

AD-A246 410



AD _____

2

GRANT NO: DAMD17-90-Z-0008

**TITLE: LOW POWER LASER IRRADIATION EFFECT WITH EMPHASIS ON
INJURED NEURAL TISSUES**

**PRINCIPAL INVESTIGATOR: Michal Schwartz, Ph.D.
Michael Belkin, Ph.D.**

**CONTRACTING ORGANIZATION: The Weizmann Institute of Science
Office of Research Contracts
and Projects
Rehovot, 76100, Israel**

REPORT DATE: April 1, 1991

**DTIC
ELECTE
FEB 27, 1992
S B D**

TYPE OF REPORT: Midterm Report

**PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012**

**DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited**

**The findings in this report are not to be construed as an
official Department of the Army position unless so designated by
other authorized documents.**

92-04664



2 2 24 074

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 1 April 1991	3. REPORT TYPE AND DATES COVERED Midterm Report (3/1/90 - 3/31/91)	
4. TITLE AND SUBTITLE Low Power Laser Irradiation Effects with Emphasis on Injured Neural Tissues			5. FUNDING NUMBERS Grant No. DAMD17-90-Z-0008 62787A 3M162787A878.BA.211 WUDA346100	
6. AUTHOR(S) Prof. Michal Schwartz Prof. Michael Belkin				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Weizmann Institute of Science Office of Research Contracts and Projects Weizmann Institute of Science, Rehovot 76100, Israel			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick Frederick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) A few years ago, we have initiated the use of low power helium-neon (He-Ne) laser (632.8 nm) for the treatment of injured nerves of the central nervous system (CNS) and of the peripheral nervous system (PNS) (Rochkind <i>et al.</i> , 1987; Schwartz <i>et al.</i> , 1987; Assia <i>et al.</i> , 1989). In those early studies we demonstrated that the low energy He-Ne laser treatment, if commences immediately after injury, delays the posttraumatic degeneration but does not prevent it (Assia <i>et al.</i> , 1989) nor does it initiate axonal growth (Schwartz <i>et al.</i> , 1987).				
14. SUBJECT TERMS Low Power Laser, He-He; Optic Nerve Injury; Central Nervous System; RA 3; lab animals			15. NUMBER OF PAGES	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.



Accession For	
NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

A few years ago, we have initiated the use of low power helium-neon (He-Ne) laser (632.8 nm) for the treatment of injured nerves of the central nervous system (CNS) and of the peripheral nervous system (PNS) (Rochkind *et al.*, 1987; Schwartz *et al.*, 1987; Assia *et al.*, 1989). In those early studies we demonstrated that the low energy He-Ne laser treatment, if commences immediately after injury, delays the posttraumatic degeneration but does not prevent it (Assia *et al.*, 1989) nor does it initiate axonal growth (Schwartz *et al.*, 1987).

We have, therefore, suggested that the use of such treatment might be beneficial in: (i) cases of marginally injured axons which do not degenerate as a result of the primary lesion, but will degenerate as a secondary event; (ii) cases of severe injuries, in combination with a treatment modality which induces or facilitates growth. Indeed, we have demonstrated the most successful growth of injured adult rabbit optic nerve (a nonregenerative system) into their own degenerative environment, which is hostile to growth when treatment is omitted. A daily irradiated for 10-14 consecutive days combined with application at the time of the injury of soluble substances, shown to facilitate growth, resulted in abundant growth of axons from the retinal ganglion cells of the injured fibers of optic nerves, as well as from the cut tips of the injured fibers. The growing axons were characterized by parameters defined for growing axons and they traversed the site of the injury by a significant distance (Lavie *et al.*, 1990). During the last years, under the support of the U.S. Army, we have made progress mainly along two lines of research related to the use of laser for the treatment of injured CNS:

1. Calibration of dose and temporal parameters in mitigating injured optic nerve degeneration by low energy He-Ne laser irradiation.
2. Establishment of physiological measurements for the newly growing axons facilitated by the use of laser, in combination with daily irradiation with low energy He-Ne laser.

Our results along these two lines of research are as follows:

1. Calibration of dose and temporal parameters in mitigating injured optic nerve degeneration by low energy He-Ne laser irradiation

1.1. *Description of the experiments*

The experiments were performed on 232 3-4-month old male Sprague-Dawley rats, weighing 200-300 gm each. Anesthesia was induced with 40 mg/kg ketamine and 8 mg/kg xylazine, injected intraperitoneally before crushing the optic nerves, as well as prior to each irradiation, and with pentobarbitone 70 mg/kg, before the excision of the optic nerves.

The electrical activity of the nerves was determined by measuring the CAP, 2 weeks after injury. The reference CAP amplitudes of normal nerves (noninjured and nonirradiated) was measured from the 232 nerves of the left eyes (Table I). The control group included 38 rats (group A), in which the right optic nerve was crushed but not irradiated. Noninjured optic nerves of 10 rats were subjected to He-Ne laser irradiation for 14 days, and then the CAP was measured to evaluate the effect of laser irradiation on normal nerves (group B).

Optic nerves of 170 rats of the test groups C-R were subjected to a calibrated crush injury and then irradiated with low energy He-Ne laser for 4, 7 or 14 days, once or twice a day, beginning before or after the crush injury (Tables II and III). Noncoherent infrared light was used in groups S and T (Table IV). The Student's t-test was used for statistical analysis of the results.

a. *Crush injury*

Crush injury to the right optic nerve was performed under a binocular microscope as follows: after lateral canthotomy and incision of the conjunctiva, the lateral rectus and retractor bulbi muscles were displaced and the optic nerve was exposed by blunt dissection for 3 mm

distal to the eyeball. The nerve was then crushed for 30 sec, 2 mm distal to the eyeball, using a modified Castroviejo cross-action forceps, which was adjusted for a moderate compression power by the screw attached to its handle^{1,8}. Finally, the canthotomy was sutured with 6/0 nonabsorbable virgin silk suture, and 5% chloramphenicol ointment was applied to the wound.

b. *Irradiation*

The He-Ne irradiated rats (groups C-R) were irradiated by a Spectra Physics Stabilite model 124-B He-Ne laser (632.8 nm, 35 mw). The laser beam, 1.1 mm in diameters, was aimed by a mirror (Physik Instruments), perpendicularly on the middle of the right cornea. For irradiations of lower intensities, the energy of the laser beam was reduced to 10.5 mW and 3.5 mW by neutral density filters. The irradiation regimens were as detailed in Tables II and III. The first irradiation was given as follows: 1 h before injury (group C); 30 min before injury (group D); immediately before injury (group E); and immediately after the injury (groups F-N). The durations of the daily He-Ne laser irradiations were as follows: 1 min (group F); 2 min (groups C-E, G-H, L and O-R); 3 min (groups I and M); 4 min (group N), 5 min (group J); and 10 min (group K). The irradiations were performed once daily in groups C-P. Groups Q and R were irradiated twice daily for 2 min each time, with an interval of 10-12 h between the two daily irradiations.

For the infrared irradiation, we used a LED system of Amcor Electronics (904 nm). Irradiations were initiated immediately after injury and powers of 10 mW (group S) or 15 mW (group T) were used for 2 min (Table IV).

c. *Electrophysiological measurements*

After 2 weeks of daily irradiations, the optic nerves were removed for electrophysiological measurements. For this purpose, the skin was removed from the rat's skull with straight scissors and the optic nerves were detached from the eyeballs with curved

scissors. Total decapitation was performed and the skull opened with a rongeur. The brain was displaced laterally, exposing the intracranial portion of the optic nerves. Dissection at the level of the chiasm made possible the removal of the whole length of the optic nerves, which were then transferred to vials containing fresh cold Krebs' solution (125 mM NaCl; 5 mM KCl; 1.2 mM KH_2PO_4 ; 26 mM NaHCO_3 ; 0.6 mM MgSO_4 ; 2.4 mM CaCl_2 ; 11 mM D-glucose), aerated with 95% O_2 and 5% CO_2 . Nerves can be kept viable, while in these conditions for at least 3-4 h. After 1 h of recovery in the cold Krebs' solution, the nerves were immersed in Krebs' solution at 37°C for the electrophysiological recordings. The nerve ends were then connected to two Ag-AgCl suction electrodes immersed in the bathing solution. The stimulating pulse was given through the electrode on one end, and the action potential was recorded on the other end. A Grass SD-9 stimulator was used for electrical stimulation (2 volts, 50 microseconds). The signal was transmitted to a Medelec PA-63 preamplifier and then to AA-7-T amplifier and a Medelec MS-7 electromyograph. The solution, stimulator and amplifier had a common ground. The average amplitude of 8 recorded action potentials was photographed with a Polaroid camera. The recording was made essentially from the portion of the nerve distal to the injury site, i.e., between the site of injury and the optic chiasm, since the proximal portion was too short.

1.2. Results

The results are detailed in Tables I-IV. The compound action potential amplitude of the normal (noninjured) rat optic nerve was found to be $4585.3 \pm 1995.1 \mu\text{V}$, as recorded from 232 (left) nerves (Table I). The mean CAP of the crushed and nonirradiated nerves as measured 2 weeks after the injury in 38 rats (group A), was very low ($505.5 \pm 303.5 \mu\text{V}$), as compared with the normal contralateral left optic nerves ($p < 0.000$). He-Ne laser (35 W) irradiation of 10 noninjured nerves for 2 min for 14 consecutive days (group B) had no statistically significant effect on the action potential ($4850.0 \pm 1415.2 \mu\text{V}$).

He-Ne laser irradiation of 35 mW for 2 min daily and of 10.5 mW for 2 and 3 min daily (Table II – groups G, H and I, respectively) led to significantly higher retention of CAP amplitudes 2 weeks after the injury, as compared with the control (crushed and unirradiated) group ($p < 0.000$). Such laser irradiation caused significantly higher CAP retention, as compared with the nonirradiated injured nerves, also when it was initiated immediately before the crush injury (Table II – group E). However, when the first daily irradiation was performed either 1 h or 30 min before the crush injury it had no effect (Table II – groups C and D, respectively). Reduction of length of the daily irradiation for only 1 min (10.5 mW He-Ne laser irradiation, group F), instead of 2 or 3 min (groups H and I) or reduction of power to 3.5 mW instead of 10.5 mW for 2, 3 or 4 min daily (groups L, M and N, respectively), beginning immediately after injury, had no effect on the CAP recorded 2 weeks after injury, as compared to that of the injured nonirradiated rats (Table I – group A). By contrast, extending the length of the daily irradiation by a 10.5 mW He-Ne laser to 5 and 10 min daily (groups J and K, respectively) caused aggravation of the damage as evident by almost complete disappearance of the CAP. When the irradiations were given for 4 or 7 days, twice a day (Table III – groups Q and R), the CAPs recorded 14 days after injury were smaller, as compared to the groups which were treated once a day for such periods (groups O and P), and not significantly different ($p = 0.25$) from the results in the injured nonirradiated control group (group A).

Treatment with noncoherent infrared light of 10 mW and 15 mW led to an apparent change in the CAPs, which were lower than those of the nonirradiated injured nerves (Table IV – groups S and T).

1.3. Discussion

Low energy He-Ne laser irradiations of moderately crushed optic nerves of rats, for 14 days, was found to result in the distal part of the nerve in higher electrical activity measured by the CAP, as compared to the injured nonirradiated nerves^{1,20}. However, this effect was

temporary and eventual degeneration always occurred, even when the irradiations were continued¹.

In the present study, we also examined several parameters related to the laser irradiation, including length of daily irradiation, power and frequency, and noncoherent infrared light irradiation on the electrical activity of injured optic nerves of rats²⁰. This information complete the data obtained by previous experiments^{1,20}.

The results confirm that 10.5 mW He-Ne laser irradiation for 2 and 3 min, and of 35 mW He-Ne laser irradiation for 2 min once daily for 2 weeks, lead to maximal delay of the degeneration in the moderately crushed optic nerves of rats. Irradiations shorter than 2 min are less effective, and irradiations longer than 3 min cause aggravation of the optic nerve damage (Table II). Irradiations twice a day for 4 and 7 days, not only were not better than a repeated single daily irradiation, but also did not have any effect in delaying the degeneration of the injured nerves (Table III).

Previous investigations showed that the time at which the treatment commenced in relation with the injury is critical. The degeneration delaying effect is maximal when the irradiation is initiated either immediately before or immediately after the injury. Irradiation initiated 2 h after the crush were about half as effective as immediate irradiation, and no apparent effect was evident when the laser was applied for the first time 5 h or more after the injury¹. In the present study, we confirmed this observation and showed that the same treatment is significantly less effective when initiated 30 min or more before the injury.

The length of the treatment period is important as well. It was shown in a previous experiment¹ and confirmed in the present one, that this 14-day irradiation was slightly more effective than a treatment lasting 7 days, and significantly more effective than 4 days of irradiation.

Low energy laser irradiation was reported to have various effects on cells, tissues and organs, manifested in biochemical, morphological and physiological phenomena, and alteration

of growth and regenerative parameters. The effects seem to be particularly prominent on the hematological and immune systems, and on connective and neural tissues^{3,5,16}. There are few reports of the biochemical and morphological effects of low level irradiation on the neural system. Direct and indirect irradiations of the rat's brain caudate nucleus by either He-Ne or nitrogen ultraviolet laser caused significant changes in the concentration of various monoamines and amino acids in the corpus striatum²⁵. Irradiation of mice retinae with a 0.5 mW laser caused increase in protein synthesis¹⁸, photoreceptor outer segment shedding, and in the interreceptor matrix³¹. Laser irradiation was shown to stimulate neurons directly in animals^{10,17,27}, as well as in men²⁸. Different irradiation regimens, however, did not influence human nerves¹¹. The neural effects of low energy irradiation was used to relieve pain²⁹ and clonus³⁰ in various disease states, although some of these results were not confirmed¹¹.

The biological effects of low energy laser irradiation are not well defined. These effects seem to require an energy flux above a threshold, which is specific to each laser/tissue combination and the effect generally consists of tissue specific, dose-dependent stimulation or depression of biochemical, physiological or proliferative activity.

The mechanism underlying this effect is poorly understood. The most reasonable hypothesis is that the low energy laser, which can penetrate only few millimeters of tissue⁷, is absorbed by chromophores specific to each wavelength, without an appreciable rise in overall tissue temperature. When these chromophores are enzymes²³, organelles or cell membrane molecules, their activity is altered and, thus, the metabolism or membrane permeability to specific molecules are altered⁴. The action spectra required to prove that this is a photochemical phenomenon has been demonstrated for enzymes¹³.

In our model, the primary events induced by the laser irradiation probably occurred at the optic nerve rather than the retinal cell bodies, although the laser beam traversed the entire

eye. This assumption is based on the fact that the diameter of the irradiation beam on the retina is very small, due to its focusing by the refractive media of the eye. The light of the He-Ne laser penetrates a few millimeters behind the eye, and its scatter in the retrobulbar tissues accounts for diffuse irradiation of the optic nerve, including the crush site and its vicinity. Furthermore, direct irradiation of the damaged region through the skin behind the eye of rabbits, after orbitotomy, yielded similar histological results like those caused by irradiation via the eye²².

According to our experiments, it seems that the 632.8 nm wavelength of the He-Ne laser is fortuitously suitable for inducing the desired effect of delaying the posttraumatic degeneration in our rat CNS model. This is probably a result of this wavelength being capable of penetrating behind the eyeball and absorbed in the appropriate chromophore, possibly in the glial cells. The results also demonstrated the dose-dependent effect of the laser irradiation with high doses, resulting either from long daily irradiation (5 min and 10 min), from high frequency (twice a day) being injurious, and lower doses (low power or short duration) being more effective. While the noncoherent infrared irradiation failed to alter the course of the posttraumatic degeneration of the nerve, it is not conclusive that this wavelength is unsuitable for the purpose, since energy densities irradiated were much lower than that of the He-Ne laser irradiation.

Viability of neuronal tissue demands morphological integrity. When the integrity of a CNS axon is anatomically or morphologically interrupted, the distal segment of the axon which was disconnected from its cell body, degenerates (Wallerian degeneration). Consequently, the cell body fails to reestablish the contact with its end organ and, subsequently, degenerates as well. The posttraumatic degeneration delaying effect of the low energy laser irradiation can be potentially utilized to facilitate regeneration before irreversible degeneration and scarring sets in²¹. This beneficial effect has been exploited successfully in treatments aiming at regeneration¹⁴. More research is required to find ways to use the degeneration delaying effect

of low energy laser in a combined treatment to induce regeneration.

2. Establishment of physiological measurements for the newly growing axons facilitated by the use of laser, in combination with daily irradiation with low energy He-Ne laser

2.1. *Description of the experiments*

The experiments were performed on 42 adult rabbits (albinos, Weizmann Institute Animal House) 5-12-month old, weighing 2.2-3 kg each. The study included four groups (see Table V).

a. *Surgery for injury of the optic nerve and implantation of conditioned medium*

Animals were anesthetized using 35 mg/kg ketamine and 6 mg/kg xylazine injected intramuscularly.

The left optic nerve was exposed under a dissecting microscope, as was described previously^{24,25}, and injured at a distance of 4-6 mm from the eyeball by complete transection, using a sharpened dissecting needle. The meningeal membrane was intentionally partially spared to ensure continuity of the nerve and to permit placement and retention of the nitrocellulose film, described below. A nitrocellulose film, 2-3 mm long by 1 mm wide, was inserted at each site of injury. In the injured control group (Table V – B) and in the injured group, which received irradiation only (Table V – C), the nitrocellulose was soaked in serum-free medium (DMEM, Gibco) for 1 h and inserted into the lesion site. In the experimental group, which was treated by the combined method (Table V – D), the nitrocellulose was soaked in CM (100 mg protein/ml) for 1 h before being inserted into the lesion site.

b. *Preparation of conditioned medium*

Carp (*Cyprinus carpio*, 800-1200 gm) were purchased from Tnuva, Israel. The fish were deeply anesthetized with 0.05% tricaine methanolsulfonate (Sigma). Optic nerves were crushed intraorbitally, as previously described²² and the fish replaced in their tanks. Eight days later, the fish were reanesthetized. The crushed regenerating nerves were dissected out, transferred into serum free medium (DMEM, Gibco) and incubated for 1 h at room temperature. CM was then collected and stored at -20°C²¹. The protein content of the medium was determined by the Bradford method. The medium was diluted to 100 mg protein/ml. From this stock solution, 5-10 µl were used for each piece of nitrocellulose.

c. *Irradiation*

The He-Ne laser irradiated rabbits (Table V – C and D) were irradiated transocularly by a Spectra Physics Stabilite model 124-B He-Ne laser (632.8 nm, 35 mw). The laser beam, 1.1 mm in diameter, was directed at the pupil of the left eye. The first 5 min irradiation was given within 30 min after the injury. Repeated irradiations were given to groups C and D once a day, for 10 consecutive days²¹.

d. *Electrophysiological measurements*

Eight weeks after injury, the optic nerves were removed for electrophysiological measurement. Lateral canthotomy was performed on deeply anesthetized animals. The skin was removed from the rabbit skull; the skull opened with a rongeur and the bone between the eyes was removed; the brain was then displaced from above the orbits and the intracranial portion of the optic nerves were exposed from the optic foramen to the optic chiasm. Using the rongeur, the orbital roof was then removed, so that the whole length of the optic nerve from the eyeball to the chiasm could be taken out. After the conjunctiva and the muscles from the eyeball were dissected away starting from the limbus, the eyeball was elevated with the optic

nerve which was then transected from the chiasm. The inevitable bleeding during this procedure caused considerable difficulty during surgery. The optic nerve, still connected to the eyeball, was immersed in fresh cold Krebs solution (125 mM NaCl; 5 mM KCl; 1.2 mM KH_2PO_4 ; 26 mM NaHCO_3 ; 0.6 mM MgSO_4 ; 2.4 mM CaCl_2 ; 10 mM D-glucose, aerated with 95% O_2 and 5% CO_2). While in the solution, the tissues surrounding the optic nerve were removed and the nerve was cut from the eyeball. After 1 h of recovery in the cold Krebs solution, the nerves were transferred to a vial of warmed (37°C) Krebs solution where cleaning of the optic nerve was completed, using a dissecting microscope. The nerve ends were then connected to two Ag-AgCl suction electrodes immersed in the bathing solution. A stimulating pulse was given through the electrode on one end, and the action potential was recorded on the other end. A Grass SD-9 stimulator was used for electrical stimulation (4 volts, 50 μsec). The signal was transmitted to a Medelec KS-7 preamplifier and then to an AA-7-T amplifier and a Medelec KS-7 electromyograph. The electrodes, stimulator and amplifier had a common grounding. The average amplitude of eight recorded potentials was photographed with a Polaroid camera.

2.2. Results

Nerves from three control groups and one experimental group were excised and measured electrophysiologically using the suction electrodes. Two parameters were measured: the amplitude of the action potential and its delay period.

Uninjured control nerves (Table V – A) were subdivided into two groups: (i) optic nerves of naive untreated animals, whose mean action potential was $1676 \pm 859 \mu\text{V}$ ($\pm\text{SD}$; $n=13$) [Table VI – A(a)]; and (ii) right optic nerves of animals, belonging to groups B-D in Table I, whose left optic nerves were treated. This group mean action potential was $1824 \pm 1009 \mu\text{V}$ ($\pm\text{SD}$; $n=19$) [Table VI – A(b)]. The mean CAPs of these two subgroups were not different. Measurements of CAPs of injured animals belonging to all operated groups was

carried out 8 weeks after injury. All measurements of the injured controls resulted in values which were below detectable levels (Table VI – B). The same results were obtained from injured nerves which received irradiation only (Table VI – C). In group D, which included 17 experimental animals, injured and treated with the combination of laser irradiation and the soluble substances derived from the regenerating fish optic nerves, different results were obtained. In 5 out of 17 examined animals, a positive, though low, compound action potential was measured (20-30 μ V). This activity was further characterized by a long delay period (mean=1.7 msec) (Table VI – D), which is significantly longer ($p<0.01$) than of the control groups.

2.3. Discussion

We have previously shown that treatment of injured adult rabbit optic nerves, with low energy He-Ne laser irradiation combined with application of soluble substances derived from regenerating fish optic nerves, leads to growth of axons within their own degenerative environment¹⁵. In the present study we show that these axons are electrophysiologically active, which provides further support to the notion that they are newly growing axons.

We showed in our previous work that in injured adult rabbit optic nerves treated with low energy He-Ne laser and soluble substances derived from regenerating fish optic nerves, there are abundant nonmyelinated and thinly myelinated axons, which traverse the site of injury and extend a significant distance distally. We provided morphological and immunocytochemical evidences that these are newly growing axons, originating from the retinae and embedded in CNS glial cells. In the present study, 5 out of the 17 treated nerves exhibited electrophysical activity. It is possible that more than 5 were active but that the others escaped detection; the nerve segments used for measurements included parts of the nerve both proximal and distal to the site of injury. There may have been cases in which successfully growing nerves traversed the site of injury but did not extend as far as where the recording

electrode was placed. With the available data we can conclude that at least 5 nerves were electrophysiologically active.

The low CAP of the 5 electrophysiologically active nerves of the experimental group is reasonable, considering the small number of viable axons which one can expect to be at the far distal region of the treated nerves. In previous studies¹⁵, we observed that about 5% of the number of axons in a normal uninjured nerve traversed the site of injury and were present 2 mm distal to the site of injury. The number of such axons gradually decreased upon increasing distance from the site of injury. As normal nerves gave a mean compound action potential of 1600-1800 μ V, one could not expect more than was found in the treated nerves which had, at the most, 5% of the axons present in an intact nerve. The uniqueness of these axon CAPs, however, is in their prolonged delay time period, which is typical of nonmyelinated and thinly myelinated axons, and not heavily myelinated axons, indicating that these are not spared axons but newly growing axons.

These results provide encouragement that the approach taken in this study, of induction of growth of axons within their own environment, is a valid one. Further studies are currently being carried out using *in vivo* electrophysiological recordings.

REFERENCES

- 1 Assia, E., Rosner, M., Belkin, M., Solomon, A. and Schwartz, M., Temporal parameters of low energy laser irradiation for optimal delay of posttraumatic degeneration of rat optic nerve, *Bran Res.*, 476 (1989) 205-212.
- 2 Attardi, D. G. and Sperry, R. W., Preferential selection of central pathways by regenerating optic nerve, *Exp. Neurol.*, 7 (1963) 46-64.
- 3 Basford, J. R., The clinical status of low energy laser therapy in 1989, *J. Laser App.*, 2 (1990) 57-62.
- 4 Belkin, M. and Schwartz, M., New biological phenomena associated with laser irradiation, *Health Phys.*, 56 (1989) 687-690.
- 5 Belkin, M., Zaturunsky, B. and Schwartz, M., A critical review of low energy laser bioeffects, *Lasers Opth.*, 2 (1988) 63-71.
- 6 Clemente, C. D., Regeneration in the vertebrate central nervous system, *Int. Rev. Neurobiol.*, 6 (1964) 257-301.
- 7 Doiron, D.R., Svaasand, L.O. and Profio, A.E., Light dosimetry in tissues, *Adv. Exp. Med. Biol.*, 160 (1981) 67-76.
- 8 Duvdevani, R., Rosner, M., Belkin, M., Sauter, J., Sabel, B. A. and Schwartz, M., Graded crush of the rat optic nerve as a brain injury model: Combining electrophysiological and behavioral outcome, *Rest. Neurol. Neurosci.*, in press.

- 9 Erlich, M., Kaplan, S., Ben-Bassat, S., Belkin, M. and Schwartz, M., Low energy laser irradiation and the consequences of neural trauma, *Laser Life Sci.*, 2 (1988) 3-5.
- 10 Fork, R. L., Laser stimulation of nerve cells in *Aplysia*, *Science*, 171 (1971) 907-908.
- 11 Greathouse, D. G., Currier, D. P. and Gilmore, R. L., Effects of clinical infrared laser on superficial radial nerve conduction, *Phys. Ther.*, 65 (1985) 1184-1187.
- 12 Guth, L. and Windle, W., The enigma of central nervous system regeneration, *Exp. Neurol. (Suppl.)*, 5 (1970) 1-43.
- 13 Karu, T. I., Photobiological fundamentals of low power laser therapy, *IEEE J. Quantum Elect.*, 10 (1987) 1703-1717.
- 14 Kiernan, J. A., Hypothesis concerned with axonal regeneration in the mammalian nervous system, *Biol. Rev.*, 54 (1979) 155-197.
- 15 Lavie, V., Murray, M., Solomon, S., Ben-Bassat, S., Belkin, M., Rumelt, S. and Schwartz, M., Growth of injured rabbit optic axons within their degenerating optic nerve, *J. Comp. Neurol.*, 298 (1990) 1-22.
- 16 Mester, E., Mester, A. F. and Mester, A., The biomedical effects of laser application, *Laser Surg. Med.*, 5 (1985) 31-39.
- 17 Olson, J. E., Schimmerling, W. and Tobias, C. A., Laser action spectrum of reduced excitability in nerve cells, *Brain Res.*, 204 (1981) 436-440.

- 18 Poon, A. M. L. and Yew, D. T., The effect of low dose laser on the functional activities of the mice retina, *Anat. Anz. (Jena)*, 148 (1980) 236-244.
- 19 Rochkind, S., Barrnea, L., Razon, N., Bartal, A., Nissan, N. and Schwartz, M., Stimulatory effect of He-Ne low dose laser on injured sciatic nerves of rats, *Neurosurgery*, 20 (1987) 843-847.
- 20 Rosner, M., Belkin, M., Erlich, M., Friedman, J. and Schwartz, M., Low energy laser irradiation prevents the early morphological and electrophysiological effects of optic nerve injury. In W. Waidelich and R. Waidelich (Eds.), *Laser Optoelectronics in Medicine, Proceedings, 7th Congress, International Society for Laser Surgery and Medicine*, Springer-Verlag, New York, 1988, pp. 703-705.
- 21 Schwartz, M., Belkin, M., Harel, A., Solomon, A., Lavie, V., Hadani, M., Rachailovich, I. and Stein-Izsak, C., Regenerating fish optic nerves and regeneration-like response in injured optic nerves of adult rabbits, *Science*, 228 (1985) 600-603.
- 22 Schwartz, M., Doron, A., Erlich, M., Lavie, V., Ben-Bassat, J., Belkin, M. and Rochkind, S., Effect of low-energy He-Ne laser irradiation on posttraumatic degeneration of adult rabbit optic nerve, *Laser Surg. Med.*, 7 (1987) 51-55.
- 23 Shen-Zeng, Xiao-Jian, Shu-Zhi, and Li-Hua, W., Effects of low power laser beam guided by optic fiber in rat brain striatal monoamines and amino acids, *Neurosci. Lett.*, 32 (1982) 203-208.
- 24 Solomon, A., Belkin, M., Hadani, M., Harel, A., Rachailovich, I., Lavie, V. and

- Schwartz, M., A new transorbital surgical approach to the rabbit's optic nerve, *J. Neurosci. Meth.*, 12 (1985) 259-262.
- 25 Solomon, A., L vie, V., Ben-Bassat, S., Belkin, M. and Schwartz, M., A new surgical approach to creating conditions conducive for regeneration of injured mammalian axons within their own degenerative environment, *J. Neural Transpl.* (1990) submitted.
- 26 Spector, W. S., *Handbook of Biological Data*, Saunders, Philadelphia, 1956, p. 34.
- 27 Vizi, E. S., Mester, E., Tisza, S. and Mester, A., Acetylcholine effects of laser irradiation to Auerbach plexus, *J. Neurol. Transm.*, 40 (1983) 305-308.
- 28 Walker, J. B., Relief from chronic pain by low power laser irradiation, *Neurosci. Lett.*, 43 (1983) 339-344.
- 29 Walker, J. B., Temporary suppression of clonus in human by brief photostimulation, *Brain Res.*, 340 (1985) 109-113.
- 30 Walker, J. B. and Akhanjee, L. K., Laser induced somatosensory evoked potentials: Evidence of photosensitivity in peripheral nerves, *Brain Res.*, 344 (1985) 281-285.
- 31 Yew, D.T., Ling Wong, S.L. and Chan, Y.A., Stimulatory effect of the low dose laser – a new hypothesis, *Acta Anat.*, 112 (1982) 131-136.

Table I. CAP as recorded 2 weeks after crush injury, without any treatment, and after 2 weeks of He-Ne laser irradiations of noninjured optic nerves, as compared to the CAP of normal rat optic nerves

Group	Rats	Irradiation		Action potential	Significance	
		mW	min	amplitude	(p)	
	Normal, noninjured					
	nonirradiated	232	—	—	4585.3±1995.1	—
A	Injured, nonirradiated					
	(control)	38	—	—	505.5± 303.5	p<0.000
B	Noninjured,					
	irradiated	10	35	2	4850.0±1415.2	p=0.679

The results are expressed by average (μV) $\pm$ SD of the amplitudes of the action potentials. The significance of the difference from the normal group of noninjured nonirradiated nerves is expressed by p value of the Student's t-test.

Table II. *The timing and duration parameters of He-Ne irradiation and their effect on the CAP measurements, 2 weeks after injury to optic nerves of rats*

Group	Number of rats	Irradiation		Action potential		Significance
		Power (mW)	Duration (min)	amplitude (μV)		(p)
C*	3	10.5	2	226.7±	25.2	p<0.25
D**	3	10.5	2	166.7±	28.9	p<0.1
E***	4	10.5	2	1700.0±	627.2	p<0.000
F	5	10.5	1	260.0±	114.0	p<0.1
G	10	35.0	2	1950.0±	1183.0	p<0.000
H	25	10.5	2	1784.0±	717.5	p<0.000
I	8	10.5	3	1950.0±	711.1	p<0.000
J	4	10.5	5	nonrecordable		—
K	5	10.5	10	40.0±	89.4	p<0.001
L	4	3.5	2	375.0±	150.0	p<0.5
M	5	3.5	3	320.0±	175.4	p<0.25
N	3	3.5	4	150.0±	86.6	p<0.05

Irradiation was initiated immediately after injury in all groups except C-E. The results are expressed by average (μ V) $\pm$ SD of the amplitudes of the action potentials. The significance of the difference from the injured nonirradiated nerves (Table I – group A) is expressed by the p value of the Student's t-test. * 1 h preceding the injury; ** 1/2 h preceding the injury; *** immediately before the injury.

Table III. *The effect of the frequency of He-Ne irradiations on the CAP measurements, 2 weeks after injury to optic nerves of rats*

Group	Number of rats	Irradiation		Action potential amplitude (μ V)	Significance (p)
		Days	Times per day		
O	14	7	1	1089.3 $\pm$ 215.7	p<0.001
P	16	4	1	1004.7 $\pm$ 264.0	p<0.001
Q	11	7	2	336.4 $\pm$ 130.6	p<0.25
R	11	4	2	631.8 $\pm$ 485.9	p<0.25

Irradiation was initiated immediately after injury. The results are expressed by average (μ V) $\pm$ SD of the amplitudes of the action potentials. The significance of the difference from the injured nonirradiated nerves (Table I – group A) is expressed by the p value of the Student's t-test.

Table IV. *CAP measurements 2 weeks after noncoherent infrared light irradiation of injured optic nerves of rats*

Group	Number of rats	Irradiation		Action potential amplitude (μ V)	Significance (p)
		Power	Duration (min)		
S	5	10	2	nonrecordable	—
T	5	15	2	76.9 $\pm$ 169.9	p<0.01

Irradiation was initiated immediately after injury. The results are expressed by average (μ V) $\pm$ SD of the amplitudes of the action potentials. The significance of the difference from the injured nonirradiated nerves (Table I – group A) is expressed by the p value of the Student's t-test.

Table V. *Animals studied in this work*

Group	Treatment	Number of animals
A	Normal uninjured control	32 *
B	Injured untreated control	8
C	Injured irradiated only	4
D	Combined treatment	17

* 13 out of the 32 nerves in this group were of naive untreated animals [see Table II – A(a)], while 19 were the right optic nerves of animals whose left optic nerves were treated [see Table II – A(b)].

Table VI. *Electrophysiological activity of axons in injured nerves treated with low energy laser, which slows down degeneration, and application of factors which facilitate regeneration*

Group	Treatment	Compound action potential (mean) (μ V)	Delayed response time * (mean) (msec)
A(a)	Intact nerve	1676.0 $\pm$ 858.9 (n=13)	0.52 $\pm$ 0.18 ^a
A(b)	Contralateral (intact)	1823.6 $\pm$ 1008.6 (n=19)	0.42 $\pm$ 0.12
B	Injured nontreated (control)	Nondetectable (n=12)	
C	Injured irradiated only	Nondetectable (n=4)	
D	Injured treated	20-30 (5 out of 17)	1.72 $\pm$ 0.45 ^b *

* ^a is different from ^b with p value < 0.01.

FUTURE PLANS

In the future, we are planning to extend our studies along the following lines of research:

- a. Establish the morphological manifestations of the laser effect on the injured nerves and thereby get an insight as to how it contributes to facilitation of growth, when combined with soluble substances.
- b. Examine noncoherent light, as well as coherent light, at other wavelengths.
- c. Further extend our studies related to regeneration in which laser is combined with the application of the soluble substances. Hopefully, to find out a combined treatment cascade, which will bring the growth into completion.
- d. Get a biochemical insight to the mechanism underlying the effect of the laser on the injured tissues. Enzymes involved with clearance of free radicals, will be examined. This issue will be studied biochemically and histologically.