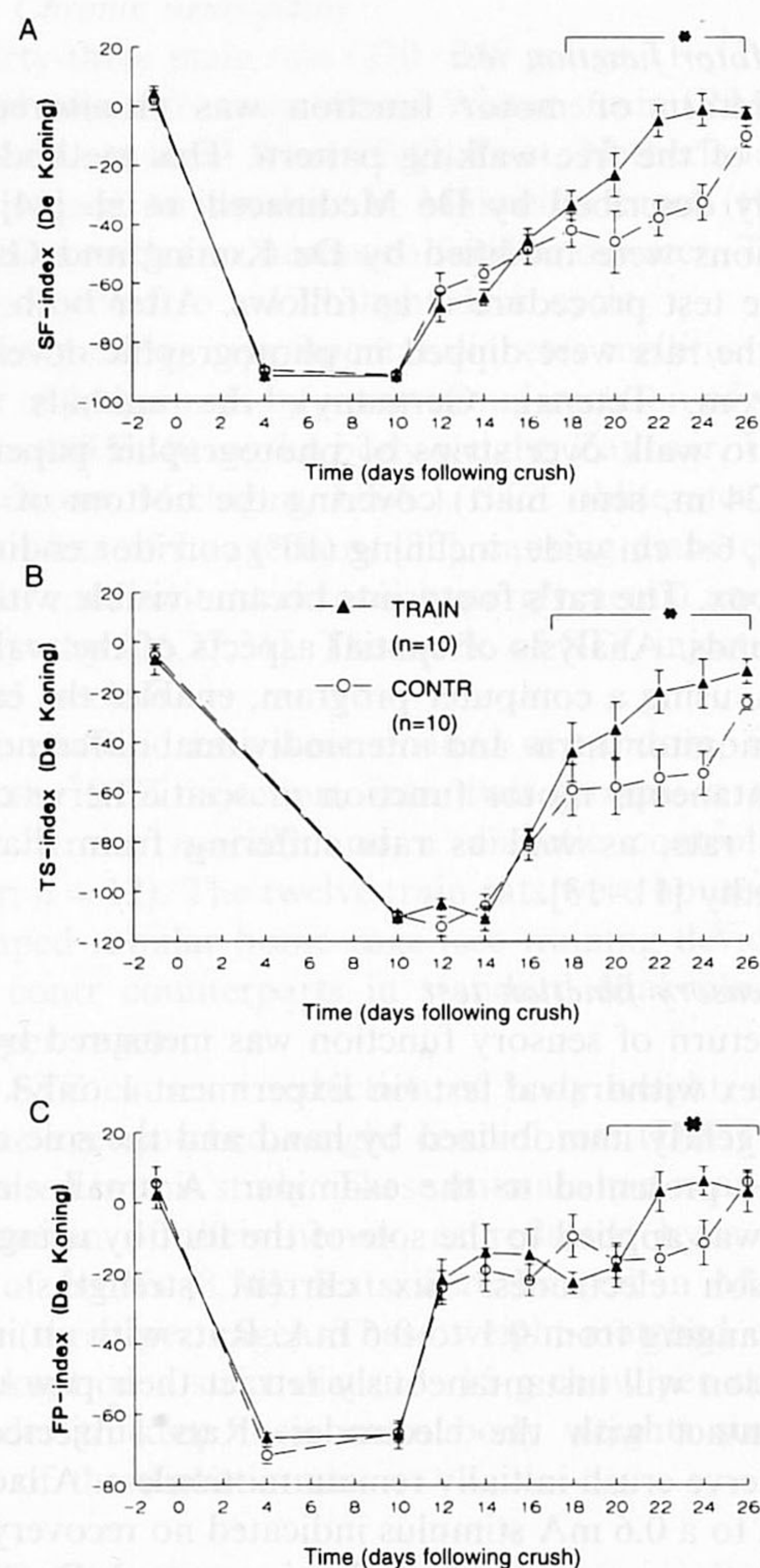


Fig. 1. The effect of exercise training on the recovery of sensorimotor function of sciatic crush-lesioned rats. Overall sciatic function (SF-index; graph A), toespreading (TS-index; graph B), and footprint (FP-index; graph C) were calculated from the free walking pattern in both spontaneously recovering rats (Contr) and exercise-trained rats (Train). Training started immediately after the rats recovered from anaesthesia. (A) MANOVA over the days 18–26: $F_{(1,18)} = 13.36$; $P < 0.01$. (B) MANOVA over the days 18–26: $F_{(1,18)} = 6.05$; $P < 0.05$. (C) MANOVA over the days 20–26: $F_{(1,18)} = 5.50$, $P < 0.05$.

¹ Two rats, one from the TRAIN group and one from the CONTR group, were found to be non-diabetic with glucose levels of 5.2 and 8.8 mmol/l, respectively. Data from both rats were excluded from the data analysis. During the study a diabetic control rat died, and all data from this rat were also excluded from analysis.

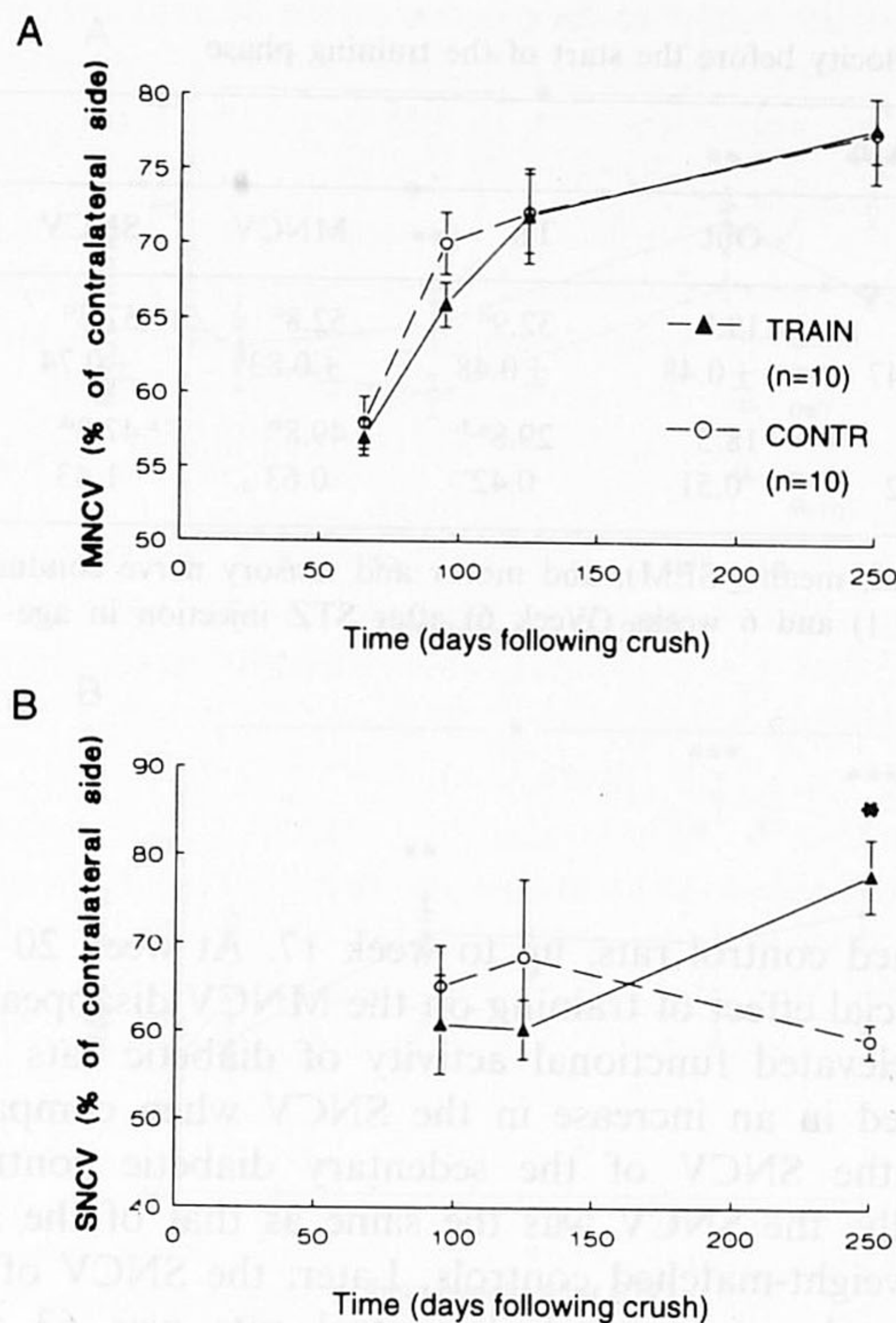


Fig. 2. The effect of 24 days of training on the late phase of recovery of motor and sensory nerve conduction velocity (MNCV and SNCV; graph A and B, respectively) following sciatic crush lesion. Both variables are expressed as a percentage of those of the contralateral intact nerve from the same rat. (A) No effects of exercise training on the MNCV values could be detected. (B) Training significantly enhanced the recovery of SNCV of the trained group at 250 days, when compared to the SNCV of the Contr group. Levine's test for equality of variances: $F = 5.440$, $P < 0.05$; Students t -test (corrected for variance) over Control vs. Train at day 250: $t = 4.13$, $DF = 12.75$, $P < 0.001$.

3.1.4. Late phase effect of training on nerve conduction velocity

At day 60 after the crush, MNCV (Fig. 2A) and SNCV (Fig. 2B) of the injured right sciatic nerve were approximately 58% and 63%, respectively, of the conduction velocities of the contralateral, intact left paw. The MNCV did not differ significantly between the two experimental groups. However, the SNCV of exercise-trained rats differed significantly from that of control rats at day 250, indicating that daily exercise enhanced the recovery of the SNCV.

3.2. Experiment 2: chronic neuropathy:

3.2.1. Body weight and blood glucose measurements

The gain in body weight normally seen in young rats is less in STZ-injected rats [26,27]. Up to week 13, no significant differences were found in body weight between the three experimental groups. From then on the

weight-matched diet group showed a gradual and small weight gain when compared to the contr and train groups. Training did not alter this decrease in body weight (Table 1).

The rats developed high blood glucose levels within 48 h after STZ injection. During the entire experiment the blood glucose levels remained significantly elevated, exceeding 15 mmol/l. No influences of training were found on blood glucose levels (Table 1).

3.2.2. Effect of STZ-induced diabetes mellitus on sensorimotor function

In a pilot study, food-restricted rats showed a gradual increase in inner- and outer-toespreads, whereas the toespreads of STZ-induced diabetic rats declined steadily (data not shown). In this experiment the age- and weight-matched control rats showed an increase in toespreads, calculated from the inner- and outer-toespreads, at week 6 (Table 2). From week 6 the diet-group rats demonstrated a continuous increase in toespreads, up to the twelfth week (Fig. 3). In diabetic rats toespreads decreased during the first 6 weeks (Table 2). Thereafter no further deterioration was seen (Fig. 3).

3.2.3. Effect of exercise training on sensorimotor function in STZ-diabetic rats

After 6 weeks, when twelve diabetic rats (train group) were introduced to the 'U'-shaped home cage, the toespreads showed an instantaneous increase and this increase paralleled the increase in the toespreads of the age- and weight-matched control rats (Fig. 3).

Table 1

Comprehensive overview of blood plasma glucose levels and body weights

	Week 0		Week 6	Week 20	
	Glucose	BW	BW	Glucose	BW
Diet	6.0 ±0.6	235 ±4	290 ±7	6.6 ±0.8	336 ±8
Contr	26.2* 1.9	238 3	293 18	27.9* 1.6	280** 8
Train	26.0* 1.7	235 3	297 16	28.7* 1.1	303** 17

Blood plasma glucose level (mean mmol/±SEM) and body weight (g; mean ±SEM) 48 h after STZ injection (week 0) and at the end of the experiment in age- and weight-matched control rats (Diet), diabetic control rats (Contr), and exercise trained rats (Train). No significant differences were found between the contr and train groups on these parameters.

* $P < 0.001$ as compared to the diet group.

** $P < 0.01$ as compared to the diet group.

Table 2

Comprehensive overview of data concerning toespreading and nerve conduction velocity before the start of the training phase

	Week 1					Week 6				
	Inn	Out	TS	MNCV	SNCV	Inn	Out	TS	MNCV	SNCV
Diet (n = 9)	12.4 ±0.35	18.9 ±0.37	31.3 ±0.34	45.5 ±1.05	46.4 ±0.93	13.6 ±0.47	19.7 ±0.48	32.9 ^a ±0.48	52.8 ^c ±0.83	57.3 ^c ±0.74
Diab (n = 24)	12.1 0.36	19.1 0.29	31.2 0.40	45.4 0.79	46.0 0.86	11.7 0.52	18.3 0.51	29.8 ^{a,b} 0.42	49.8 ^b 0.63	47.8 ^d 1.43

Inner (Inn) and outer (Out) toespread (mm; mean ± SEM), toespreading (TS; mm; mean ± SEM), and motor and sensory nerve conduction velocity (MNCV and SNCV respectively; m/s; mean ± SEM) 24 h before (week 1) and 6 weeks (Week 6) after STZ injection in age- and weight-matched control rats (Diet), diabetic rats (Diab).

^a $P < 0.05$ as compared to week 1.

^b $P < 0.05$ as compared to the DIET group.

^c $P < 0.001$ as compared to week 1.

^d $P < 0.001$ as compared to the DIET group.

3.2.4. Effect of STZ-induced diabetes mellitus nerve on conduction velocity

Weight- and age-matched control rats showed an increase in MNCV and SNCV, which is normally seen in maturing rats (Table 2). In diabetic rats this increase in conduction velocity vanished and even, in case of the MNCV, reversed: 6 weeks after STZ administration, the MNCV (Fig. 4A) and SNCV (Fig. 4B) of diabetic rats were significantly different from those of the age- and weight-matched control rats (Table 2).

3.2.5. Effect of exercise training on nerve conduction velocity in STZ-diabetic rats

Exercise training started at week 6 after STZ injection and resulted in an elevation of the MNCV, which initially paralleled the increase in the age- and weight-

matched control rats, up to week 17. At week 20 this beneficial effect of training on the MNCV disappeared. The elevated functional activity of diabetic rats also resulted in an increase in the SNCV when compared with the SNCV of the sedentary diabetic controls. Initially, the SNCV was the same as that of the age- and weight-matched controls. Later, the SNCV of the age- and weight-matched control rats was 63 m/s, whereas the trained diabetic rats had an SNCV of approximately 54 m/s. The mean SNCV of the trained group was significantly different from that of their sedentary diabetic counterparts.

4. Discussion

In order to evaluate the effects of exercise training on acutely as well as chronically impaired nerve function we used two existing, abundantly documented models of neuropathy: sciatic nerve crush lesion and STZ-induced diabetes mellitus in the Wistar rat. The former causes acute functional sensorimotor deficits [10,14] and impaired NCV's [11], and the latter causes chronic hyperglycaemia and EDN [32], as evinced by, amongst others, deterioration of NCV's [26–28] and progressive impairment of locomotor paw function [Van Meeteren, unpublished observations] [12,13].

4.1. Experiment 1: acute neuropathy

The results of the first experiment are, in part, in line with those of our previous reports [45,47]. In the present study, the SF-index, which is a measure of the overall use of paw muscles, and both the TS-index and the FP-index, which are measures of the use of distal and proximal calf muscles, respectively, recovered at a faster rate in the exercise-trained rats than in the control rats (Fig. 1A, B and C). These observations demon-

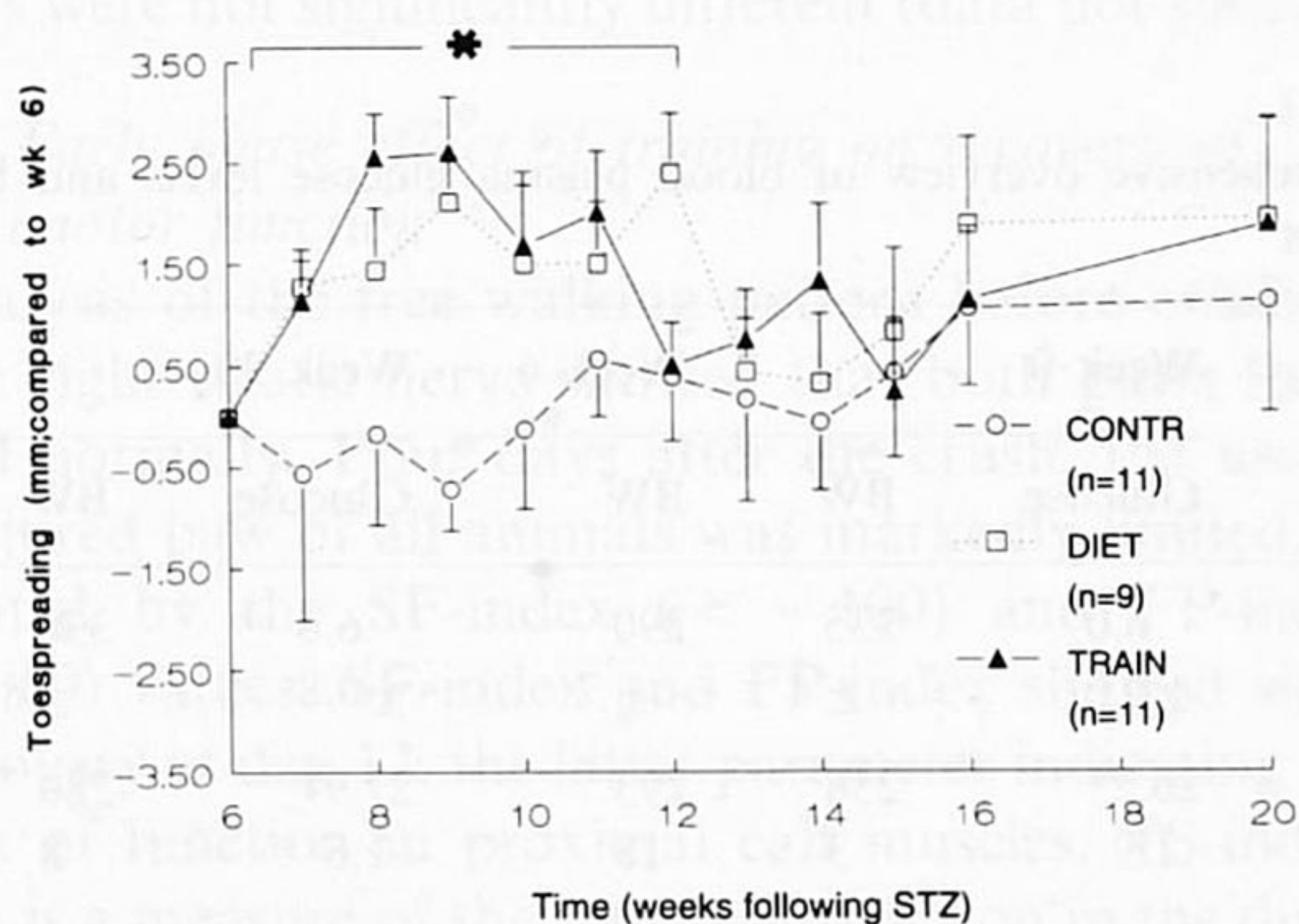


Fig. 3. The effect of STZ-induced diabetes mellitus (Contr) on coordinated sensorimotor function, calculated from the free walking pattern of the rat. Toespread is expressed as the gradual change in distance (mm) between the inner toes and the outer toes, starting from the moment when exercise training began (6 weeks after STZ injection). These data were compared to values for age- and weight-matched control rats (Diet). Toespread in STZ-induced diabetic rats housed in the training device was initially spared from the deterioration seen in untrained rats. MANOVA over the weeks 7–12: $F_{(2,22)} = 4.67$, $P < 0.05$; One way ANOVA $F_{(2,25)} = 4.03$; $P < 0.05$.

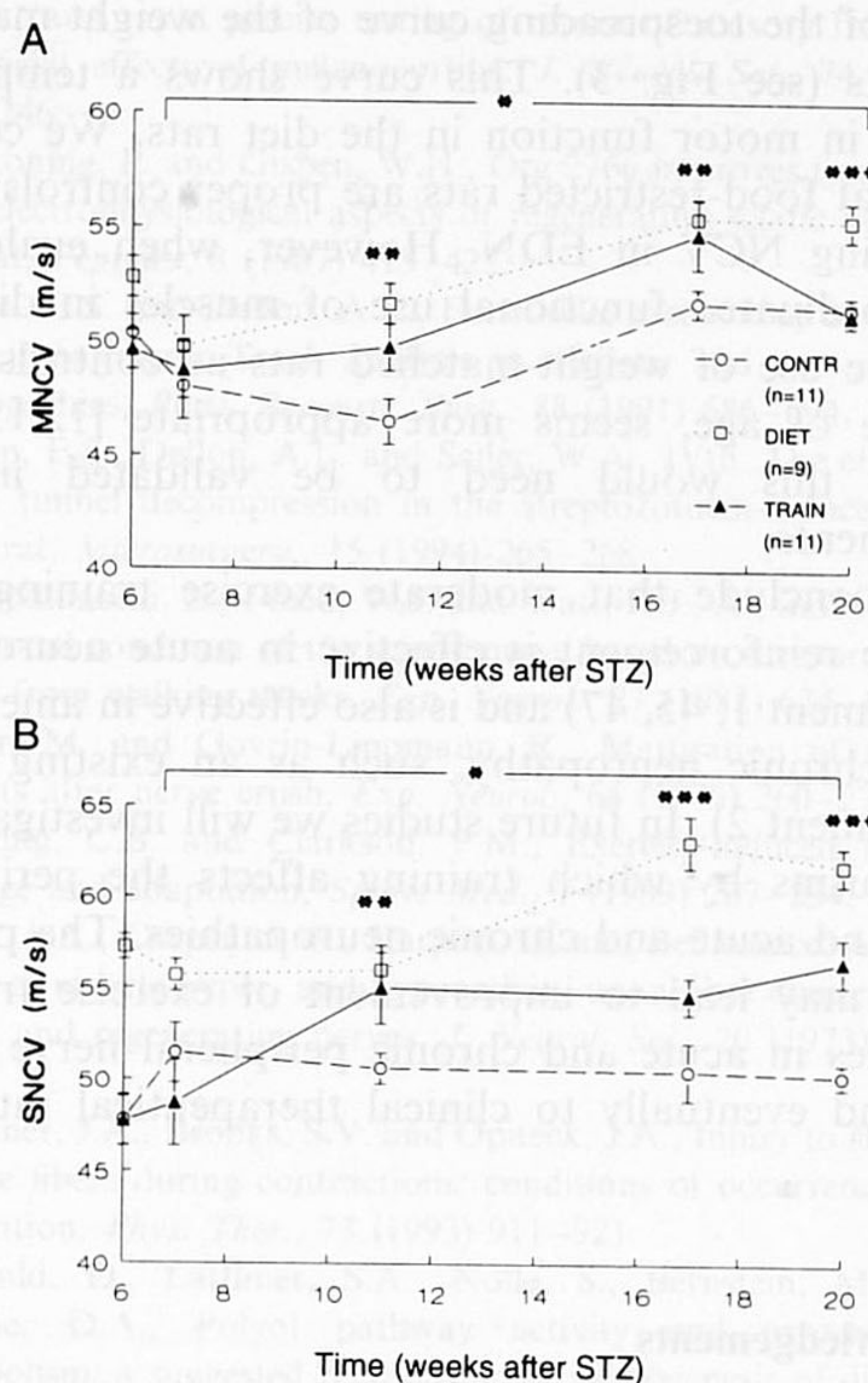


Fig. 4. Motor and sensory nerve conduction velocity (MNCV and SNCV respectively) in diabetic (Contr, $n = 11$), age- and weight-matched control rats (Diet, $n = 9$) and trained (Train, $n = 11$) rats. Rats were trained from 6 weeks after the induction of diabetes mellitus with STZ. (A) MANOVA week 7–20: $F_{(2,21)} = 15.86$, $P < 0.001$; Student-Newman-Keuls test week 11 and 17: Contr vs. Diet, Contr vs. Train: $P < 0.05$ and Train vs Diet: ns. Student-Newman-Keuls test week 20: Contr and Train vs Diet: $P < 0.05$ and Contr vs Diet ns. (B) MANOVA $F_{(2,21)} = 51.19$, $P < 0.001$. Student-Newman-Keuls test week 11 (ANOVA: $F_{(2,25)} = 4.52$, $P < 0.05$): Contr vs Diet, Contr vs Train: $P < 0.05$ and Train vs Diet ns. Student-Newman-Keuls test week 17 (ANOVA: $F_{(2,22)} = 15.39$, $P < 0.001$) and 20 (ANOVA: $F_{(2,22)} = 31.42$, $P < 0.001$): Contr vs Train, Contr vs Diet and Train vs Diet: $P < 0.05$.

strate that exercise enhances functional recovery from acute crush neuropathy. These beneficial effects are restricted to the injured paws, as no beneficial effects could be detected when comparing data for the intact contralateral paws of both trained and sedentary rats (data not shown).

The effect of physical exercise on intact peripheral nerves is debated: the diameter of nerve fibres is either increased or decreased as a consequence of experimentally induced hyperactivity of muscles [17]. Nerve fibre diameter affects electrophysiological variables, for instance the NCV [15,25,30,34]. The beneficial effects of 24 days of training in the early phase of recovery seem to remain during the late phase, as indicated by the enhanced recovery of the SNCV after 250 days. Phasic exercise training after a crush lesion results in a profound amelioration of MNCV and a slightly, but insignificantly, enhanced SNCV at 150 days after crush lesion

[47]. Measurement of H-latencies, from which the SNCV is calculated, show insufficient reliability at 100–150 days after the crush [11,47]. Therefore, we assume that late phase electrophysiological measurement of H-latencies must be extended to at least 250 days, in order to monitor the potential effects of therapeutical interventions on the SNCV.

The different effects of training on the FP index, the return of the foot withdrawal reflex, and MNCV in our present and previous study [45,47] need further consideration. These contrasting results indicate that different exercise devices and training strategies have different consequences on the healing of an injured nerve. A nerve 'function-structure relationship', as suggested by Young in 1946 [51], might result in specific morphological, histological, physiological, and biochemical adjustments in the nerve, induced by the specific functional demands made during the period of regeneration. This function-structure theory is consistent with what is known about the effect of diverse training programmes on striated muscle [18,23,35,36]. Therefore future studies will require detailed monitoring and description of the type and parameters of the training protocols used.

4.2. Experiment 2: chronic neuropathy

The exact pathogenesis of diabetic neuropathy is debated. A combination of neurovascular and neurochemical abnormalities jeopardize nerve function in diabetes mellitus [3,22,48], as reflected by a reduced endoneural blood flow [42] and a dysregulation of the glucose metabolism in the nerve [19]. Diabetic neuropathy is characterized on electrophysiological, biochemical or histological grounds [1]. Slowing of MNCV and SNCV occurs in human diabetic neuropathy [40] and in EDN [26–28]. In addition to these characteristics of EDN, a deterioration of distal hind paw function in STZ-induced diabetic rats was recently observed by Dellon and colleagues [12,13]. This observation offered the possibility of a combined evaluation of functional and electrophysiological aspects of therapeutical interventions in EDN.

In diabetic rats there is considerable nerve demyelination, as indicated by a steady decrease in the NCVs [26,27,44]; as can be seen in Fig. 4. The deterioration in MNCV and SNCV in hyperglycaemic, diabetic rats and comparison of toespreading data from diet and diab rats after 6 weeks suggests that the hyperglycaemia undermines normal nerve function in diabetic rats, and indicate that EDN was established (Table 2). The impairment of toespreading in diabetic rats seen in this experiment and our pilot study differs from the results obtained by Dellon and his colleagues, as they demonstrated an increased inner toespread and unchanged outer toespread 45 days after STZ-injection [12,13]. We can only speculate on this issue and it remains to be elucidated in the future.

The initial improvement in toespreading in STZ-induced diabetic rats (train group), the transient increase in MNCV, and the lasting increase in the SNCV demonstrate that the 'U'-shaped training device is effective in attenuating EDN in these relatively young rats. Additionally, training could have attenuated the diabetic myopathy, as atrophy is counteracted by functional use or training [35]. When diabetic myopathy is well established, there is a slowing of contractions, a reduction of oxidative capacity [9], and an increased vascular resistance of skeletal muscle [31]. These variables are affected by training in healthy animals [16,23,36]. We speculate that these effects were reversed in the trained diabetic rats by our exercise training protocol.

In mildly diabetic rats, training improves glucose homeostasis as a consequence of an increased quantity of circulating insulin [20,21], and a recovery of glucose homeostasis by insulin supplementation restores NCVs [40,43]. The amount of STZ injected in the present experiment caused severe hyperglycaemia. Exercise training did not affect the diabetic state (see Table 1), whereas the NCVs showed a clear improvement. Consequently, these results rule out the possibility that training ameliorated the hyperglycaemia as an explanation for the improvement in the NCVs. Alternatively, diabetes mellitus causes abnormal endoneurial capillaries [50], reduced basal nerve blood flow and a related NCV deficit [5,28], and decreased tissue oxygenation [52]. Cameron applied artificial chronic electrical impulse activity (10 Hz, 8 h/day) to rats with STZ-induced EDN, a strategy which partly restored NCV, resistance to hypoxic conduction failure, and basal nerve blood flow [4,7]. The diabetic rats housed in the 'U'-shaped home cage had to traverse 8 m to get to their food and water. Freely moving rats show an enormous variation in the frequency of motor unit discharges [24]. Consequently, the 'U'-shaped home cage may have caused an increased physiological nerve activity when compared to that of the controls. In line with the explanation of Cameron and colleagues, we hypothesize that, as a result of the training-induced increase in nerve function, EDN-associated abnormalities were partly improved. We are currently investigating the effects of exercise training on basal nerve blood flow and nerve metabolism.

Thomas and Tomlinson [40] argued that the validation of the diabetic animal model is closely related to the parameter under study. As STZ causes a reduction in body weight, age- and weight-matched control rats (diet group) were used in this experiment. Food-restricted young rats express near-normal maturation of their nerves, as evidenced by a regular gain in NCVs [8]. Food deprivation is known to cause a deterioration of muscle mass and changes in ultrastructure in the long term [49], which might be reflected in the later

stages of the toespreading curve of the weight matched diet rats (see Fig. 3). This curve shows a temporary decline in motor function in the diet rats. We considered that food-restricted rats are proper controls when evaluating NCV in EDN. However, when evaluating the coordinated functional use of muscles in diabetic rats, the use of weight-matched rats as controls, irrespective of age, seems more appropriate [12,13], although this would need to be validated in our experiments.

We conclude that moderate exercise training with positive reinforcement is effective in acute neuropathy (Experiment 1; 45, 47) and is also effective in ameliorating a chronic neuropathy, such as an existing EDN (Experiment 2). In future studies we will investigate the mechanisms by which training affects the peripheral nerve and acute and chronic neuropathies. The present results may lead to improvement of exercise training strategies in acute and chronic peripheral nerve disorders and eventually to clinical therapeutical interventions.

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