Acta Neurochir (Wien) (1986) 83: 125–130

Acta Neurochirurgica

Electrophysiological Effect of HeNe Laser on Normal and Injured Sciatic Nerve in the Rat *

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Summary

The effect of low energy CW HeNe laser irradiation on normal and dissected nerves in the rat was examined. The methods are described. Results are compared to the laser effect on other living tissues. HeNe irradiation was found to increase significantly the action potentials of the nerves. It was found to be a long-lasting effect, keeping an increase in the nerves action potential for more than eight months after irradition has been stopped. A possible explanation for the way the irradiation acts on the nerve is suggested.

Keywords: HeNe laser; nerve regeneration; action potential.

Indroduction

The use of lasers as cutting tools in industry, medicine and research is well known. On the other hand, the low energy laser is making its first appearance in biological and medical research as a stimulating and therapeutical tool. Pioneering work on patients habe been performed mainly in eastern Europe. Epithelial growth was enhanced for gynaecological purposes [6] and ENT purposes [10], deep cutaneous ulcers were treated nonsurgically [8]. The influence of low energy laser irradiation—LELI—on wound healing was tested in animals as reported in various works [2, 4, 8, 10, 13]. The effect of LELI on the nervous system has been studied by some authors [9, 11, 14] with growing interest. Its effect on normal brain and gliomas in rats has been reported recently [7].

The most important problem in the nervous system, either PNS or CNS, is how to prevent anterograde degeneration and maintain the target organs viable following a nerve injury as well as to preserve the proximal stump from retrograde degeneration, thus minimizing disability. The enhancement of faster peripheral nerve regeneration is another major medical goal in neurological disciplines. In the work presented in this paper we are trying to reach toward these two goals using HeNe LELI.

In order to asses the effect of HeNe LELI on living nerve we measured Action Potentials (APs) in the vicinity of normal sciatic nerves in rats before and after laser irradiation (the normal group N 1). A definite increase in APs was observed on the irradiated side. In another group of rats the sciatic nerves on both sides were cut (Paralytic group P 2), and a sharp decrease in APs was measured. Irradiation of one side caused the APs in this limb to decrease to a rate slower than in the non irradiated limbs. This effect was found to be long-lasting in both groups, keeping the beneficial effect of HeNe LELI at least for 8 months.

One of the major problems discussed in all works dealing with HeNe LELI is the way or ways in which it affects living tissues. We made two further contributions toward the understanding of these processes. We found the time/energy dependance of HeNe LELI effect, and we measured the penetration of HeNe radiation into living tissues.

Method

The experiments were conducted at the Tel-Aviv Medical Center, using white Sprague-Dawley rats. All rats were healthy 2–4 months old, weighing circa 300 g.

The rats were anaesthetized using intraperitoneal diluted Nembutal 30 mg/100 g weight. They were all shaven on both thighs along the area covering the sciatic nerves.

The rats were divided into 4 normal and 2 paralysed (cut) groups. The normal groups consisted of 12 rats that did not have any further treatment prior to radiation. The cut groups consisted of 6 rats in

* This work was supported by a joint grant from the Ministry of Industry and Commerce and Tel-Aviv University, Israel.

which the sciatic nerve was cut and dissected bilaterally creating a 0.5 cm gap between the stumps. The nerves were exposed through a minimal skin opening and the cutting was executed using a surgical scalpel (no. 15). The skin wound was sutured with atraumatic 3/0 catgut sutures.

Once a rat was ready for testing, two bipolar platinum needles (Medelec Co.) were introduced transcutaneously to the vicinity of the sciatic nerves using the same needle length (depth of needle penetration) in all animals. The proximal needle, in the hip, was connected to a Grass stimulator (Grass Co., Quincy, Mass. Model Sye) delivering 1V pulses to the rat at 1 Hz frequency. The distal needle was in the Gastrocnemius muscle, connected to a Dynograph type RM Recorder 700 (Beckman Co.) working at the 1000 μV/cm range.

In one normal group (N 1, 3 rats) and one Paralytic group (P1, 3 rats) the nerves in both legs were stimulated and recorded prior to irradiation. The stimulation (1 V, 1 Hz) and recording lasted 20 seconds, followed by 100 seconds rest. This sequence was repeated 5 times. The readings served as normal recording, base lines for the following tests.

The right legs of all rats of both groups (N1, P 1) were irradiated using a 16 mW CW HeNe laser (Aerotech Inc.) delivering approximately 8 J/cm^2 to the closed skin, overlying the sciatic nerve between the needles. The left legs of all rats were not irradiated and were used as selfcontrol.

The same radiation procedure was repeated every day for the first 20 days. The nerves activity was recorded on the 1st, 3rd, 7th, 14th, 20th, 30th, 60th, 120th, 180th and 240th days following the first irradiation day, with the rats anaesthetized on each radiation and/or recording session.

A second normal group (N 2, 4 rats) was used for the analysis of the accumulative effect of laser energy caused by a single continuous irradiation session lasting up to 30 minutes. The anaesthesia and shaving were identical to the other groups, as was the technique and equipment used. Only the right leg was monitored in each rat. The recording started with 10 minutes preradiation as in the other rats. The rats in this group N 2 were irradiated for one half hour, while recording the APs after 1, 3, 7, 10, 14, 21, and 30 minutes.

Another normal group (N 3, 3 rats) and another paralytic group (P 2, 3 rats) were tested as controls. The normal rats did not have any treatment at all except for shaving and anaesthesia, while the "cut" rats had their sciatic nerves cut on both sides in a procedure identical to the first paralysed group P1. The rats in both groups were anaesthetized and action potentials were recorded at the same time intervals as in group P 1, *i.e.* the 1st, 3rd, 7th, 14th, 21st and 30th days, with no irradiation at all.

Each record in all rats was analysed manually and the average value for 5 consecutive pulses was used as the representative result of the experiment.

Two normal rats were tested as a special group (N 4) in order to assess the penetration of LELI. The rats were anaesthetized and shaven as all other rats. The skin and quadriceps muscles covering the sciatic nerves on both limbs were dissected; the thickness was measured. The HeNe LELI was applied to the skin, and a power meter (Coherent, Model 212) was used to record the intensity of the radiation penetrating the skin and muscle.

Results

The data was collected and analysed in the Tel-Aviv Medical Center and the results are presented in the following.

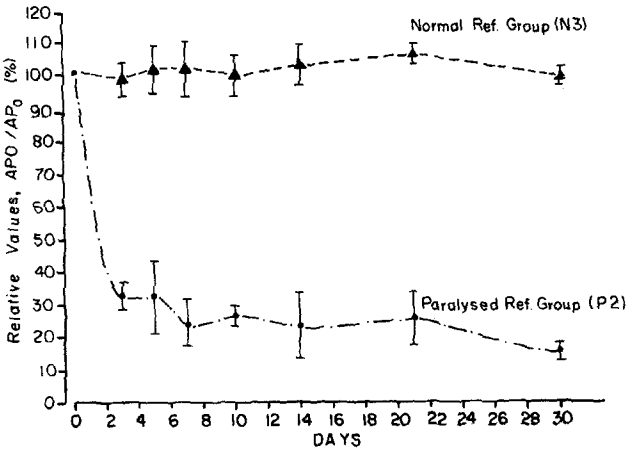

Fig. 1. APs compared to the Norm (AP) in percentage for both normal reference group N 3 and paralysed reference group P 2

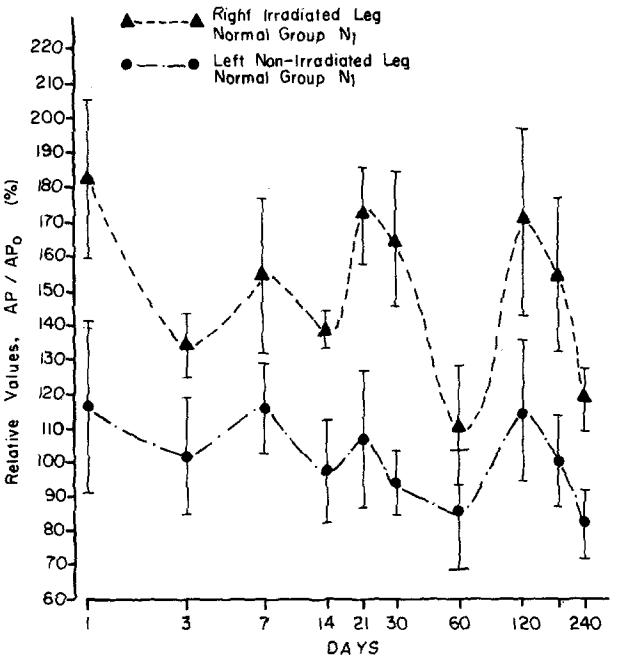

Fig. 2. APs compared to the norm (AP') in percentage, for both legs in the normal irradiated group N 1

Fig. 1 presents the results from the two reference groups: 3 normal rats (N 3) and 3 rats with their sciatic nerves cut on both sides (P 2). The two groups did not have any irradiation. The results are compared with the APs recorded in the same rats on the very first occasion (day 0), taken as the norm. The repeatability is indicated by the results in the normal group, while the paralysed group is demonstrating the expected decline of APs of the nerves.

The results from the normal irradiated group N 1 are recorded in Fig. 2. The APs were measured and com-

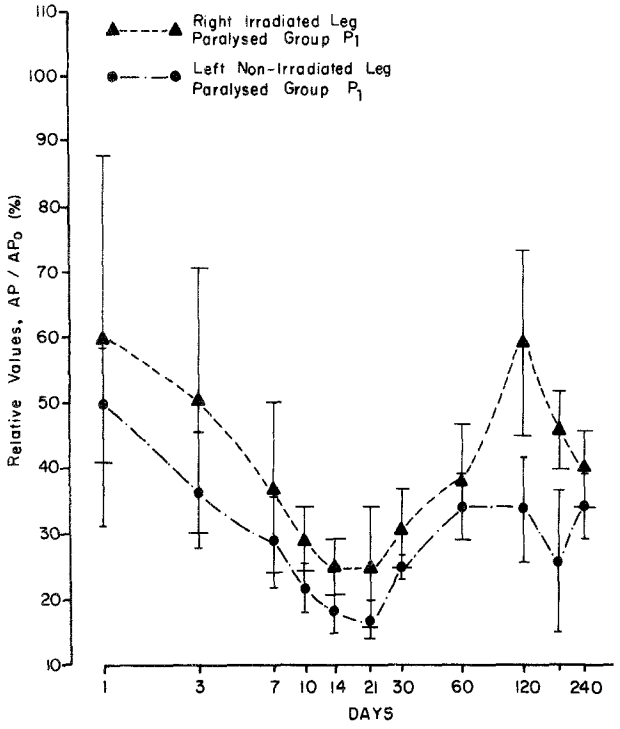

Fig. 3. APs compared to the norm (AP) in percentage for both legs in the paralysed group P 1

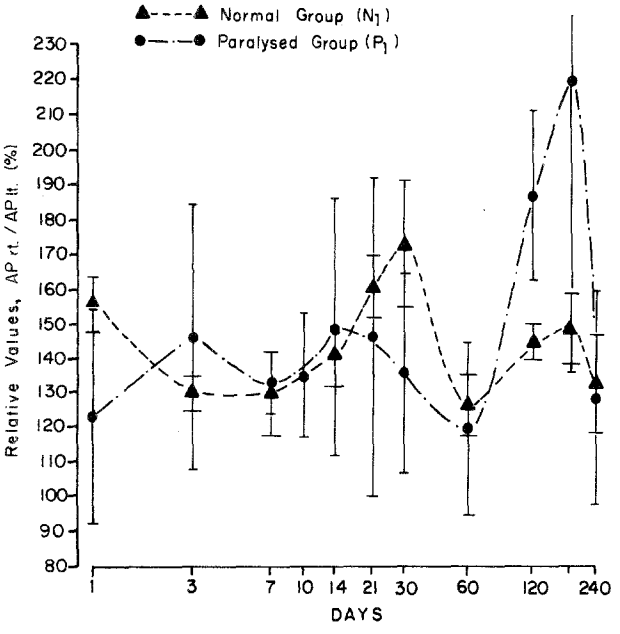

Fig. 4. APs in right, irradiated leg compared to APs in left, nonirradiated leg in percentage, for both normal and paralysed groups N 1 and P 1

pared to the APs taken before the first irradiation—the norm. The results in the left non-irradiated limb are similar to the normal, while in the right irradiated limb they are significantly higher.

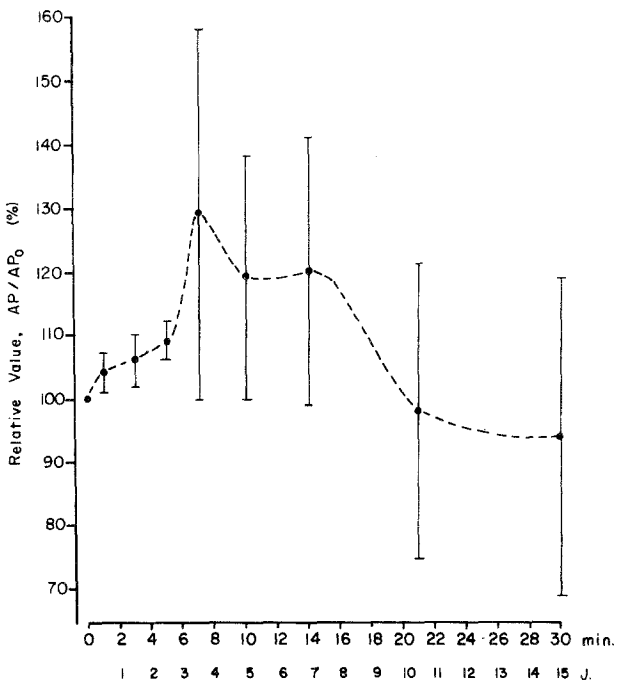

Fig. 5. APs following irradiation are compared to APs before irradiation (AP_0) in the same rats in percentage. The irradiation is continuous, for up to 30 minutes (equivalent to 15 J)

In Fig. 3 we present the results from the irradiated paralysed group P 1. The APs are compared again to the norm. The APs decline in both nerves as expected, but the APs in the right irradiated leg were higher than those in the left, nonradiated leg.

For each rat in group N 1 and P 1 we compared APs in the right irradiated leg to those in the left non-irradiated one, both recorded at the same session. The results are presented in Fig. 4, indicating clearly a higher AP in the right leg in all animals (values bigger than 1.0).

Fig. 2, 3 and 4 indicate that the beneficial effect of HeNe LELI lasts for 8 months at least.

In Fig. 5 we present the change in AP in normal rats (group N 2) following different times of radiation. Since the amount of energy delivered per minute is constant in our experiments, 0.5 J/minute, the time base can be changed to energy delivered. As one can see, there is a certain time/energy threshold below which there is no change in AP, and there is another threshold above which the beneficial effect disappears and there is even a suppression effect.

Low energy laser irradiation (LELI) has a bio-stimulating effect on various living tissues. Results and Methods published in recent years are given in Tab. 1.

Table 1. *Summary of Some of the Results Published Concerning Laser Irradiation*

Author (year)	Laser	Wavelength	Energy output	Delivered energy	Method	Effect	Notes
Rochkind (1978)	HeNe	632.8 nm	2 mW 4 mW 25 mW	— — —	every day during 15 days	positive	dogs
Kana (1981)	HeNe Ar Ne-YAG	632.8 nm 514.5 nm 1,060 nm	25 mW — —	4–20 J/cm^2 — —	45 mW/cm^2 45 mW/cm^2 45 mW/cm^2	positive positive —	on collagen synthesis, rats
Kovacs (1981)	HeNe	632.8 nm	5 mW	1 J	3′ 15″ irradiation every day	positive	women, portio surface
Mester (1981)	Rubi	694 nm	—	1 J/cm^2	3′, twice daily	positive	human and animal skin
Olson (1981)	dye laser	490–685 nm	—	0–3.5 mJ	1 μsec pulses.	negative	threshold at 60 μJ, cerebellar neurons in culture, rats
Ribari (1981)	HeNe	632.8 nm	1.3 mW	390 mJ	5 μ every day	positive	guinea-pigs, timpanic membrane
	HeNe	632.8 nm	1.3 mW	390 mJ	5 μ every day	positive	humans, 16 patients
Finsterbush (1982)	CO_2	10,600 nm	15 mW	—	incision, 30 nm	positive	rabbits, fascia incisions
Yew (1982)	HeNe	632.8 nm	0.5 mW	300 mJ	10′, once	not defined	pigment epithelium in culture, chicks
Surinchak (1983)	HeNe	632.8 nm	—	1.1 J/cm^2 2.2 J/cm^2	30′ exposure every 3rd day + 3′ exposure, twice daily	temporarily positive	rabbits skin
Man-Kai Cheng (1984)	dye laser	632 nm	50–200 mW	338–2,204 J/cm^2		negative	rats, brain tissue.

Key to effect assessment: positive—higher AAs, faster healing etc.; negative—lower APs, healing depression; not defind—mixed results.

Discussion

The use of low energy laser irradiation (LELI) as a biostimulator device is a new method, recently under investigation in various centres. We were interested in LELI effect upon peripheral nerve injuries, a major medical problem following trauma.

We started by analysing the recordings of action potentials (APs) from the nonirradiated normal sciatic nerves in the rat. These were taken regularly for 30 days and the results indicate (Fig. 1) that in both limbs the readings are almost equal to the norm, repeatable and similar to each other, with standard deviation (SD) less than 8%. In the paralysed nonirradiated group the APs diminished significantly during the 30 days of measurements. The APs are more variable in this case, with SD getting bigger (30% in some rats), probably because of the experiment being done in vivo. Our results justify the use of the norm as reference in our work, as well as the use of the left leg as a self-control for the right one. This is more accurate in the normal groups, but can be used with certain reservation in the paralysed groups as well. The significant decrease in AP following the cut (Fig. 1) justify our belief that our system is measuring

mainly the AP of the nerve itself, even though our needles are only in its vicinity (as accepted for human EMG).

Irradiating the right normal limb of the rats in the first normal group N1 every day while using the left limb as a control and recording APs on both sides leads to the results in Fig. 2. As one can easily see, LELI has a pronounced positive effect increasing AP of the irradiated nerve, while the contralateral nerve remains normal.

The very low AP in the P 1 group indicates that practically the sciatic nerve does not function following cut injury. But even in this situation, comparing the irradiated side to the other, nonirradiated one, it is possible to identify an increase in activity in the irradiated limb, Fig. 3.

The effect of HeNe LELI is even more pronounced when comparing the irradiated and nonirradiated limbs in the same rats in both N 1 and P 1 groups, Fig. 4. The ratio is always bigger than one with yet unexplained peaks and troughs.

The effect of LELI on AP depends on the energy delivered to the nerve in a way described in Fig. 5. We found two thresholds: if the energy delivered to the skin was under 3 J, there was no change in AP, and once the energy is higher than 8–9 J, there is an inhibitory effect. Between the two limits there is a pronounced increase in AP. We believe this energy-dependent effect to cause the contradictory results published by various researchers[8–10, 13, 14] (Tab. 1). A similar effect was reported when electric currents were used for nerve regeneration[12]. It was found that currents of 1–20 nA stimulate regeneration while bigger currents had an inhibitory effect. Jaffe and Nucetelli[3] using electric fields have found that a field of 0.7–1.4 V/cm^2 enhanced regeneration, while smaller fields did not have any effect, and bigger fields suppressed nerves activities.

Another finding in our work was that the LELI effect lasted for a very long time following irradiation. The increase in AP was present up to the end of the test, 8 months and more after the irradiation ceased (Figs. 2–4). This result disagrees with Finsterbush[2] who indicated in his work that the enhancing effect of wound healing persists only during the first 21 days after irradiation, and later disappeared. The use of a different laser (CO_2) and the different model—wound healing—could be the reason for this disagreement.

The laser radiation delivered to the skin penetrated the living tissues and a significant part of it reached the nerve, lying some 4–8mm under the skin and muscle. We found 0.5 mW or 6% to reach the nerve. This finding is in good agreement with known physical data[1], indicating the existence of an "optical window" in the skin for HeNe light.

Some of the recent publications concerning the effects of LELI on various tissues are summarized in Tab. 1. One can see that the variability in tissues, lasers used and methods is enormous, resulting in a commensurate variability in results. But still, in most cases, a definite positive result is reported.

A major problem, tackled by many researchers but with no conclusive results so far, is the question of how: how does LELI affect the living tissues?

Kovacs[6] and Mester[8] found an increase in blood supply and neovascular formation of the epithelial tissues irradiated. We observed a similar effect in the sciatic nerve of the rat (data not published). Kiernan[5] emphasized the importance of the diffusion of plasma proteins at the site of injury in nerves. This fact coupled with the assumption that the newly formed vessels are more permeable to plasma proteins than the normal vessels, could be part of the explanation for LELIs effects on tissues.

Another possible explanation was hinted at by Olson[9]. He showed that neurons could become more sensitive under the influence of LELI, but this effect diminished above a certain threshold energy flux.

Olson's results could explain some of our findings. It is possible that the activity threshold of some of the sciatic nerve fibres, nonactive usually, decrease because of the LELI we used. In this way the electric stimulation will cause a bigger number of fibers to fire, thus increasing the APs. However, this is only one possible educated guess and the full answers are not known yet.

The long-lasting and cummultative effects caused by LELI cannot be explained by any known method. There are some hints that the answer is in Molecular Biology and Biochemistry. However, the results are promising enough for further investigations.

Conclusions

1. HeNe LELI increases action potentials in normal sciatic nerves in rats.
2. HeNe LELI increases action potentials in cut sciatic nerves in the rats as compared to nonirradiated cut nerves.
3. HeNe LELI effect lasts for a very long time following irradiation.
4. There are optimal times for HeNe LELI.
5. There is a minimum threshold of energy flux, under which there is no effect, and a maximum threshold beyond which the effect is reversed.

While working on this paper, the 6th Congress of the International Society for Laser Surgery and Medicine took place in Jerusalem, Israel (October 8th). In the congress some groups (K. Atsumi, Y. Abe, G. Boussignac, K. Kamikawa, Y. Oyamada, R. P. Abergel, L. S. McKibbin, M. A. Trelles) and others presented their results. The overall conclusions were similar to ours: the HeNe laser is producing a biostimulating effect with positive results when in an energy range similar to the one found by us. No one could explain the methodology causing the effects found.

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