CHAPTER 3

REVIEW OF LITERATURE

3.1. Low level laser therapy

The action of visible light on the specialized cells of the eye underlies sight. Nevertheless, the problem of the possibility of action of visible light on other human and animal cells and tissues was for a long time left unexplored. The appearance of lasers, sources of intensive visible and infrared radiation, gave a new impulse to this problem (Tuner et al 1999,Karu et al 2003).Lasers became widely used in surgery and therapy, and the question of whether visible light has an effect was solved by itself. The mechanism of action of laser light can, nevertheless, vary in different situations and for the most part is poorly studied.

Activation of life processes under laser radiation, often called “Biostimulation”, is of most interest (Karu et al 1984, Karu et al 1989) .Under large doses of laser radiation, its positive action changes, as a rule, into inhibition of vital activity processes, which is a main hindrance to a successful application of laser therapy and a cause of disappointment.

Irradiation by low-intensity lasers (LIL) is widely used by physiotherapists to reduce neuropathy pain, treatment of edema, eczema, and dermatitis, treatment of persisting ulcers, burns, “diabetic foot” to relieve pain or treat chronic diseases like arthritis and arthrosis and by therapists, in veterinary medicine, sports medicine, and rehabilitation centers (Baxter et al 1994, Tuner et al 1999, Siminovic et al 2000,Karu et al 2003,)

The most effective irradiation is that in the red and near infrared range of the spectrum. The most commonly used sources are the helium-neon laser (He-Ne) (radiation at

632.8 nm), the gallium-aluminum laser (Ga-Al) (630-685 nm), the helium-neon-arsenate laser (He-Ne-As) (780-870 nm), and the gallium-arsenate laser (Ga-As) (904 nm), as well as light emitting diodes whose emission band lies in a wide region of the spectrum (670 to 950 nm) (Karu et al 2003).The main reason for using the sources radiating in the red and near infrared spectral region is the fact that hemoglobin does not absorb in this region and light can penetrate deep into living tissue.

Due to its pain-relieving and wound-healing properties, LLLT has many uses in hospitals and aged-care homes, such as for the treatment of pressure sores in bed-ridden patients, and for enhanced post-operative wound healing and pain relief. The effect of LLLT is such that it can accelerate remodelling of scar tissue, and "give a more cosmetically-acceptable result" (Baxter, et al 1994) to post-operative scarring.

Trelles et al (1987) reviewed the use of local irradiation with Low-Level Laser in therapy. The stimulus was applied mainly to local lesions to elicit the following types of effect:

- Biostimulatory effects in ulcers, granulomas, burns, septic wounds and trauma to superficial tissues.
- Stimulation of local cell metabolism in damaged tissues in vivo and in vitro.
- Stimulated activity of local tissue enzymes.
- Enhanced scar formation and tissue regeneration, mitogenic activity, and osteogenic activity.

Trelles et al (1987) and Muxeneder (1987) also reviewed the effects of LLLT in vertebral pain, headaches and local immune responses. Other recorded therapeutic effects of LLLT (Illarionov et al, 1993) are:

- Analgesic, Antiexudative, Antihaemorrhagic;
- Anti-inflammatory;
- Antineuralgic, Antioedematous, Antiseptic;
- Antispasmodic;
- Vasodilatory.

According to Laakso et al (1994), “the analgesic response to phototherapy may be mediated through hormonal/opoid mechanisms and responses to LLLT which are dose and wavelength dependent."

Comparison of the therapeutic effects of a coherent (LIL) and an incoherent (LED) source showed no significant difference (Sazonov et al 1985,Karu et al 1989, Karu et al 2003,) This is also relevant to the action of light on the level of cells where coherent and incoherent sources had the same effect at the same wavelengths, intensities, and radiation times (Karu et al 1982, Bertoloni et al 1993).

For a given wavelength of light, energy density is the most important factor in determining the tissue reaction" (Baxter 1994). Research indicates that Energy Densities in the range 0.5 to 4 Joules/cm^2 are most effective in triggering a photobiological response in tissue (Mester & Jaszagi-Nagy, 1973,Haina, et al 1982, Mashiko et al, 1983;), with 4 Joules/cm^2 having the greatest effect on wound healing (Mester et al, 1985).

Australian research suggests that this 'therapeutic window' of Biostimulation may be extended to include 5 Joules/cm^2 (Laakso et al, 1994), and has applications in other areas of practice, such as Myofascial Trigger Point therapy and pain control. Dosages of 8 - 12 J/cm^2 and higher, and the resulting bioinhibition, may also have therapeutic applications, such as in the treatment of keloid scarring and pain management.

Baxter et al (1991) recommends a number of treatment parameters for common musculoskeletal disorders in terms of both Joules and Joules/cm^2 dosages, such as 1 - 2 Joules (8 - 16 J/cm^2) for ligament strains. The energy and energy density combination in this case can only be achieved with a specific laser beam power to spot size ratio of 1:8. Not all laser devices will incorporate this specific ratio, and so a generalised representation of dosage parameters in this way is Limited in its usefulness.

However, it can be seen that by knowing and understanding the relationship between the Joules dosage and its corresponding Joules/cm^2 dosage and the resultant Biomodulative effect, allows more accurate specification and delivery of a particular treatment dosage. It also makes a successful treatment easier to replicate, and to share results with other practitioners and researchers.

3.2. EFFECT OF LOW LEVEL LASER THERAPY IN TREATING VARIOUS CONDITIONS

A number of papers have shown a reduction of pain with laser treatments directed over acupuncture points. Altered skin resistance with a reduction of pain was also noted in subjects who receive low level laser therapy (LLLT) over muscular trigger points. A group of subjects with chronic tendinopathies that had been previously treated unsuccessfully with physical therapy, NSAIDS, local injections, and or surgery had an 87 percent success rate in pain reduction following the application of LLLT. In a study involving over 4,000 subjects who had suffered from conditions such as degenerative arthritis, muscle pain, tendinitis and tension myalgia. More than 80 percent of the subjects found a marked lessening of their symptoms following irradiation with an Infrared laser (White et al 2002).

In a study involving total of 69 subjects and more that 80 percent of the subjects with chronic Radiculopathies and over 90 percent of the subjects with chronic neuropathies

experienced a greater than 50 percent of total pain relief following LLLT. In a similar study involving 60 total patients and 111 total laser treatments, it was shown that LLLT produced an immediate reduction of pain in 79 percent of the subjects.

In a study involving over 100 subjects and over 500 laser treatments, it was observed that acute soft tissue pain syndromes showed a dramatic response following the initial laser treatment with a marked reduction in tissue swelling, bruising and good pain relief. Subsequent treatments (2-3) produced further improvement. It was also noted that chronic pain syndromes were slower to respond to LLLT (average of eight treatments), around 75 percent of the subjects were noted with significant pain relief. A two-stage survey of 116 chartered physiotherapists in Northern Ireland, who utilized LLLT as part of their clinical practice, ranked LLLT effective for the treatment of myofascial and postoperative pain syndromes; rheumatoid arthritis; muscle tears; hematomas; tendinitis; shingles; herpes simplex; scarring; burn and would healing. In this same survey, LLLT was ranked first, on the basis of relative effectiveness, when compared with four other modalities (interferential therapy, shortwave diathermy, ultrasound, and pulsed electromagnetic therapy), for use in pain relief and wound healing (White et al 2002).

Effect of Low Level laser irradiation in nerve regeneration

Low-intensity laser therapy (LILT) has demonstrated the ability to stimulate the healing process and reduce the pain associated with peripheral nerve damage. At the cellular level, LILT has been shown to improve Schwann cell proliferation and reduce scar tissue formation, in addition to reversing the process of progressive nerve degeneration post-injury. Furthermore, a positive influence on axonal growth and re-myelinization has been noted (Rockind et al 2006).

A double-blind randomized study found an almost 70 percent improvement in positive somatosensory-evoked responses and better quality (larger-diameter axons) of the nerve regeneration process with LILT after complete surgical transection and direct anastomosis of the sciatic nerve (Shamir et al 2001) .

Clinically, a study by Iijima, et al (1991) using LILT reduced pain levels by 45 percent in 18 patients with severe post-herpetic neuralgia. A clinical double-blind, placebo-controlled, randomized study compared the effectiveness of LILT on patients who had been suffering (six months to several years) from incomplete peripheral nerve and brachial plexus injuries. This study revealed that LILT significantly improved motor function in addition to recruitment of voluntary muscle activity in the partially paralyzed limbs of brachial plexus injuries compared to the placebo group, which failed to show any improvement.

More specifically, a randomized controlled trial carried out(Anderson et al 1995) at General Motors Company found that carpal tunnel patients treated with low-intensity laser had better functional recovery and a higher back-to-work percentage (72 percent active laser vs. 41 percent sham). Similarly, a more recent study showed that LILT improved both the sensory and distal motor latencies of the median nerve in carpal tunnel patients compared to controls. (Evick et al 2007) In short, by safely an and effectively healing damaged nerve tissue, restoring function and alleviating pain, low-intensity laser has been found to be a key therapeutic component in the treatment of peripheral nerve injuries.

Schwartz et al (2002), conducted a study to elucidate the mechanism by which the light might induce therapeutic effects. Skeletal muscle cultures were chosen as a target for light irradiation and nerve growth factor (NGF) was the biochemical marker for analysis.

It was found that there is a transient elevation of intracellular calcium in the myotubes immediately after irradiation ($P<0.001$). Preincubation of the myotubes with either the photosensitizers 5-amino-levulinic acid (5-ALA), or with hematoporphyrin (Hp) enhanced the elevation of cytosolic calcium ($P<0.001$) after helium/neon irradiation (633 nm) with an energy of 3 J/cm. In addition, helium/neon irradiation augmented the level of NGF mRNA fivefold and increased NGF release to the medium of the myotubes. Thus, it is speculated that transient changes in calcium caused by light can modulate NGF release from the myotubes and also affect the nerves innervating the muscle. The NGF is probably responsible for the beneficial effects of low-level light.

Nissan et al (1986) conducted experiments with low energy laser irradiation (LELI) on living tissue they used HeNe laser on rats. The exponential absorption was reaffirmed in the living tissues overlying the sciatic nerve. An optimal range of energy between 3.5 and 7 J--associated with energy concentration of 4-10 J/cm2 delivered transcutaneously was found to cause a significant increase in action potential in the sciatic nerve. The effect lasted for more than 8 months after the irradiation session.

Benato et al (2004) objective was to investigate the effects of postoperative laser therapy on nerve regeneration after end-to-side neurorrhaphy, an innovative technique for peripheral nerve repair. After complete transection, the left median nerve was repaired by end-to-side neurorrhaphy on the ulnar "donor" nerve. The animals were then divided into four groups, one placebo group, and three laser-treated groups that received laser therapy three times a week for 3 weeks starting from postoperative day 1. Three different types of laser emission were used: continuous (808 nm), pulsed (905 nm), and a combination of the two. Functional testing was carried out every 2 weeks after surgery by means of the grasping test. At the time of withdrawal 16 weeks postoperatively, muscle mass recovery

was assessed by weighing the muscles innervated by the median nerve. Finally, the repaired nerves were withdrawn, embedded in resin and analyzed by light and electron microscopy. Results showed that laser biostimulation induces a statistically significant faster recovery of the lesioned function, a statistically significant faster recovery of muscle mass, and a statistically significant faster myelination of the regenerated nerve fibers.

Rochkind et al conducted experiments on the possible mechanism of action of phototherapy on the nervous tissue with respect to peripheral nerve regeneration has been provided by in vitro studies, which showed that phototherapy induced massive neuronal sprouting and outgrowth in cultured neuronal cells as well as Schwann cell proliferation. It has also been suggested that phototherapy may enhance recovery of neurons from injury by altering mitochondrial oxidative metabolism, and guiding neuronal growth cones in vitro, perhaps due to the interaction with cytoplasmic proteins and, in particular, to the enhancement of actin polymerization at the leading axon edge. A possible molecular explanation was provided by demonstrating an increase in growth-associated protein-43 immunoreactivity in the early stages of rat sciatic nerve regeneration after phototherapy. Another study by Rockind et al showed that application of phototherapy up regulates calcitonin gene-related peptide mRNA expression in facial motor nuclei after axotomy. By altering the intensity or temporal pattern of injury-induced calcitonin gene-related peptide expression, phototherapy may thus optimize the rate of regeneration and target innervations and neuronal survival of axotomized neurons.

Rockind et al suggested that Laser phototherapy significantly improves recovery of the injured peripheral nerve and in addition, decreases posttraumatic retrograde degeneration of the neurons in the corresponding segments of the spinal cord. Previous studies

investigating the effects of low-power laser irradiation on an injured peripheral nerve in rats have found that it provides

- Immediate protective effects that increase the functional activity of the injured peripheral nerve
- Maintenance of the functional activity of the injured nerve over time
- A decrease or prevention of scar tissue formation at the site of injury
- Prevention or decreased degeneration in corresponding motor neurons of the spinal cord
- An increase in the rate of axonal growth and Myelinization.

Shin et al (2003) used Low power laser irradiation (LPLI) in the treatment of peripheral nerve injury. In this study, they verified its therapeutic effect on neuronal regeneration by finding elevated immunoreactivities (IRs) of growth-associated protein-43 (GAP-43), which is Up-regulated during neuronal regeneration. Twenty Sprague-Dawley rats received a Standardized crush injury of the sciatic nerve, mimicking the clinical situations accompanying partial axonotmesis. The injured nerve received calculated Low Power Laser Irradiation(LPLI) therapy immediately after injury and for 4 consecutive days thereafter. The walking movements of the animals were scored using the sciatic functional index (SFI). In the laser treated rats, the SFI level was higher in the laser treated animals at 3-4 weeks while the SFIs of the laser treated and untreated rats reached normal levels at 5 weeks after surgery.

In immunocytochemical study, although GAP-43 IRs increased both in the untreated control and the LPLI treated groups after injury, the number of GAP-43 IR nerve fibers was much more increased in the LPLI group than those in the control group. The elevated numbers of GAP-43 IR nerve fibers reached a peak 3 weeks after injury, and then declined in both the untreated control and the LPLI groups at 5 weeks, with no

differences in the numbers of GAP-43 IR nerve fibers of the two groups at this stage. This immunocytochemical study using GAP-43 antibody study shows for the first time that LPLI has an effect on the early stages of the nerve recovery process following sciatic nerve injury.

Shi et al (1997) The purpose of their experiment was to elucidate the influence of the low-energy He-Ne laser on the function of regeneration of peripheral nerve. Forty-four rabbits about 2.5 kg body weight were used in the experiment. The animals were divided into 4, 8, 12, 16 weeks groups according to the observation period. Six animals were used in each irradiated group and in the control group 5 rabbits were used in each observation period. Regeneration of the axon and myelin sheath, the latent rate of the common peroneal nerve, the conditions of the anterior tibital muscle and the toe expansion test were all observed systematically in both groups. The experimental results was: A few thin regenerated axon was seen at 4 weeks in the irradiated group, while in the control group it might be seen at 8 weeks, the P value was < 0.01. A low amplitude latent rate of the common peroneal nerve is determined at the peroneal side of the anterior tibial muscle in a few animals at 4 weeks of the irradiated group, and it is not observed in the control group, from 12 to 16 weeks. The latent rate of the common peroneal nerve was improved in the irradiated group than in the controlled. The P value was < 0.01. The regeneration of the myelin sheath was evident in the irradiated group, and also the section of the muscle fibers anterior tibial muscle was clearly visible than the controlled. 16 weeks postoperatively, the toe expansion test was normal in the irradiated group, while in the control group it was the same as seen at 12 weeks after operation in the irradiated group. Now it was certain that the low-energy He-Ne laser could promote the function of the spinal motor nerve cells and accelerate the axonal regeneration.

Shi et al (1997) The objective of his experimental study was to investigate the influence of low-energy He-Ne laser on the motor nerve cells of the spinal cord. The experimental study included as follows: Four rabbits were used in this experiment. The L5-6 spinal cord segment was irradiated by He-Ne laser percutaneously, the nerve velocity of the comon peroneal nerve was measured in order to determine the function of the spinal motor nerve cells when the peripheral nerve was intact. The common peroneal nerve was transected on one side without repair, two weeks after laser irradiation the grey matter of the spinal cord of L5-6 segment was procured for electronic microscopic examination. The common peroneal nerve on the contralateral side was transected and followed by end-to-end anastomosis, and laser irradiation was done on the same spinal cord segment. Two weeks after irradiation, the nerve velocity of the common peroneal nerve and the toe expanding test were investigated. The results were: the He-Ne laser can influence the spinal motor nerve cells function as expressed by latent rate when the peripherial nerve is intact. i.e. the nerve velocity is slower than normal, and the amplitude is markedly decreared. The change of the microstructure of the spinal motor nerve cells is comparatively slight in the 10 and 15 minutes groups. The recovery of the nerve velocity and the toe expansion are earlier in the 15 min group. In short, the low-energy He-Ne laser can influence the function of the spinal motor nerve cells.

Khullar et al (1995) conducted experiments with albino rats, Twenty animals received a standardized injury (axonotmesis) to the right sciatic nerve using a time, load and length sequence (10 min, 150 N, 5 mm) known to cause extensive axonal degeneration of the rat sciatic nerve. The Low Level Laser treatment was administered using a hand-held laser probe in light contact with the skin on the dorsal aspect of the hind leg overlying the site of the axonotmesis injury to the sciatic nerve. A group of 10 animals were treated with 6J of LLL (GaAlAs 830 nm) daily for a period of 28 days. Ten more animals were treated

daily with a sham exposure setting and served as controls. Nerve function was assessed by a recognised method of walking tract print analysis; the "Sciatic Functional Index" (SFI), and nerve regeneration was assessed by recording the evoked compound action potentials (cAP) in the common peroneal nerve. At 21 days post-injury, the laser-treated group had a significantly lower median SFI than the sham laser-treated group, indicating that the real laser treatment had improved functional recovery in the nerve. However, no differences were found between the evoked cAP parameters that were measured in the laser-treated and sham laser-treated groups. Histological examination reiterated the lack of difference between the two groups. Consequently, they concluded that the effects of Low Level Laser on recovery must have occurred more peripherally to the point measured.

Miloro et al (2002) surgically created defects in the rabbit inferior alveolar nerve. The purpose of this investigation was to determine the effects of low-level laser (LLL) irradiation on neural regeneration. Five adult female New Zealand White rabbits underwent bilateral exposure of the inferior alveolar nerve. A 6-mm segment of nerve was resected, and the nerve gap was repaired via entubulation by using a Gore-Tex conduit. The experimental side received 10 postoperative LLL treatments with a 70-mW gallium-aluminum-arsenide diode at 4 sites per treatment. At 15 weeks after surgery, the nerve segments were harvested bilaterally and prepared for light microscopy. Basic fuchsin and toluidine blue were used to highlight myelinated axons. The segments were examined histomorphometrically by using computer analysis to determine mean axonal diameter, total fascicular surface area, and axonal density along the repair sites. Gross examination of all nerves showed intact neural bundles with variable degrees of osseous remodeling. Light microscopic evaluation revealed organized regenerated neural tissue in both groups with more intrafascicular perineural tissue in the control group.

Histomorphometric evaluation revealed increased axonal density in the laser treated group as compared with the control. They concluded that LLL irradiation may be a useful noninvasive adjunct to promote neuronal wound healing in surgically created defects

Snyder et al (1988) The purpose of this randomized, double-blind study was to determine the effect of a helium-neon (He-Ne) laser on latency of peripheral sensory nerve. Forty healthy subjects with no history of right upper extremity pathological conditions were assigned to either a Laser or a Placebo Group. Six 1-cm2 blocks along a 12-cm segment of the subjects' right superficial radial nerve received 20-second applications of either the He-Ne laser or a placebo. We assessed differences between pretest and posttest latencies with *t* tests for correlated and independent samples. The Laser Group showed a statistically significant increase in latency that corresponded to a decrease in sensory nerve conduction velocity. Short duration He-Ne laser application significantly increased the distal latency of the superficial radial nerve. This finding provides information about the mechanism of the reported pain-relieving effect of the He-Ne laser.

3.3. ALLOXAN INDUCED DIABETIC NEUROPATHY IN RATS

Alloxan exerts its diabetogenic action when it is administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse (Eizirik 1994). The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg b.w. (Gruppuso et al. 1990, Boylan et al. 1992). When alloxan is given intraperitonealy or subcutaneously its effective dose must be 2-3 times higher. The intraperitoneal dose below 150 mg/kg b.w. may be insufficient for inducing diabetes in

the rat (Katsumata et al. 1992, 1993). Fasted animals are more susceptible to alloxan (Katsumata et al. 1992, Szkudelski et al. 1998).

Szkudelski (2001) Alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their action in Beta cells of the pancreas has been intensively investigated and now it is quite well understood. The cytotoxic action of both these diabetogenic agents is mediated by reactive oxygen species, however, the source of their generation is different in the case of alloxan and streptozotocin. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. There after highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells. Streptozotocin enters the B cell *via* a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore,streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage.As a result of the streptozotocin action, B cells undergo the destruction by necrosis.

Manjunath et al (2010) used albino rats weighing approximately 200-250 g for the study. Diabetes was induced by a single dose (150 mg/kg, i.p) of freshly prepared solution of alloxan monohydrate 5% (dissolved in normal saline). The induction of diabetes was

confirmed after 48 hr by blood glucose estimation and rats with blood glucose levels between 250-350 mg/dl were selected for the study. Blood sample for glucose estimation was collected from rat tail tip. About 1 mm of its end was cut and the drop of blood was collected directly on the strip placed in the Glucometer.

Vogel, Gang (2002). In Drug discovery and evaluation of pharmacological assay studied methods to induce experimental diabetes mellitus. The experimental rats were on fasting for 24 hours prior to experimentation and were rendered diabetic by a single dose of intra- peritoneal injection of Alloxan 150 mg/kg body weight by dissolving in normal saline and rats with more than 200mg/dl were considered to be diabetic .

Deshmukh et al (2010) studied the Antidiabetic activity of ethanol extract of Colocasiaesculenta leaves they used albino rats weighing 150-200 g of either sex which were allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intraperitoneal injection of alloxan 120 mg/kg body weight.15 After 18 hours of injection of alloxan, diabetes was confirmed by testing blood sugar level more than 200 mg/dl were selected for the further study.

3.4. NCV-TESTSTING

NCV measures different characteristics of action potentials traveling along the axons, and is invaluable in diagnosing diseases that primarily affect nerve function. NCV uses electrodes similar to those used in electrocardiograms placed on the skin over a nerve. A mild electrical shock delivered to the nerve causes action potential which is recorded by other electrodes as it travels through the nerve. The speed of nerve conduction is influenced by a coating around axons, called myelin. Myelin insulates each axon and forces action potentials to "jump" quickly along the axon. Action potential travels slower when myelin is damaged. Healthy axons provide a strong action potential. If axons

degenerate the action potential becomes weaker. Different diseases preferentially either affect myelin sheathing or damage axons. This is why the type of nerve damage detected by NCV is so important in making the right diagnosis.

Nerve conduction studies are widely employed in evaluating patients with peripheral nerve disease and are often used serially to measure disease progression or to assess a therapeutic intervention. Chaudhry et al (1991) determined the inter- and intra-examiner reliability of electrophysiological data by performing serial nerve conduction studies on 7 normal subjects. A high degree of inter-examiner reliability was present, but significant inter-examiner differences were found. Their results suggest that if nerve conduction studies are to be used longitudinally, they should optimally be performed by a single examiner to minimize the degree of variability associated with different examiners.

Kanaya (1996) conducted study to determine which parameters was the best measure of nerve regeneration, assuming that the sciatic functional index (SFI) represented the "gold standard." Three different sciatic functional indexes and11 commonly used electrophysiologic and morphologic indicators of regeneration were all determined in 24 rats 12 weeks after one of three lesions was createdin the sciatic nerve. With linear regression analysis, only fiber/axon diameter ratio (D/d) and myelin thickness/axon diameter ratio showed statistically significant correlations with sciatic functional index ($r = 0.55$ and 0.53, respectively). The other 11 parameters had poorer correlation. Therefore, if sciatic functional index is the best measure of comprehensive nerve function, then other parameters are not. It is probable that each parameter measures some different component of the regeneration process. A stepwise multiple linear regression analysis produced a model that included D/d, nerve conduction velocity, and nerve action potential amplitude that gave a slightly better correlation ($r = 0.67$). The relatively poor correlation between

sciatic functional index and the other parameters of nerve function indicates that all nerve regeneration studies must be interpreted carefully before comparisons are made. Furthermore, the best measure of nerve function remains unproved or undiscovered in the experimental animal.

Motor nerve conduction velocity was measured in vivo by the method described by Sharma and Thomas (1975). The animals were anaesthetized with ether and placed on an electrically heated pad in a warm room. Skin temperature was measured on the medial aspect of the upper thigh and, in some experiments, intramuscular temperature in the posterior thigh and calf muscles was recorded with a thermistor probe. The right hind limb was held in full extension by strapping. A sterile concentric needle electrode (Disa type K051 1) was inserted into the muscles of the first interosseous space of the foot. The sciatic nerve was stimulated through a needle electrode at the sciatic notch and the tibial nerve posterior to the medial malleolus with just supramaximal stimuli delivered from a Devices isolated stimulator at the rate of one per second. The evoked muscle action potentials were suitably amplified and displayed on an oscilloscope. Conduction time between upper and lower stimulation sites was measured from photographs. The distance between the two stimulating points measured on the skin with calipers was taken as the conduction distance and divided by the conduction time to obtain conduction velocity.

Biessels et al (1999) Neurophysiological changes in the central and peripheral nervous system of streptozotocin diabetic rats MNCV and SNCV were measured in the sciatic nerve according to the method described by Dekoning and Gispen (1987). In short, the sciatic and tibial nerve were stimulated at the sciatic notch and ankle, respectively. The latencies of the responses of the musculature of the foot were measured. The MNCV and SNCV were calculated by dividing the distance between the two stimulation points by the

differences in latencies of the M response and the H reflex after proximal and distal stimulation.

Gerbi (1999) After 8 wk of diabetes induction motor nerve conduction velocity was recorded in 10 animals of each group from the left sciatic tibial nerve in temperature-controlled environment under ether anaesthesia. The rectal temperature was maintained at 36–37°C with heating lamp and pad. The left sciatic nerve was stimulated proximally at the sciatic notch and distally at the ankle via bipolar electrodes with supramaximal stimuli (6 mA) done by rectangular pusle, 0.3 ms in duration, from a stimulator at 10 Hz on Neuropack 2 EMG (Nihon Kohden). The muscle action potential was recorded from the first interosseous muscle of the left hind limb by unipolar pin electrodes. The latencies were measured from the stimulus artifact to the onset of the negative M-wave deflection. The nerve conduction velocity (NCV) was calculated by the ratio distance between the two sites of stimulation in mm divided by the difference between proximal and distal latencies in ms, giving a value for MNCV in meters per second (m/s)

Wiggin (2008) NCVs were recorded For the sciatic nerve, the recording electrodes were placed in the dorsum of the foot, and the stimulating electrodes at the knee and sciatic notch. For the sural nerve, the anode was placed on the third toe of the foot, and the cathode was placed on the heel of the foot. The cathode and anode were placed 5-mm apart. The frequency band was inclusive of two, 10 Hz muscle potential recordings (orthodromic, motor) and 10, 2-Hz potential recordings (antidromic, sensory).

Watanabe O(1998) The purpose of this study was to observe functional recovery and motoneuron death after nerve transection-and-repair in neonatal versus young animals. One hundred nine Lewis rats underwent posterior tibial nerve transection-and-repair at 6 or 22 days of age. Fifty-two and fifty-seven nerves at the 6- and 22-day times were used

for endpoint analysis at 1, 3, 10, and 14 months. These assessments included serial functional walking track analysis,electrophysiologic studies, muscle mass evaluation, motoneuron counts with retrograde horseradish peroxidase tracing, and histologic and morphometric nerve analysis. Walking track analysis and nerve conduction velocity indicated significantly poorer functional regeneration in the 6-day-old group than in the 22-day-old group. Muscle mass in the 6-day-old group did not recover as well as in the 22-day-old group. Motoneuron numbers stained with horseradish Peroxidase were less in the 6-day-old group than in the 22-day-old group. In contrast, Morphometric analysis did not reach significance. This study suggests that the same nerve injury sustained in a neonatal rat is less likely to demonstrate functional recovery than one sustained in a young rat.