SHOCK WAVE TREATMENT IMPROVES NERVE REGENERATION IN THE RAT

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ABSTRACT: *Introduction*: The aims of the experiments were to: (1) determine whether low-energy shock wave treatment accelerates the recovery of muscle sensitivity and functionality after a nerve lesion; and (2) assess the effect of shock waves on the regeneration of injured nerve fibers. *Methods*: After compression of a muscle nerve in rats the effects of shock wave treatment on the sequelae of the lesion were tested. In non-anesthetized animals, pressure pain thresholds and exploratory activity were determined. The influence of the treatment on the distance of nerve regeneration was studied in immunohistochemical experiments. *Results*: Both behavioral and immunohistochemical data show that shock wave treatment accelerates the recovery of muscle sensitivity and functionality and promotes regeneration of injured nerve fibers. *Conclusion*: Treatment with focused shock waves induces an improvement of nerve regeneration in a rodent model of nerve compression.

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The therapeutic application of extracorporeal shock waves includes a multitude of disorders and diseases. These include calculi of the kidney and other organs,[1] calcific tendonitis,[2] tennis elbow,[3] osteoarthritis,[4] and plantar fasciitis,[5] to name a few.

Focused high-energy shock waves (with a focal energy flux density of 0.6 mJ/mm^2 and higher[2,6,7]) cause a marked and steep pressure increase in tissues followed by cavitation forces that can destroy stones in the kidney, lower urinary tract, and gall bladder. Low- to moderate-energy shock waves (with an energy flux density from 0.08 to 0.28 mJ/mm^2)[2] are believed to exert their effects through other (possibly additional) mechanisms, such as improved circulation or increased angiogenesis.[8,9] It has to be pointed out, however, that this classification into low- and high-energy shock waves is not generally accepted.[10] Besides energy flux levels, the frequency and repetition rates of shock waves are factors that influence the results of the treatment.

Relatively few reports have dealt with the effects of shock waves on nerve fibers. Recently, a selective loss of unmyelinated nerve fibers in peripheral nerves has been reported as a possible mechanism for the long-lasting analgesia that sometimes follows high-energy treatment of the musculoskeletal system.[6,7,10,11] Other groups have described a loss of the neuropeptide substance P (SP) and calcitonin gene–related peptide (CGRP[12–14]) in nerve fibers treated with shock waves. SP is assumed to be present exclusively in nociceptive fibers, and many of the fibers that express CGRP also supply nociceptors. Thus, the loss of these peptidergic fibers likewise could explain the analgesia or hypoalgesia after shock wave treatment, although there are also nociceptive fibers that do not express neuropeptides but are IB4-positive (isolectin subtype B4[15]).

Of particular interest in the context of this study are publications that address the reinnervation of tissues as a sequel of shock wave treatment. In 2001, Ohtori and colleagues[13] reported reinnervation of sensory fibers, and in 2006 another Japanese group[11] stated that shock waves induce the expression of growth-associated protein-43 (GAP-43) in rat dorsal root ganglia. GAP-43 is a protein that occurs particularly in growth cones of nerves.[16]

These reports have prompted us to test the effect of low-energy shock waves on the regeneration of damaged nerve fibers in the rat. In a model of nerve compression, the time course of nerve regeneration was determined in behavioral and histological experiments.

METHODS

The experiments were performed on a total of 26 adult male Sprague-Dawley rats (body weight 320–440 g). The experimental design was approved by the local ethics authority responsible for animal experimentation. All experiments were carried out in accordance with German law on the protection of animals and with the ethics proposals of the International Association for the Study of Pain (IASP).[17] At the end of the experiments the animals were killed with an intraperitoneal overdose of thiopental sodium (Trapanal).

Study Design. Two series of experiments were performed to investigate the effects of focused shock wave treatment on the functional and structural regeneration of injured peripheral nerve fibers.

Abbreviations: ATF3, activating transcription factor 3; CAP, compound action potential; CGRP, calcitonin gene related peptide; DRG, dorsal root ganglion; GAP 43, growth associated protein 43; GS, gastrocnemius sol eus; IB4, isolectin subtype B4; NGF, nerve growth factor; PBS, phos phate buffered saline; PGP 9.5, protein gene product 9.5; PPT, pressure pain threshold; SP, substance P; SU, sural nerve; TGF β, transforming growth factor β1; VEGF, vascular endothelial growth factor

Key words: focused shock waves, muscle, nerve regeneration, pain related behavior, PGP 9.5 immunohistochemistry

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In the first series, the time-course of sensory nerve regeneration was studied in behavioral experiments. The pain-related behavior and motor coordination of 10 rats before and after crushing of the lateral and medial branches of the left gastrocnemius–soleus muscle (GS) nerve were determined (referred to as "GS crush group" in what follows). The animals were divided into 2 subgroups: 5 animals treated with shock waves ("GS crush treated" group); and 5 animals in which the GS nerves were crushed but were not treated with shock waves ("GS crush untreated" group). The aims of these experiments were to: determine whether the shock wave treatment had an effect on the behavioral sequelae of the nerve lesion; and to identify the right time window for the histological experiments of the second series. In all animals, the GS muscle of the right leg served as a control ("GS control side").

In the second series, structural regeneration of injured nerve fibers distal to the lesion site was studied by visualizing all nerve fibers with protein gene product 9.5 (PGP 9.5) immunohistochemistry. PGP 9.5 is commonly used as a universal marker for all neural elements.[18] The experiments were carried out on the sural (SU) nerve, because the GS nerve is not straight but instead follows a circuitous course and is therefore not suited to studying distances of nerve regeneration. Five animals with a crushed SU nerve were treated with shock waves ("SU crush treated"), and 5 animals with the SU nerve crushed were not treated ("SU crush untreated"). The aim of these experiments was to determine whether the shock wave treatment effects observed in series 1 could be linked to changed nerve regeneration speed.

Pilot experiments. In 3 animals with intact GS nerves, the effect of shock wave treatment on the sensitivity of the GS muscle to painful stimulation was tested, and in 3 further animals the compound action potentials of the GS and SU nerves were recorded across the nerve lesion.

Crushing of Gastrocnemius–Soleus or Sural Nerve. The nerves were crushed under deep ketamine/xylazine anesthesia (100 mg/kg/7.5 mg/kg intraperitoneally). Under sterile conditions, both branches of the left GS nerve (series 1) or the left SU nerve (series 2) were exposed surgically at the level of the popliteal fossa. The nerves were crushed using a hemostatic forceps with polythene-tube–covered tips. The forceps were left in place for 5 min. The crush caused a macroscopically visible nerve contusion over a length of about 1 mm. It damaged the nerve fibers, but left the nerve sheath intact (Fig. 1A and B).

In the immunohistochemical studies of series 2, the nerve sheath at the lesion site was marked with a black permanent marker immediately after removing the forceps. Thus, the site of the nerve crush was visible in the longitudinal tissue sections that were processed for PGP 9.5 immunohistochemistry 6 days after nerve injury (Fig. 1C).

Shock Wave Treatment. From the vast (mainly clinical) literature on shock wave treatment, a medium level of energy flux density and a medium number of impulses (see ref. [2]) were selected for the experiments. The main requirements for the treatment were absence of pain during treatment and avoidance of tissue damage,[19] because high energy densities have been shown to destroy nerve fibers.[6] During treatment, the rats were not anesthetized. The animals were treated with focused shock waves starting 1 day after nerve crush. In each treatment session, the GS nerve and muscle (series 1) or the SU nerve (series 2) were treated at 8 sites linearly arranged from the lower thigh to the Achilles tendon (Fig. 1F). The first and second treatment sites were located proximal to the nerve lesion; the others were distal in order to treat the sprouting axons. The electromagnetic shock wave transmitter (Duolith SD1, Model F-SW with stand-off II; Storz Medical) was placed perpendicularly on the shaved skin of the lateral popliteal fossa and lower leg. The circular tip of the shock wave transmitter has a diameter of 2.3 cm. To couple the transmitter to the skin, the tip was covered with transmission gel (Sonavelle; Meditec-Elefant-Chemie, Germany). At each treatment site, 10 impulses were applied at an energy flux density of 0.2 mJ/mm^2 and a repetition rate of 4 Hz. The focus of the shock waves had the shape of an ellipsoid; its total penetration depth was 30 mm with the maximum energy flux density at a depth of 15 mm.

In series 1, eight treatment sessions were performed in the first and second week after nerve crush. The sessions were grouped in 2 blocks of 4 treatment sessions per week. A first block was administered at days 2, 3, 4, and 5 after nerve crush, and a second block was administered at days 8, 9, 10, and 11 (Fig. 1E). Care was taken not to include the skin incision covering the popliteal fossa in the treatment. All animals tolerated the shock wave treatment well. They never showed withdrawal reflexes or other forms of pain-related behavior.

In series 2, only 1 block of 4 treatment sessions could be administered (at days 2, 3, 4, and 5), because regeneration of the axons was studied histologically at day 6 after nerve injury. The time-point of 6 days after crush was chosen for the histological evaluation, because, at this time-point, there was still a clear effect of the crush in the behavioral experiments, indicating that the

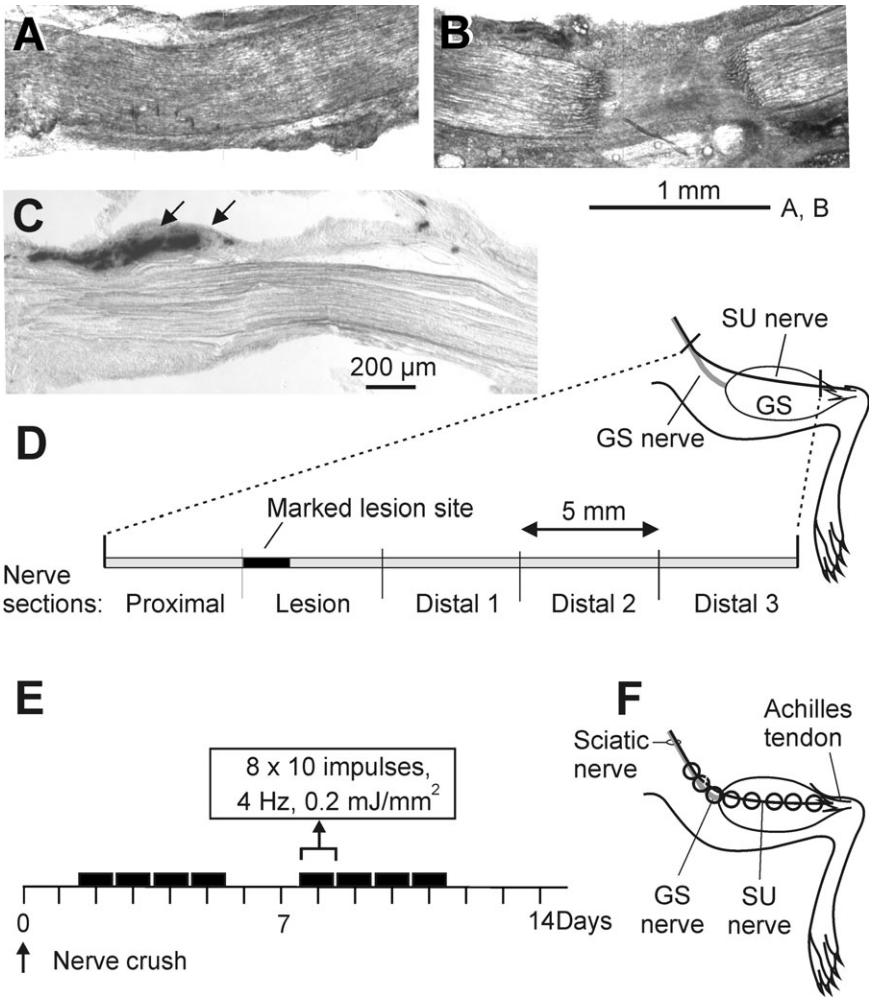

FIGURE 1. (A, B) Unfixed whole mount preparations of the intact GS nerve (**A**) (medial branch) and after crushing of the nerve (**B**). (**C**) Longitudinal histological section (thickness 25 μm) processed for PGP 9.5 immunohistochemistry 6 days after SU nerve crush. At the lesion site ink particles of the marker are visible in the nerve sheath (arrows). (**D**) Scheme of the 5 SU nerve sections that were used for histological analysis. Longitudinal tissue sections of these nerve sections were processed for PGP 9.5 immunohistochemistry (see (**C**)]. (**E**) Time schedule of shock wave treatment after GS nerve crush. Eight treatment sessions were performed in 2 blocks of 4 treatment sessions each (black bars). The animals in which the SU nerve was histologically studied 6 days after nerve crush received the first 4 treatment sessions only. (**F**) In each treatment session [see (**E**)], the GS and SU nerves were treated at 8 sites, as indicated by the circles. At each site, 10 impulses were applied (0.2 mJ/mm^2, repetition rate of 4 Hz). GS, gastrocnemius–soleus muscle.

regenerated nerve fibers had not yet reached the muscle (see later). In the untreated animals, the shock wave transmitter was placed on the same 8 treatment sites, but no shock waves were applied.

The pilot experiments on 3 animals with intact GS nerves had shown that the shock wave treatment of up to 100 impulses per day at energy flux densities of 0.2 mJ/mm^2 had no effect on the pressure pain threshold of rats when the GS muscle was stimulated. Likewise, the treatment did not cause behavioral changes of animals with intact GS nerves.

Recordings of Compound Action Potentials. In 2 animals, the compound action potentials (CAPs) were recorded electrophysiologically across the lesion site before and 30 min after nerve crush to determine the extent of the loss in nerve conduction (1 GS nerve crush, 1 SU nerve crush). In 1 additional animal, CAPs across the lesion site were recorded 15 days after GS nerve crush to test for the recovery of nerve conduction.

CAPs were recorded in deeply anesthetized animals [thiopental sodium (Trapanal), 100 mg/kg intraperitoneally, followed by 10–20 mg/kg each hour]. The sciatic, SU, and GS nerves were exposed surgically. For electrical stimulation of the nerve fibers a stimulating electrode was placed under the sciatic nerve 10 mm proximal to the nerve crush (stimulus intensity up to 50 V, stimulus duration 0.3 ms). CAPs of the nerves were recorded from the GS or SU nerve, about 5 mm distal to the lesion. The nerves were crushed between stimulating and recording electrodes.

Behavioral Experiments. Three behavioral tests were performed: determination of the pressure pain threshold; an open field test; and a motor coordination test on a rotating rod.

Test 1: Determination of Pressure Pain Threshold. In non-anesthetized animals, the sensitivity of the GS before and after GS nerve crush was tested by determining the hindlimb withdrawal threshold. Pressure stimuli were applied to the muscle with an electronic von Frey anesthesiometer (IITC 2390 Series; Life Science Instruments). The specially made blunt tip with a 3.46-mm^2 surface area[20] was pressed with increasing force to the medial head of the GS muscle in the mediolateral direction on the intact skin. The lower leg was fixed manually to prevent passive movements during pressure stimulation. In all tests the tip of the von Frey anesthesiometer was placed at the same position in the middle of the muscle belly about 20 mm distal to the GS nerve lesion. It was shown previously that, by using a blunt tip, the pain sensitivity of muscles can be determined separately without eliciting nociceptive input from the skin.[21] The stimulus intensity (in grams) required to elicit withdrawal of the hindlimb was measured before and after nerve crush. An increase in withdrawal threshold after nerve crush indicated that sensory nerve fibers were blocked or destroyed by the lesion. The time to restoration of the initial pressure pain threshold (PPT) was used as a behavioral parameter of sensory nerve regeneration. As control measurements, in each animal, the right GS muscle (no nerve crush) and the skin overlying the left GS muscle (nerve crushed) were tested with the same probe. For testing the overlying skin the probe was pressed to a small skin fold at the level of the GS muscle.

Test 2: Open Field Test. The influence of the nerve crush on locomotion was examined by observing the spontaneous exploratory behavior of the rats in a large cage. The animals were placed individually in an open field arena (100 cm × 60 cm), the bottom of which was divided into 15 squares (20 cm × 20 cm each). Locomotion was quantified by counting the number of squares crossed by a rat within 5 min.[20]

Test 3: Motor Coordination Test on a Rotating Rod. Crush-induced changes in motor coordination were determined by placing the rats on a rotating rod driven by an electric motor, which was controlled by a programmable power supply (HM8142; Hameg). The rod has a diameter of 12 cm. The speed of rotation increased gradually from 0 to 16 rotations/min within 4 min. Fifteen seconds after starting the rotation, the animals were placed on the rod, and the latency until they fell off the rod (latency to fall) was measured. In each test period, 3 tests of latency to fall were made with a pause of 1 min in between. The mean of the 3 tests was used for further evaluation.

The behavioral tests were carried out 1 day before (baseline) and 1, 4, 9, 12, 15, 18, and 21 days after crushing of the GS nerve. The person who performed the tests was blinded to the experimental condition (shock wave treated or untreated).

PGP 9.5 Immunohistochemistry. Six days after crushing of the SU nerve the animals were killed by an intraperitoneal overdose of thiopental sodium (Trapanal). The SU nerve was removed, placed elongated on a glass slide, and fixed for 1.5 h with 4% paraformaldehyde. The fixed nerve was cut in 5 sections of 5-mm length each (Fig. 1D). The first nerve section was just proximal to the marked lesion site (proximal), the second 1 at the lesion site (lesion), and the others distal to the lesion site (distal 1, 2, and 3). The proximal end of each nerve section was marked with a fine needle. The nerve sections were rinsed in phosphate-buffered saline (PBS) containing first 10% and then 30% sucrose, and snap frozen. Longitudinal cryostat sections (thickness 25 μm) were cut from each nerve section.

The tissue sections were processed for PGP 9.5 immunohistochemistry as follows: (a) primary antiserum: rabbit anti–PGP 9.5 (Biotrend), dilution 1:1000 in PBS, and incubation for 24 h at room temperature; (b) secondary antiserum: biotinylated anti-rabbit IgG (Vector), 1:200, 60 min at room temperature; and (c) immunoreactivity was visualized using the avidin–biotin complex method and 3,3-diaminobenzidine tetrahydrochloride as the chromogen.

In each nerve section, PGP 9.5–immunoreactive (IR) nerve fibers were evaluated in 3 randomly selected tissue sections. PGP 9.5-IR fibers were visualized with a Zeiss microscope (40×) and a digital camera (ColorView) on a computer display. All PGP 9.5-IR nerve fibers crossing a straight line perpendicular to the nerve axis in the middle of each tissue section were marked by the investigator and counted with imaging software (analySISb; Olympus). The investigator was blinded to the experimental conditions.

Data Analysis. Data were analyzed using the Mann–Whitney *U*-test and the Wilcoxon test of paired data. $P < 0.05$ (two-tailed) was considered significant.

RESULTS

Pilot Experiments. To test the efficacy of the nerve crush method, the CAPs across the nerve lesion were recorded in 3 rats. Before crushing of the GS or SU nerve, a CAP component evoked by

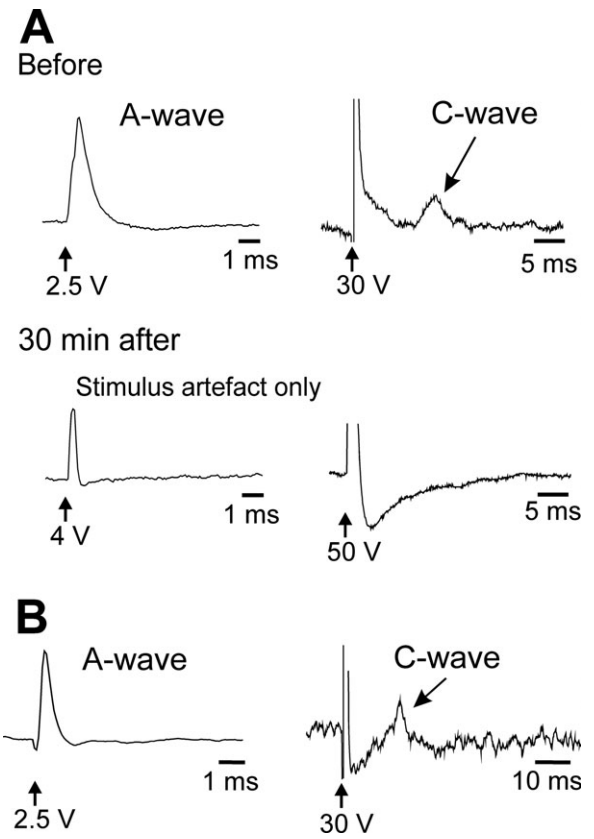

FIGURE 2. (**A**) Recordings of compound action potentials (CAPs) across the lesion site (pilot experiments on 3 rats). Before nerve crush, a CAP evoked by myelinated, fast-conducting nerve fibers (A-wave) as well as a CAP evoked by unmyelinated, slowly conducting fibers (C-wave, arrow) were visible. Thirty minutes after crush, no CAPs could be recorded across the lesion site; only the stimulus artifacts were visible. The stimulus intensity was higher than in the upper panel to ensure that no CAP had been overlooked because of a possible increase in electrical threshold. (**B**) Recordings of the CAPs 15 days after GS nerve crush showing recovery of nerve conduction (without shock wave treatment). Both CAPs (A- and C-wave) were visible, thus indicating that the nerve fibers had regenerated—at least partly—within that time.

myelinated fast-conducting nerve fibers (A-wave; Fig. 2A) and a component evoked by unmyelinated slowly conducting fibers (C-wave) were visible. Thirty minutes after nerve crush no CAPs could be recorded across the lesion site, indicating that the crush had caused a complete conduction block of the nerve. The recordings made 15 days after crushing of the GS nerve showed an almost complete recovery of nerve conduction. At that time, both CAP components (A- and C-wave) were visible, thus indicating that the fibers had at least partly regenerated within the 15 days (Fig. 2B). Quantitative evaluation of the size of the CAP components was not undertaken.

Series 1: Sensory Nerve Regeneration. ***PPT.*** Com-

PPT. Compared with the GS muscle on the intact control side ("GS control side," right leg) the mean PPT of the GS muscle with the crushed nerve ("GS crush untreated," left leg) was higher (Fig. 3A). This difference was significant at days 1, 4, and 9 after GS nerve crush ($P < 0.01$). At day 12 after crush, the mean pain threshold of the GS

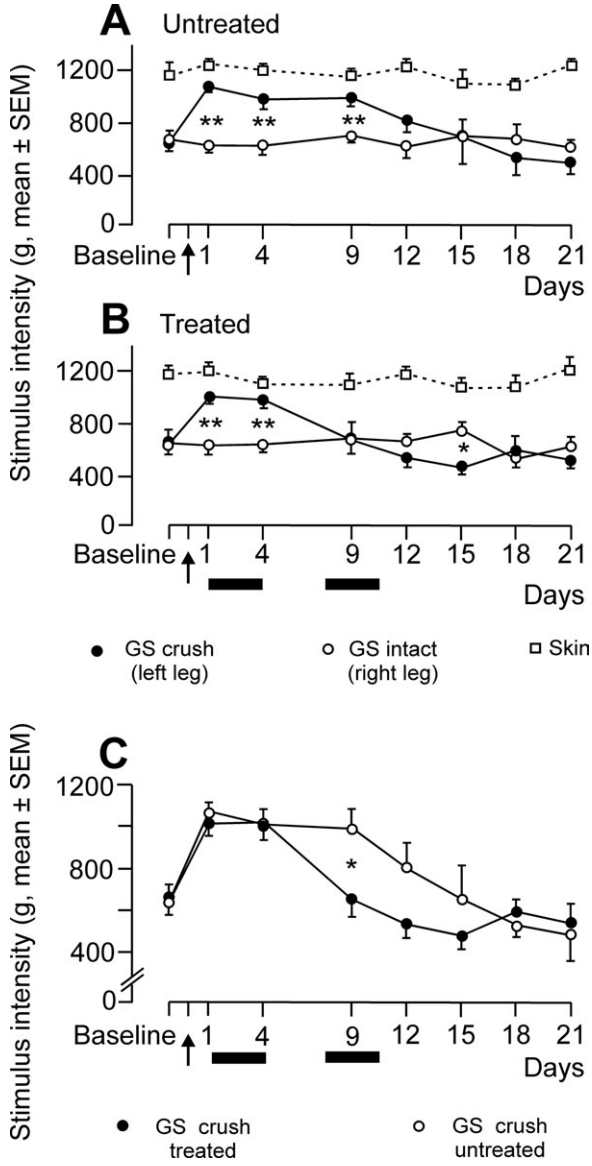

FIGURE 3. Determination of the pressure pain threshold (PPT). Y axis in all panels: mean pressure applied to the left and right GS muscle that led to hindlimb withdrawal. (**A**) Animals with a crushed left GS nerve, but not treated with shock waves (untreated). (**B**) Shock wave treated animals with a crushed left GS nerve (treated). Filled circles in (**A**) and (**B**) (GS crush): muscle with crushed nerves of the left hindlimb; open circles in (**A**) and (**B**) (GS intact): intact control muscle of the right hindlimb. Open squares in (**A**) and (**B**): PPT of the skin overlying the left GS muscle. (**C**) Comparison of untreated (open circles) and treated (filled circles) animals with the GS crushed. Upward pointing arrows indicate the time of crushing of the GS nerve. The bars underneath the x-axis in (**B**) and (**C**) mark the blocks of shock wave treatment. Asterisks show statistically significant differences between left and right muscle in (**A**) and (**B**), and between treated and non-treated animals in (**C**) ($^{*}P < 0.05$ and $^{**}P < 0.01$, Mann–Whitney *U*-test).

muscle no longer differed significantly from the intact control side, and at day 15 after crush the PPT had fully recovered.

The "GS crush treated" group still showed significantly higher PPTs compared with the "GS control side" at days 1 and 4 ($P < 0.01$), but at day 9 after GS nerve crush the PPT was the same as that of the "GS control side" (Fig. 3B). Fifteen days after nerve crush the "GS crush treated" animals even exhibited a significantly lower PPT. This indicated slight hyperesthesia in the shock wave treated rats compared with the "GS control side." The PPT of the skin overlying the GS muscle was never affected, either by the GS nerve crush or the shock wave treatment (Fig. 3A and B, uppermost curve in both panels).

The direct comparison between the "GS crush treated" and "GS crush untreated" groups showed a significant PPT difference at day 9 (Fig. 3C; $P < 0.03$), indicating that, in the treated rats, the PPT of the injured muscle had recovered faster. No significant differences were found when the PPTs of the GS muscles of the untreated and treated animals on the intact right side ("GS control side") were compared (see Fig. 3A and B, open circles).

Open Field Test. Both treated and untreated rats showed a drop in exploratory locomotor activity 1 and 4 days after nerve crush. After this period, the locomotor activity of the untreated rats remained at a low level, whereas the activity of the treated animals showed (not significant) signs of recovery (Fig. 4A).

Motor Coordination. Surprisingly, the performance on the rotating rod was not strongly affected by the nerve lesion. One day after nerve crush both the treated and untreated animals showed a trend toward better performance on the rod than before the crush. During the following days the untreated animals performed better than the treated ones. At day 12 after nerve crush this difference was significant ($P < 0.04$; Fig. 4B).

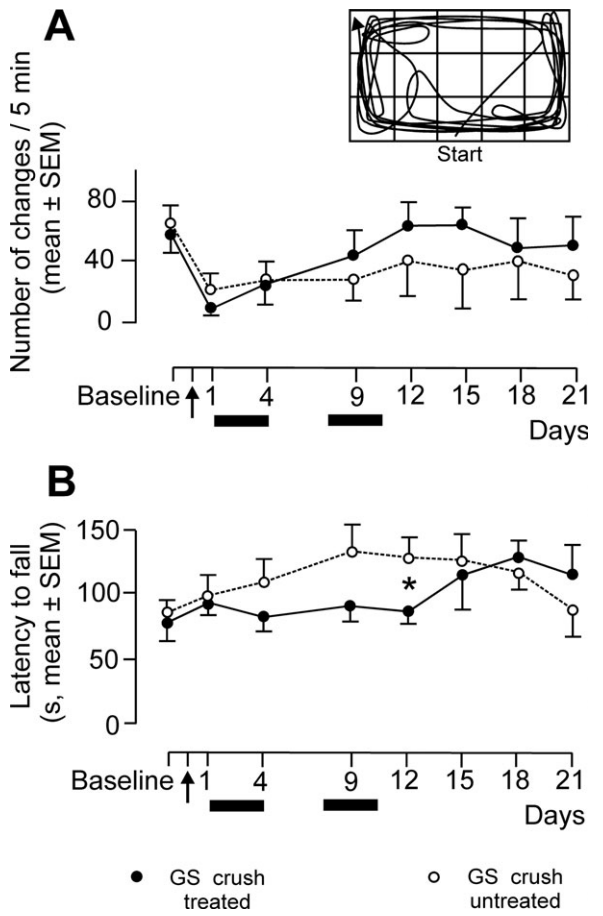

FIGURE 4. Behavioral tests. (**A**) Exploratory locomotor activity. Mean number of squares crossed during 5-min exploratory locomotion in the open field. The inset shows the open field arena with the path of an animal of the 'GS crush untreated' group 12 days after GS nerve crush. The bottom of the open field arena was divided into 15 squares of 20 × 20 cm^2 each. (**B**) Motor coordination on the rotating rod. y-axis: latency to fall from the rod. Upward pointing arrows in (**A**) and (**B**) indicate the time of GS nerve crush, and the bars underneath the abscissa indicate the 2 blocks of focused shock wave treatment. The asterisk indicates a significant difference between shock wave treated and non-treated animals (*$P < 0.05$, Mann–Whitney *U*-test).

Series 2: PGP 9.5-IR 6 Days after SU Nerve Crush. Figure 3B shows that, in the "shock wave treated" group, the PPT had fully recovered at 9 days after nerve crush, indicating that most of the regenerating nerve fibers had reached the muscle and resumed their function. To detect a possible difference in the speed of nerve regeneration between treated and untreated animals, a time window well before 9 days had to be chosen for the histological evaluation. A time of 6 days after nerve crush appeared to be appropriate for this purpose. Compared with the nerve section proximal to the lesion site (Fig. 5), in both the "SU crush treated" und "SU crush untreated" groups, smaller numbers of PGP 9.5-IR fibers were found in the nerve sections "distal 1," "distal 2," and "distal 3." In the most distal nerve section, "distal 3," the mean number of PGP 9.5-IR nerve fibers per tissue section was close to zero at 6 days after crush (Fig. 6). In the section "distal 1," the drop in fiber number was smaller in the "SU crush treated" group, but, compared with the proximal section, the difference was not significant ($P < 0.08$). In the "SU crush untreated" group this difference was significant ($P < 0.002$). When treated and untreated animals were directly compared, the "distal 1" section contained a statistically higher number of PGP 9.5-IR nerve fibers per tissue section in treated animals ($P < 0.02$). This finding indicates faster nerve regeneration after shock wave treatment.

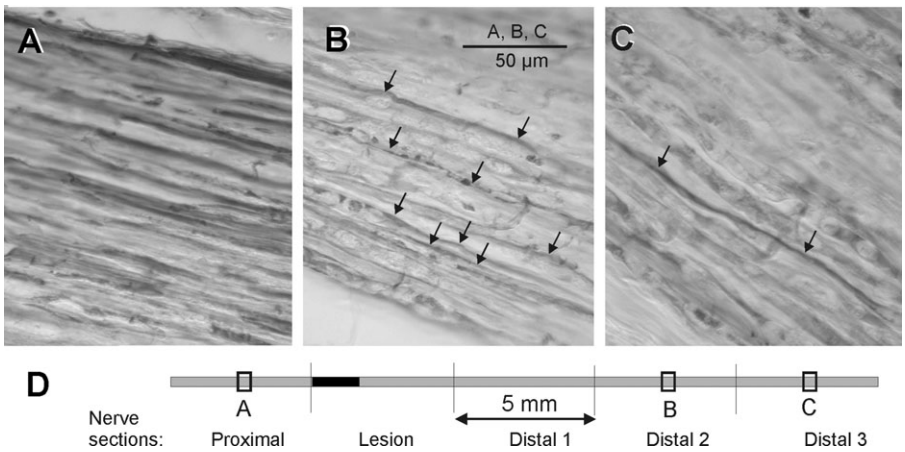

FIGURE 5. (**A**)–(**C**) PGP 9.5 IR nerve fibers 6 days after SU nerve crush and shock wave treatment. Histological sections from the same SU nerve proximal to the lesion (**A**), and 10 mm (**B**) and 15 mm (**C**) distal to the lesion [see scheme in (**D**)]. (**A**) Section is full of nerve fibers. Arrows in (**B**) and (**C**) indicate single nerve fibers labeled with PGP 9.5; in (**C**), only 1 nerve fiber is visible. (**D**) Location of the sections shown in (**A**)–(**C**).

DISCUSSION

These experiments show that shock wave treatment at a relatively low energy flux density can be beneficial for the treatment of a severe nerve lesion. The speed of nerve regeneration was higher and the recovery from functional deficits faster after treatment. The level of energy flux density appears to be crucial for the effect, because high-energy shock waves are known to destroy nerve fibers.[6]

The slight but significant hyperesthesia (drop of PPT) of the "GS crush treated" group 15 days after onset of treatment (Fig. 3B) was unexpected. A possible explanation is that the regenerating nerve fibers within the GS muscle were hyperexcitable to mechanical stimuli. Hyperexcitability of regenerating nerve fibers in the tongue was found starting 15 days after lesion of the lingual nerve in rats.[22] Likewise, the hyperexcitability of regenerating nerves associated with an increased level of nerve growth factor (NGF) has been reported in cutaneous nerve sprouts.[23] In this case, the hyperexcitability was found to be due to subthreshold membrane oscillations in the regenerating nerves.

The results of the behavioral experiments are hard to explain. In the open field test (Fig. 4A), the animals exhibited less spontaneous locomotor activity directly after GS nerve crush, and they recovered slowly. The initial drop in exploratory activity may have been due not only to the nerve crush but also to the wound in the popliteal fossa. No significant effect of the shock wave treatment could be found.

Interpretation of the results of the motor coordination test on the rotating rod poses even more problems. Surprisingly, locomotion on the rotating rod was not impaired by the nerve compression, although the animals could no longer contract the left gastrocnemius–soleus muscle. One possible explanation is that the animals, when forced to move by the rod, were able to compensate for the motor deficit by activating other muscles of the nerve-crushed or contralateral hindlimb.

For the finding that untreated animals stayed longer on the rotating rod than shock wave treated animals there is a less speculative interpretation. As stated in the Results section, 12 days after nerve lesion, when the functional deficits in the "GS crush treated" rats had resolved (at least with respect to the PPT; see Fig. 3B), this group performed worse on the rotating rod (the latency to

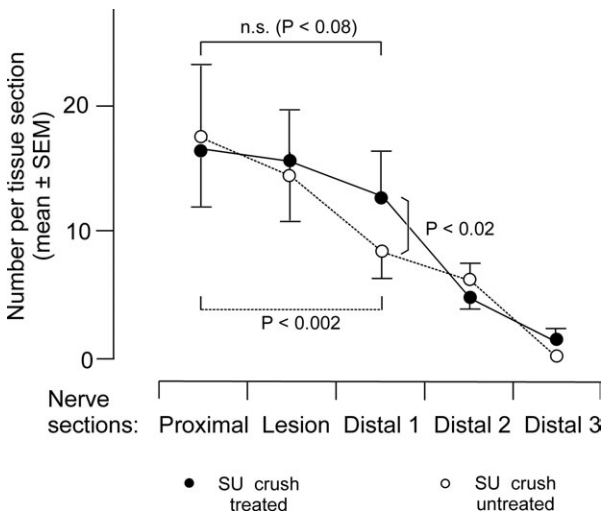

FIGURE 6. Comparison of the number of PGP 9.5–labeled nerve fibers 6 days after SU nerve crush. At each counting site (cf. Fig. 1D), 3 tissue sections per animal were evaluated (15 sections per data point). Open circles: 'SU crush untreated' group; filled circles: 'SU crush treated' group. Horizontal brackets indicate the level of significance between the proximal nerve section and section 'distal 1' (Wilcoxon test of paired data; n.s., not significant), the vertical bracket a statistically significant difference between treated and untreated animals in section 'distal 1' (Mann–Whitney *U*-test).

fall was significantly shorter; Fig. 4B). This finding may relate to the hyperexcitability seen at about the same time (15 days after GS crush) in the treated group. The hyperexcitability may have caused dysesthesia that irritated the animals, so they were less capable of fulfilling the complicated task of balancing on the rotating rod. However, it is also clear that the behavior of rats depends on a multitude of factors, not all of which can be controlled in our experiments.

The exact mechanisms underlying the accelerated regeneration and functional recovery seen in the treated animals are unknown. Because the treatment was directed at the popliteal fossa and lower leg, the main mechanisms were probably peripheral. Dorsal root ganglion (DRG) cells may have been indirectly involved, because there is axonal transport of signal molecules from the lesion site to the DRG and back.

Among the effects of shock waves on nerve tissue are membrane hyperpolarization and formation of free radicals,[24] but nothing is known about the possible role these changes may play under the conditions in our study.

More likely factors for the faster regeneration may be the changes in the microcirculation of the damaged nerve. In a case of femoral head necrosis, improved blood supply after shock wave treatment has been reported.[25] This effect may be due simply to vasodilation. A more powerful mechanism, particularly for severed nerve fibers, is enhanced neovascularization in the regenerating nerve. Such a shock wave effect has been reported by Wang and colleagues.[9] Enhanced neovascularization has been reported as a possible mechanism for the increased resorption of calcium deposits in tendons and joints after shock wave treatment.[8,9] In parallel with this effect, amelioration of pain has been described.[14]

At the molecular level, *de novo* angiogenesis appears to be associated with an increase in vascular endothelial growth factor A (VEGF[26]) and transforming growth factor-β1 (TGF-β[27]). Interestingly, these factors were found to increase growth and differentiation of mesenchymal cells after shock wave treatment in osteogenesis experiments. Shock waves have also been reported to induce expression of activating transcription factor 3 (ATF3) and growth-associated protein-43 (GAP-43) in DRGs.[11] ATF3 promotes neurite outgrowth in DRGs when the peripheral axon is injured.[28] Together with *de novo* angiogenesis, this latter mechanism could lead directly to faster regeneration of damaged nerve fibers, as found in our study.

In conclusion, with the exception of the behavioral locomotor tests, all sets of data obtained in our animal model of nerve injury suggest that focused shock wave treatment at low-energy flux density can promote and accelerate the regeneration of injured nerve fibers. For potential clinical applications, additional studies are needed that evaluate the mechanisms underlying the faster nerve fiber regeneration after shock wave treatment. Moreover, these studies will have to show that the shock wave therapy has no adverse effects and yields the same results in humans as it did in our animal model. If so, low-energy shock waves may represent an effective and non-invasive treatment in severe cases of nerve compression where fiber regeneration is necessary.

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