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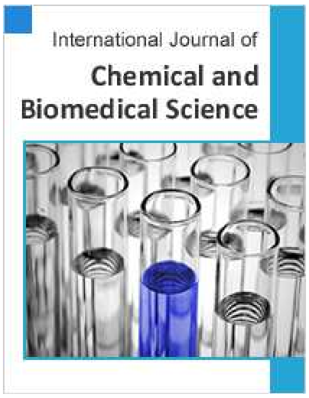

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Rabbit Retinal Function after Nd – YAG Laser Treatment

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Abstract
The aim of this work is to test the functional state of the eye retina exposed to neodymium yag (Nd:YAG) laser beam of the same intensity and duration as that used in lens posterior capsulotomy after cataract surgery. Rabbits' eyes were exposed to laser beam(1064nm, 0.01w/cm^{2}and exposuretime of 0.25 sec and 0.1 sec).Changes in the bioelectricity of the retina were investigated. The experiments were performed on light and dark adapted eyes of rabbits for control and afterNd:YAGlaserexposure. Theelectroretinogram; ERGwas recordedin both cases of dark and lightadaption. We tried to adapt the physical conditions surrounding the exposed eye during laser exposureand during the measurements. The ERG response was found to be reduced by about 50%. Selected a-wave & b-wave of the recordedERG signal,before and after exposure, were measuredin case of laser exposure times 0.25 sec, 0.1 sec in case of dark and light adaptation. The obtained results indicated a significant reductionin the a-wave& b-wave amplitudes, more significantly in case of the longer exposure time. This reduction after treatment, made slow recovery after 4 days and approached to its control values after 8 days of exposure. The late effect studies indicated a faster recovery for the shorter exposure time.

1. Introduction

Laser is widely used in all fields of scientific research, medicine, and industry. It is used for a wide variety of surgical operations [1].However, this is not without possible risks and disadvantages –as with any technology- there may be unanticipated side effects and complications. Laser exposure may affect the eye tissues by inducing biological effects, which mainly depend on the energy absorbed by the eye tissues. The absorbed energy is transmitted to eye tissues and completely transformed into heat [2-4], and the maximum reached temperature and the time during which the tissue submitted to laser lead to different biological effects [5-6]. Because of the high radiant power of many laser systems; a very short exposure time can cause damage [7]. Many factors must be taken into consideration duringlaser surgery of the eye; wavelength or spectral region of laser radiation, optical density at particular wavelengths, maximum irradiance (W/cm^2)or power(W), and also the type of laser system and kind of disease [8-10].

Nd:YAG laser has been frequently used in treatment of eye diseases. Its high gain, narrow linewidth, low threshold and physical properties make it a most versatile laser for a variety of applications spatially in eye treatment [11,7, 4]. Nd-YAG laser emits light in the near infrared spectrum at a wavelength of 1064nm.With this wavelength, Nd-YAG laser energy is less efficiently absorbed by water and more readily absorbed by other materials such as the hemoglobin in red blood cells. The energy penetrates further into the soft tissue (up to 4 mm) before being absorbed.Nd:YAG offers a wider application range, in large part because of its unique properties and because it is one of the few lasers that operates efficiently with either flashlamp or diode pump and in pulsed or continuous-wave mode[12]. Moreover, the delivery system can be a contact or a noncontact system, both of which can influence the tissue insult and the degree of post-treatment inflammation.

On applying Nd-YAG laser for eye treatment, it's necessary to consider the possibility and extent of complication [11]. The side effects that have been reported included damage to corneal endothelium, vitreous humor [13], the lens and the retina causing rupture of retinal vessels [14]. The effects may be manifested in biochemical and physiological phenomena in various enzymes, cells, tissues, organs and organisms. These effects may be due to changes in the transport of ions and various molecules across the cell membranes, changes in the characteristic biological activity and dielectric properties of various cells and tissues [15, 8, 16].

Posterior capsule opacification (PCO) is a very frequent problem after cataract surgery, which currently necessitates a posterior capsulotomy (LCT) with Nd-YAG laser in one fourth to fifth of eyes that have undergone cataract extractionandintraocularlens (IOL) implantation[17].Even though laser capsulotomy is easy to perform, itcarries risks. In addition to intraocular lens pitting, cystic macular edema and intraocularpressure rise, it is thought to increase the risk of RD four times compared with uncomplicatedextra capsular cataract extraction only[18-20].The exact mechanisms leading to RD after LCT are not known, even though several theoriesare proposed. It is commonly believed that Nd-YAG laser treatment initiates structuralchanges in the anterior vitreous, resulting in vitreous liquefaction, posterior vitreousdetachment and, if any abnormal vitreoretinal attachments exist, in retinal breaks anddetachment[21-27].

Nevertheless, some questions considering possible complications of laser treatment remain unsolved. Accordingly, in an attempt to investigatethe effect of Nd-YAG laser exposure during eye treatment, we intend to evaluate its effect on retinal functionusing theelectroretinogram (ERG) recording technique [28, 29].With proper analysis, the functional integrity of different retinal structures can be dissected out and we are able to understand information processing mechanisms and/or the sites of retinal disorders associated with laser exposure. Since the ERG reflects the electrical activity of the outer (i.e., rods and cones) and middle retina [30, 31], it provides a physiological survey of afferent visual function. Values of the a-wave & b-wave amplitude will bemeasured before and after laser exposure with the aim of testing the functional state of the eye tissues exposed to laser beam of the same intensity and duration as that used in lensposterior capsulotomy cases. We will try to adapt the physical conditions surrounding the exposed eye during exposure.

2. Materials and Methods

2.1. Animals

The experiments were performed on total twenty rabbits, weighing 1.5-2 Kg.The rabbits were divided into 4-groups, one unexposed control group and 3 other groups exposed toNd-YAG laser, all housed in separated cages with free access to water and food under 12/12h light and dark cycle. The rabbits were anesthetized by an intramuscular injection (0.l mL/ Kg body weight) of cocktail made up of ketamine hydrochloride, acepromazine male ate and xylazine. All rabbits were thenexposed toNd-YAG laser and the ERG was recorded either immediately or left for 4 or 8 days in order to study the expected repair mechanisms (recovery).

2.2. Laser Treatments

Laser irradiation was carried out at nanotechnology laboratory, Faculty of engineering in Shobra, Banha University, withpulsedNd-YAG laser (Model L A B-150 S/N, made in U.S.A) (1064nm, with intensity 0.01 w/cm^2., spot size 450µm, duration time 9 n sec., 30 pulses per sec and time exposure 0.25 and 0.1 sec.). Each rabbit eye, in each group;right and left eye, was exposed, in vivo, to laser with time exposure 0.25sec& 0.1sec.

2.3. Electroretinogram (ERG) Recording

The experiments were performed on light and dark adapted eyes of rabbits for control and exposed eyes, in-vivo conditions. After laser exposure of the anesthetized rabbit eye, the ERGwas recorded in case of controland after exposure in both cases of dark and light adaptation. Pilot experiments were carried out at Ain Shams University, Faculty of Science, Physics Department, Biophysics lab.

The ERGtechniquewas consisted of two parallel silver disc electrodes, 1cm diameter coated by non-polarizable silver chloride layer. The active electrode was directly placed on the cornea, whilst the reference one was on the sample ear (in vivo recording). The silver electrodes were frequently covered with silver chloride to reduce the electrode polarization artifacts to negligible levels as judged from measurements on physiological saline solutions and other similar saline solution. The active and the reference electrodes were connected to a computer interface; Science Work Shop 700 interference (CI-6565A). The ERG was recorded from the control samples, and laser exposed samples, immediately after exposure, or after 4daysand 8days of exposure.This recording was performed for 30 minutes, flasher every 5minuteswhile the flasher placedat a distance 25cm from the rabbit eye.

3. Results and Discussion

The recorded ERG signals, for the two selected exposure times (0.25 sec, 0.1 sec), areshown in fig (1).

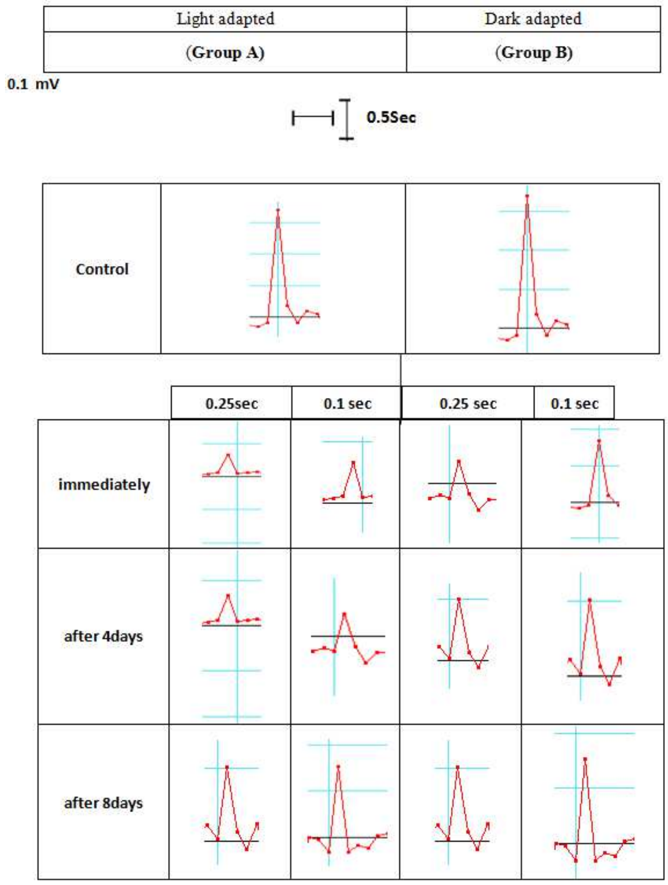

Fig. (1). Typical ERG records for light (Group A) and dark (Group B) adapted eyes before (control) , immediately and 4&8 days after laser exposure for two exposure times 0.25 & 0.1 sec respectively.

From Fig (1) It is noticeable that the recorded ERG waves, in both cases of light and dark adaptationare seen as a biphasic responseconsisted only of a- and b- waves while the c- and d- waveswere absent. This may be due to the used short flasher time (< 1sec) at which the c -wave does not appear and never outlasts light [32]. The other components of the ERG can be elicited only by using special recording techniques.

After laser exposure, there were remarkable changes in the ERG signalsdue to the noticeable variations in both the a- and b-wave amplitudes and durations in both cases of dark and light adaptation (Fig. 2). These variations were more pronounceable in case of the longer laser duration (0.25 sec). With this exposure time, the recovery of these variations was slower and took a longer timeto return back and approach their normal values, than those recorded in case of 0.1 sec exposure.

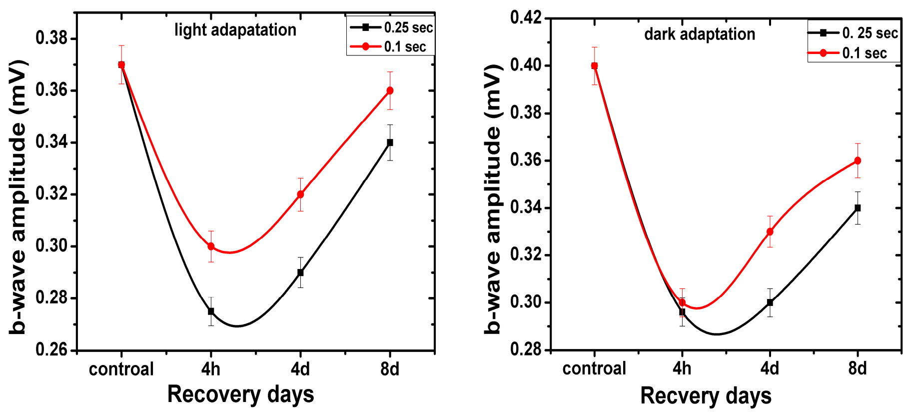

Fig. (2). b-wave amplitude and recovery days, of dark (left) and light(right) adapted eyes in case of exposure times 0.25 sec and 0.1sec.

The ERG originates from extracellular currents that are generated in response to a light stimulus. The ERG b-wave is generally not of fixed magnitude and cannot be simply predicated from the number of laser lesions and their dimensions. It varies among animals and varies with time in each animal so, in this work, we try to make the time between lesions constant. Moreover, the exact source of the ERG b-wave is still under dispute. The major contribution comes from light-induced activity in ON-center bipolar cells. The extracellular currents that generate the b-wave either originate directly in these cells or reflect potassium-induced changes in the membrane potential of Müller cells enveloping them. Regardless of the exact mechanism, the b-wave is telling us about light-induced electrical activity in retinal cells post-synaptic to the photoreceptors. The b-wave is also affected by OFF-center bipolar cells and by light-induced activity in 3rd order retinal neurons (amacrine and ganglion cells).

The effects of laser on the ERG responses showed in the figures (1-2) represent the retinal response before and for different time intervals after treatment. The ERG response was found to be reduced by about 50%. This reduction after treatment, made slow recovery after 4 days and approached to its control values after 8 days of exposure.

Table (1). *The ratio values for amplitude (b-wave /a-wave) of eye tissues for control, immediate,4 & 8 recovery days (4D &8D) in case of 0.1 & 0.25 Sec exposure times in dark and light adapted.*

A_b/A_a			
Dark		**Light**	
control	4	4	

Recovery days	0.25sec(dark)	0.1 sec(dark)	0.25 sec(light)	0.1sec(light)
Immediately	4.229	4	3.9855	4
4D	4	3.976	3.973	4
8D	4	4	3.9743	4

ERG analysis that is based only upon amplitude measurements may lead to erroneous conclusions if the pupil is not maximally dilated. Furthermore, exchange of information between laboratories that use different recordings conditions has always been problematical. One way to circumvent this difficulty is to compare a series of ERG responses for the relationship between the a-wave and the b-wave. This analysis is based upon our understanding of retinal physiology and the origin of the ERG waves. If the a-wave reflects activity in the photoreceptors and the b-wave originates from post-synaptic neurons, then normal signal transmission in the distal retina will be expressed in normal relationship between the b-wave and the a-wave. This is shown in table (1). This type of analysis can also help in understanding the site of disorder. Any disorder that is localized to the photoreceptors and does not involve more proximal sites will be expressed in abnormal a-wave amplitude but normal b-wave to a-wave ratio. In contrast, a defect in signal transmission in the outer plexiform layer will show abnormal b-wave to a-wave ratio but the amplitudes of the ERG waves may even increase.

Generally, the effectiveness of the laser lesions depends on numerous factors such as degree of pigmentation and optics of the eye. The wide range of laser effect probably reflects several contributions. These include differences in the degree of pigmentation and variety in the size of the eye ball between rabbits of different weight and differences in the time period between laser treatment and first ERG recording [33]. In addition to that, Nd-YAG vitreolysis caused signification electrophysiological changes, (34). The b-wave, which is mainly related to the bipolar cell currents and less to Muller cells, showed a reduction in its amplitude (fig. 1 & 2). The a-wave that reflects the photoreceptors activity was significantly reduced indicating that, Nd-YAG laser exerts an effect on the retina that begins particularly in photoreceptors layer and inner retinal layers. The later results clarify the affected inner nuclear layers as well as photoreceptors layers.

The ERG results in this study were in agreement with previous studies [35-37]demonstrating vitreolysis effects on the retina: these results correlated by another finding by Kolb et al [38] which clarified that the ERG of retinal detachment reduced and represents the healthy part of the retina. The observed changes on the ERG may be attributed to the ability of Nd-YaG laser photo disruption to the formation of shock waves.

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