CHAPTER: 5

Effects of Nd: YAG and HeNe laser irradiation on cockroach leg nerves
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Summary

Recently optical methods have been developed for highly precise and non-invasive control of neuronal spike timing. Laser radiation affects conduction in mainly slow conducting nerve fibers. Laser induced inhibitory effects in peripheral nerves are also reported. Infrared neural stimulation (INS) provides an alternative technique to stimulate neurons and improve spatial selectivity.

In this chapter effects of cw mode Nd: YAG (Wavelength $\lambda = 1064$ nm, Power = 150 mW) laser and HeNe (Wavelength $\lambda = 632$nm, Power = 2 mW) laser on the neuronal spikes generated from cockroach leg (Periplaneta Americana) nerves are studied using electrophysiological techniques.

Neuronal spike train or action potentials are recorded using a standard electrophysiological set up consisting of microelectrodes, micromanipulator, pre-amplifier & spike train amplifier.

A new data acquisition is used to acquire and process the neuronal spike data from cockroach leg nerve.

Spike potentials are acquired and stored through a digital signal processing software and subjected to statistical analysis for variance, power, entropy & standard deviation. Effects of laser parameter such as power, laser exposure time on the statistical parameters of neuronal spike train are measured, which is a significant contribution.

- **Vijay H. Ghadage**, Gauri R. Kulkarni. Effects of Nd: YAG laser stimulation on neuronal spike train recorded from cockroach leg nerve (manuscript under Preparation).
5.1 Cockroach leg nerve structure

Figure 5.1: A) Left Metathoracic leg of cockroach, anterior view showing sites of Electrode placement. B) Innervations of the cockroach mesothoracic leg.
The legs of all insects are made up of the same basic parts—femur, trochanter, coxa, tibia, and tarsus, but they can differ considerably. Coxa is the basal segment of the insect leg, somewhat comparable to the ball joint. Trochanter is the leg segment between coxa and femur. Femur is the third leg segment, comparable to human thigh bone. The fourth leg part of cockroach is tibia. Tarsus is the part of the leg beyond the tibia. It has spines which point backward. They are used for holding onto the surfaces. For the present experiment adult cockroach leg with typical sizes are used—length of coxa 0.9cm, femur 1.2cm, tibia 1.4 cm and tarsus 1cm.

Nervous System of Cockroach:

Nervous system of cockroach includes—

a) Central nervous system
b) Peripheral nervous system
c) Autonomous nervous system

a) Central nervous system:

It includes nerve ring, double ventral nerve cord and segmental ganglia. In cockroach nerve ring is present around the oesophagus. Nerve ring is formed by a pair of supra oesophageal ganglia, a pair of sub-oesophageal ganglia, a pair of circum oesophageal connectives which connect them. Supra oesophageal ganglia or brain is present on the dorsal side of oesophagus. The brain is mainly a sensory and an endocrine center releasing hormones into the haemolymph. A pair of suboesophageal ganglia lies below the oesophagus. It is the motor center that controls the movements of mouth parts, legs, wings.

1) Ventral Nerve Cord:

It consists of two longitudinal nerves which are solid. Hence it is described as double solid ventral nerve cord. The nerve cord extends in between sub-oesophageal ganglia and the last abdominal ganglia present in the 7th segment.
2) Segmental ganglia:
Total numbers of segmental ganglia in cockroach are nine. Among them three are present in thoracic and six in the abdominal region. In cockroach abdominal ganglia are present in 2, 3, 4, 6 and 7 segment. Abdominal ganglia are absent in 5, 8, 9 and 10 segments. The largest abdominal ganglion is 6th ganglion present in the 7th abdominal segment.

b) Peripheral nervous system:
It includes nerves which arise from central nervous system and supply to the different parts of the body. From the brain three pairs of nerves arise and supply to compound eyes, antennae and labrum and also to frontal ganglion. A sub-oesophageal ganglion gives off three pairs of nerves to mandibles, maxillae and labium. Thoracic ganglia supply nerves to legs and wings. Metathoracic ganglia send nerves to first abdominal segment. Nerves from each of the first five abdominal ganglia innervate all organs of each of the segments, two to six serially. All organs present in 7th to 10th segments receive nerves from the last abdominal ganglion. The organs include the reproductive organs, copulatory appendages besides anal cerci. Abdominal ganglia supply nerves to body wall and other parts in those segments. The last abdominal ganglion supply nerves to 7th, 8th, 9th and 10th segments including anal cerci and anal styles in male.

c) Autonomous nervous system:
It is also called somatogastric nervous or visceral nervous system. It includes four nerve ganglia and nerves which connect them and supply to visceral organs. The nerves ganglia are frontal ganglion present on pharynx, just in front of the brain. Occipital ganglion is present behind the brain above the oesophagus. Somatogastric nervous system supply nerves to alimentary canal heart and other visceral organs.
5.2.1 Experimental set up

Figure 5.2: Block diagram of Experimental setup for recording neuronal spike train

- DSP PORT UNIT
- ADC
- Spike Train Amplifier
- CRO
- PC
- Lasers (Nd: YAG, He Ne)
- Optical fiber
- Cockroach leg
- Micro Manipulator

G₁ = Ground Electrode
A₁ = Active electrode
A₂ = reference Electrode
Figure 5.3: Neurosignal Recording Systems.
Figure 5.4: Neuronal Data Acquisition System.

**Micromanipulator:**

The manipulator device consists mainly of a lever and a slide rail mechanism to convert the coarse movements of the hand into micro movements. Leitz micromanipulators were used for ensuring mechanical stability to microelectrode and positioning the microelectrodes on leg nerve.
Faraday Cage:

A Faraday cage is an enclosed conducting shell that shields from strong electric fields and electromagnetic waves. A Faraday cage or Faraday shield is an enclosure formed by conducting material or by a mesh of such material. Such an enclosure blocks out external static electrical fields. A Faraday cage's operation depends on the fact that an external static electrical field will cause the electrical charges within the cage's conducting material to redistribute them so as to cancel the field's effects in the cage's interior. This principle is used to protect electronic equipment from electrostatic noise improve signal to noise ratio.

Ground:

Ground or earth may be the reference point in an electrical circuit from which other voltages are measured, or a common return path for electric current, or a direct physical connection to the earth. Connections to ground limit the build-up of static electricity.

For measurement purposes, the Earth serves as a (reasonably) constant potential reference against which other potentials can be measured. An electrical ground system should have an appropriate current-carrying capability in order to serve as an adequate zero reference level. In electronic circuit theory, a "ground" is usually idealized as an infinite source for charge, which can absorb an unlimited amount of current without changing its potential. Where a ground connection has a significant resistance, the approximation of zero potential is no longer valid. Stray voltages or earth potential rise effects will occur, which may create noise in signals or if large enough will produce an electric shock hazard. In the present set up star grounding is used to get a good quality recording of neuronal signals.

Preamplifier:

A preamplifier for impedance matching is used with following specification.

1) Low noise: typically 20 µV
2) Input Impedance: \(10^{11}\) ohms
3) Output impedance= 100 ohms
4) Frequency response = DC to 30 KHz (variable)
5) Gain: 10, 100 and 1000

**Spike Train amplifier:**

It is used as second stage amplifier having gain up to 400 with low noise figure. Frequency range = DC to 10 KHz.

**Cathode Ray Oscilloscope:**

Agilent 1000 series oscilloscope is used for experimental purpose to observe & store the signals. Its important features are:
1) Up to 2 GSa/s sample rate and 20 kpbs memory. 2) Crisp and bright 5.7” color display with wide viewing waveform and making it easy to view signal. 3) Waveform recording and playback (sequence mode). 4) Dedicated, color-coded vertical controls make it easy to access the most common functions. 5) Built in help, menus, front panel overlay template, and manual offered in eleven languages. 6) Auto scale helps you quickly display signals, automatically setting the controls. 7) Automatic measurements or manual cursor measurements. 8) Zoomed time base display shows big picture and zoomed in view simultaneously. 9) Built in USB ports it easy to save your setups, data, and screen images, print the signals connected to PC. 10) Use of save button storage the data in USB as a PSV images.

Agilent 1000 series oscilloscope two or four channels 60 MHz, 100MHz and 200 MHz band width model is shown in the photograph.

**Specifications:**

1) Digital multimeter DC gain Accuracy: DC voltage measurements accuracy better than ± 0.1 % of reading.
2) Power supply, DC Gain Accuracy: 0V to 35 volt DC; 10mV resolution.
3) Power Sensor, Analog Bandwidth: 100 KHz to 1 GHz ± 3% accuracy.
4) BNC Cable, DC gain accuracy, Time scale accuracy and trigger sensitivity: 50 ohms characteristic impedance, BNC connectors.
5.2.2 Microelectrode Preparation

Electrode Preparation:

Electrodes are prepared by metal electrolyte etching method. Take tungsten metal wire with diameter 0.2 mm and length of 3 cm. Take 80 ml of water in beaker mix with pellets of sodium hydroxide to make a strong alkaline solution. A.C. power supply (0-3/6/9V AC) is used. A wire is connected to graphite rod to the power supply at 0 volt and inserted into the beaker. Another wire is connected to syringe needle to the power supply at 6 volt and tungsten wire is insert in the needle. After 2 to 3 minutes remove the tungsten electrode and clean with pure distilled water for one minute. The tip of electrode is seen by using the microscope. Using microscope scale (calibrated) tip diameter of each electrode is measured.
was measured. Electrodes with tip diameter of 5 μm were used for recording signals.

Parameters used to make electrodes are

1) Purity: 99.9%
2) Wire diameter: 0.2mm
3) Length of Electrode: 3cm

The terminal of few mm of tungsten wire was immersed in aqueous solution of NaOH. The process of anodic oxidation of tungsten in a NaOH solution is expressed by the following steps.

\[
\begin{align*}
W + 6OH^- & \rightarrow WO_3(S) + 3H_2O + 6e^- \\
WO_3(S) + 2OH & \rightarrow WO_2^- + H_2O
\end{align*}
\]

**5.2.3 Experimental Procedure**

**Preparation of Leg:**

Anaesthetize the cockroach with a very mild dose of chloroform soaked in cotton. Remove one of the legs of an adult cockroach (Periplaneta Americana) by cutting at the base of coxa with a pair of scissors. The length of cockroach coxa is typically 1cm and femur length is 1.2 cm and tibia length is 1.4 cm and tarsus length is 1cm. Place the cockroach leg on wax sheet. Depress the coxa and femur into the wax. With this arrangement the femorotibial joint should be freely movable and the tibia spines accessible to stimulation. Ground electrode is inserted in the coxa and active and reference electrode inserted on the femur as shown in following figure 5.4.

- Position the microelectrode on the leg with micromanipulator.
- Connect the microelectrodes to the differential inputs (G1 & G2) of preamplifier of Grass Company (model P16).
- Offset voltage adjustment
- Set the gain and Band width of preamplifier.
- Ensure grounding connections.
- Connect the output of preamplifier to spike train amplifier.
- Connect the output of spike train amplifier to the CRO/ data acquisition system.
Laser Stimulation:

CW mode Nd:YAG laser with wavelength $\lambda=1064\text{nm}$ at different power (9.13mW, 32.28mW, 51.47mW and 71.17mW) is used for stimulating the leg nerve. Laser power is varied by keeping a constant exposure time of 10 sec. Also by keeping laser power constant, exposure time is varied from 10sec, 30sec, 50sec, 70sec and 90 sec. Distance from optic fiber to cockroach leg is 5mm. This signal is stored with data acquisition system with Comport 4 and keeping sampling frequency 4000Hz and data capture time of 10sec. By using data acquisition system, text file is converted into mtx
file. This mtx file can be read by DSP view software. This data file is subjected to
statistical analysis and displayed in figure 5.8 to 5.17.

HeNe laser wavelength $\lambda = 632$nm, power $= 2$mW is used for experimental
purpose. HeNe laser with constant power $= 2$mW is used for exposure time 10sec,
30sec, 50 sec, 70sec and 90 sec. The cockroach leg signal is captured by using data
acquisition system. Distance between lasers to cockroach leg is 3cm. Following
parameters are used
1) Sampling frequency= 4000Hz
2) Comport= 4
3) Band width of preamplifier: DC to 3 KHz
4) Total Gain= 10 x 300= 3000
6) Signal Acquisition time= 10sec
5.2.4 Laser parameters and dose

Nd: YAG laser (cw mode, wavelength $\lambda = 1064$ nm and maximum power = 150mW) is used to irradiate the cockroach leg via fiber optic delivery system (inner diameter of fiber optic = 8 $\mu$m and Numerical aperture = 0.22). The exposure time was varied from 10 to 90 sec at fixed incident laser power. Then at constant exposure time incident laser power was varied from 9.13 mW to 71.17 mW. The incident laser spot diameter on the target was measured using thermal paper and was found to be $d = 1$ mm and corresponding area $A = 0.785 \times 10^{-6}$ m$^2$.

The photon energy of Nd:YAG laser is $0.018693 \times 10^{-17}$ J i.e. 1.16 eV. The incident power density was calculated using laser power and spot area and is given by 191.08 kW/m$^2$ for maximum power of 150 mW. At different laser power the power density is given as 11.630, 41.12, 65.56 and 90.66 kW/m$^2$ (Table 5.1)

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Laser power (mW)</th>
<th>Power density (kW/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.13</td>
<td>11.630</td>
</tr>
<tr>
<td>2</td>
<td>32.28</td>
<td>41.12</td>
</tr>
<tr>
<td>3</td>
<td>51.47</td>
<td>65.56</td>
</tr>
<tr>
<td>4</td>
<td>71.17</td>
<td>90.66</td>
</tr>
</tbody>
</table>

Table 5.1: Incident laser power and the power density incident on cockroach femur

The energy of Nd:YAG laser, power density and number of photons (dose) incident on cockroach leg for typical time of 10 sec is calculated as follows.

Laser photon energy: Wavelength $\lambda = 1064$ nm and Power = 150 mW, using $E = h \nu = \frac{hc}{\lambda}$, where $h = \text{Planck's constant} = 6.626 \times 10^{-34}$ J-sec

$c = \text{speed of light} = 3 \times 10^8 \text{ m/sec}$

$\lambda = \text{wavelength} 1064 \times 10^{-9} \text{ m}$

Then photon energy $E = 1.16$ eV

Power Density = incident laser power/ spot area

The beam diameter of Nd: YAG laser is = 1 mm and radius $r = 5 \times 10^{-4}$ meter

Power density = $\frac{(150 \times 10^{-3})}{[3.14 \times (0.5 \times 10^{-3})^2]} \text{ W/m}^2$

Power density = 191.08 kW/m$^2$
Number of Photons (dose) incident on target = \( \frac{\text{Power}}{h \nu} \times \text{exposure time} \)

\[ = 9.13 \times 10^{-3} \times 10 / (0.01868233 \times 10^{-17}) \]

Number of Photons incident on target = \( 4.88697 \times 10^{17} \)

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Laser power (mW)</th>
<th>Power density (kWm(^2))</th>
<th>Exposure time (Sec)</th>
<th>Photon dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.13</td>
<td>11.630</td>
<td>10</td>
<td>4.88697 \times 10^{17}</td>
</tr>
<tr>
<td>2</td>
<td>32.28</td>
<td>41.12</td>
<td></td>
<td>17.27835 \times 10^{17}</td>
</tr>
<tr>
<td>3</td>
<td>51.47</td>
<td>65.56</td>
<td></td>
<td>27.500968 \times 10^{17}</td>
</tr>
<tr>
<td>4</td>
<td>71.17</td>
<td>90.66</td>
<td></td>
<td>38.09482 \times 10^{17}</td>
</tr>
</tbody>
</table>

Table 5.2: photon dose at constant time but variation in laser power

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Laser power (mW)</th>
<th>Power density (kWm(^2))</th>
<th>Exposure time (Sec)</th>
<th>Photon dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.13</td>
<td>11.630</td>
<td>10</td>
<td>4.88697 \times 10^{17}</td>
</tr>
<tr>
<td>2</td>
<td>32.28</td>
<td>41.12</td>
<td>30</td>
<td>14.66091 \times 10^{17}</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>50</td>
<td>24.43485 \times 10^{17}</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>70</td>
<td>34.20879 \times 10^{17}</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>90</td>
<td>43.982 \times 10^{17}</td>
</tr>
<tr>
<td>6</td>
<td>32.28</td>
<td>41.12</td>
<td>10</td>
<td>17.27835 \times 10^{17}</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>30</td>
<td>51.835076 \times 10^{17}</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>50</td>
<td>86.39179 \times 10^{17}</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>70</td>
<td>120.948 \times 10^{17}</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>90</td>
<td>155.505 \times 10^{17}</td>
</tr>
</tbody>
</table>
HeNe laser (wavelength $\lambda = 632$ nm, power = 2mW) is used to irradiate the cockroach leg femur. The exposure time was varied from 10 to 90 sec at fixed incident laser power. The beam diameter of HeNe laser $d = 2$ mm & its radius is $1 \times 10^{-3}$m. The energy of HeNe laser is $0.03145 \times 10^{-17}$ Joule or 1.96 eV. The power density is 0.6369 K W/m² (table 5.4).

### Table 5.3: photon dose at constant laser power but variation in exposure time.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Laser power (mW)</th>
<th>Power density (kWm²)</th>
<th>Exposure time (Sec)</th>
<th>Photon dose</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>2</td>
<td>0.6369</td>
<td>10</td>
<td>635.93 x 10¹⁴</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>30</td>
<td>1907.790 x 10¹⁴</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>50</td>
<td>3179.65 x 10¹⁴</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>70</td>
<td>4451.51 x 10¹⁴</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>90</td>
<td>5723.37 x 10¹⁴</td>
</tr>
</tbody>
</table>

### Table 5.4: photon dose at constant laser power but variation in exposure time.
5.3.1 Neuronal Signal Acquisition system

Neuronal signal:

A neuron or nerve cell is an electrically excitable cell that processes and transmits information by electrical and chemical signaling. Neurons connect to each other to form networks. All neurons are electrically excitable, maintaining voltage gradients across their membranes by means of metabolically driven ion pumps, which combine with ion channels embedded in the membrane to generate intracellular-versus-extracellular concentration differences of ions such as sodium, potassium, chloride and calcium. Changes in the cross-membrane voltage can alter the function of voltage dependent ion channels. If the voltage changes by a large enough amounts (threshold level) an all-or-none electrochemical pulse called an action potential is generated, which travels rapidly along the cell's axon, and activates synaptic connections with other cells.

Action potential thus represents a change in membrane potential due to depolarization and repolarization. Action potentials are typically 1ms duration signals with amplitude variations. Amplitude values depend on mode of recording (extracellular or intracellular) and electrode impedance values. Action potentials are discrete and have a time sequence. However trains of action potentials are random signals and statistical methods are used to estimate the signal distribution.
Figure 5.5 shows basic block diagram of data acquisition system. It accepts input signal already amplified in the range of -10 to +10 volts and frequency from dc to 5 KHz. The WG-DSPPORT-B.2 unit digitizes input signal and sends the digitized samples to PC through RS. 232 USB link.

For higher sampling rates (e.g. 12 KHz) generates large data volumes. To transfer this data to PC requires a 1MBPS (1 mega bytes per second) serial interface. To acquire large number of data RS. 232 from BF-810 from BAFO technologies converter is used.

Neuronal data acquisition system designed by Wavelet Group Pune consists of WG-DSPPORT-B-2, Comport, USB interface, PC software & digital signal processing software DSP view 3.0.
5.3.2 Parameter setting & Procedure

**System Specifications:**
1) Power supply: (7-12 volt DC)
2) Input signal Source (0-5 KHz, +/- 10 V)
3) WG-DSPPORT-B.2 unit.
4) WG-DSPPORT-B-2 PC software.

**Technical specifications:**
1) Input Signal: DC to 5 KHz
2) Input Signal Level: -10V to +10V
3) Processor: Fixed point DSP
4) ADC: 24 bit
5) Maximum Sampling rate: 12 KHz (Programmable)
6) Host Interface: Rs-232 interface, 750 Kbps
7) Power requirement: 7-12 V DC @ 0.5 A
8) Unit Size: 255mmx140mm x66mm
9) Operating Temperature: 0°c to 50 °c

**Host PC configuration:**
1) Intel core 2 duo 2.53 GHZ CPU
2) 1 GB DDR II RAM
3) 160 GB HDD
4) DVD RW
5) Operating system- windows XP Home-OE

**Software features:**
WG-DSPPORT- B-2 GUI WIN 32 compatible with windows XP
1) WINDOWS XP based GUI
2) Selectable sampling frequency
3) Programmable data capture duration
4) Text to Mtx and vice versa conversion
5) Serial Comm. Baud rate: 750 kbps
Software limitations:

The programmable sampling frequency is limited up to 12 KHz

Neuronal Signal Acquisition Procedure:

- Tungsten microelectrodes are connected to the input of preamplifier (Grass company, model number P16) with a gain of ten and frequency response from 100Hz-2 KHz.
- Output signal from preamplifier is fed to the second stage spike train amplifier with a gain of 300 which is displayed on CRO (Agilent Technologies, Model DSO 1014A).
- Amplified signal is further fed to the input port of data processing system.
- The analog signal is converted into digital signal using WG-DSPPORT-B-2 module which consists of a 24 bit ADC card.
- Sampling frequency is programmable and typically 4 KHz sampling frequency was adjusted. Data was acquired for 10 sec duration.
- The ADC sampling rate and capture duration is user programmable from graphical user interface.
- Digitized data is given to computer via comport (Moxa company) and USB interface.
- Comport is used to select appropriate baud rate (typically 921600).
- Signals are stored in text files and further converted into mtx files which are compatible with DSP view software.
- Signals are then displayed and statistical parameters power, variance, entropy are measured.
- Signals are recorded and analysed under different conditions of laser stimulation.
5.4.1 Neuronal Spike Train display

In the following figures, typical Neuronal spike trains recorded from cockroach leg nerve are displayed.

Figure 5.8: Neuronal spike train from cockroach leg nerve (Control)

A series of signals were recorded and analyzed using ten samples of leg nerve preparations.
Figure 5.9: Neuronal spike train from cockroach leg nerve irradiated with Nd:YAG laser

(\( P = 9.13 \text{ mW}, \lambda = 1064 \text{ nm}, \text{exposure time} = 10 \text{ sec} \).)
Figure 5.10: Neuronal spike train from cockroach leg nerve irradiated with Nd:YAG laser

(P = 32.28 mW, λ = 1064 nm, exposure time = 10 sec).
Figure 5.11: Neuronal spike train from cockroach leg nerve irradiated with Nd:YAG laser

(P = 51.47 mW, λ = 1064 nm, exposure time = 10 sec).
Figure 5.12: Neuronal spike train from cockroach leg nerve irradiated with Nd:YAG laser

(P = 71.17 mW, λ = 1064 nm, exposure time = 10 sec).
Figure 5.13: Neuronal spike train from cockroach leg nerve irradiated with HeNe laser

\(P = 2 \text{ mW}, \lambda = 632 \text{ nm}, \text{exposure time} = 10 \text{ sec}\).
Figure 5.14: Neuronal spike train from cockroach leg nerve irradiated with HeNe laser

(P = 2 mW, λ = 632 nm, exposure time = 30 sec).
Figure 5.15: Neuronal spike train from cockroach leg nerve irradiated with HeNe laser

(P= 2 mW, λ= 632 nm, exposure time= 50 sec).
Figure 5.16: Neuronal spike train from cockroach leg nerve irradiated with HeNe laser

(P= 2 mW, \(\lambda\) = 632 nm, exposure time= 70 sec).
Figure 5.17: Neuronal spike train from cockroach leg nerve irradiated with HeNe laser

(P = 2 mW, λ = 632 nm, exposure time = 90 sec).
5.4.2 Statistical analysis of Neuronal Spike/Signals

The Neuronal spike trains recorded from cockroach leg nerves are analyzed using statistical methods. The neuronal signal entropy, Variance/ power, standard deviations are measured by using digital signal processing software.

**Power:**

The average square value of a signal equals its average power, adapting the convention that the signal is considered to represent an electrical voltage across one ohm resistor. The square of the mean value represents the power of the DC component of the signal.

**Variance:**

The variance is generally given the symbol $\sigma^2$, where $\sigma$ is referred to as 'standard deviation'. The variance is defined as the average or expected value of the square of the function’s departure from its mean, and is therefore a measure of signal fluctuations. The second central moment for discrete random signal having $n$ possible levels is given by

$$\sigma^2 = (y_n - y)^2 = \sum (y_n - y)^2 \cdot P_m$$

and for continuous signal is given by

$$\sigma^2 = (y - y)^2 = \int (y_n - y)^2 \cdot P(y) \cdot dy$$

**Standard Deviation:**

Standard deviation of a series is the positive square root of the arithmetic mean of the square of deviations of the various items from the arithmetic mean of the series. It is denoted by symbol $\sigma$. The standard deviation can be calculated from the following formula.

$$\sigma^2 = \frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x})^2$$

Where $\sigma$ is the standard deviation, $x_i$ is the spike train amplitude; $\bar{x}$ is the mean amplitude of the spike train, $n$ is the number of observation.
5.4.3 Entropy of Neuronal Spike train

Entropy:

Consider a source ‘S’ which is emitting a sequence of symbols,

\[ S = (S_1, S_2, S_3, \ldots, S_n) \]

The successive symbols are selected according to some fixed probability law

Now, for a simplest kind of source, we assume that successive symbols emitted from the source are statistically independent. Such an information source is termed as zero memory source and is completely described by the source alphabet ‘S’ and the probabilities with which the symbol occur.

\[ P(S_1), P(S_2), \ldots, P(S_n). \]

Calculate the average information provided by a zero memory information source as follows.

If symbols \( S_1 \) occur, we obtain an amount of information (I) equal to

\[ I(S_i) = \log \frac{1}{P(S_i)} \text{ bits} \]

The probability that this will happen is just \( P(S_i) \), so that the average amount of information obtained per symbol from the source is

\[ \sum_S P(S_i) I(S_i) \text{ bits.} \]

Where \( \sum_S \) indicates the summation over the \( n \) symbols of the source.

This quantity, the average amount of information per source symbol is called entropy, \( H(s) \) of zero memory sources.

\[ H(s) = \sum_S \log \frac{1}{P(S_i)} \text{ bits} \]
5.5.1 Graphs & Tables

This part depicts various graphs & related tables of a typical experiment on cockroach leg nerve. The neuronal signals are recorded and analysed as described earlier in this chapter. For each condition of laser stimulation fifteen data files have been recorded & analysed.

![Graph showing neuronal signal power against laser power for constant exposure time of 10 sec.](image)

**Figure 5.18:** Neuronal Signal power from cockroach leg nerve is plotted against laser power for constant exposure time of 10 sec.

<table>
<thead>
<tr>
<th>File no.</th>
<th>Condition</th>
<th>Neuronal Signal Power (Variance)</th>
<th>R.M.S Value</th>
<th>Amplitude Peak to Peak value of Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Control</td>
<td>1.0834</td>
<td>1.040</td>
<td>6.67</td>
</tr>
<tr>
<td>A2</td>
<td>Irradiated power 9.13mW</td>
<td>1.9981</td>
<td>1.413</td>
<td>7.50</td>
</tr>
<tr>
<td>A3</td>
<td>Irradiated power 32.28mW</td>
<td>0.07091</td>
<td>0.266</td>
<td>1.98</td>
</tr>
<tr>
<td>A4</td>
<td>Irradiated power 51.47mW</td>
<td>0.06887</td>
<td>0.262</td>
<td>1.97</td>
</tr>
<tr>
<td>A5</td>
<td>Irradiated power 71.17mW</td>
<td>0.03703</td>
<td>0.192</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Table 5.5: Statistical parameters of neuronal signals recorded from cockroach leg nerve after Nd: YAG laser irradiation (λ = 1064 nm) at constant exposure time (10 sec).
Figure 5.19: Neuronal Signal power from cockroach leg nerve is plotted against exposure time for different laser power.

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Time in sec</th>
<th>Signal power ( Variance)</th>
<th>Signal power ( Variance)</th>
<th>Signal power ( Variance)</th>
<th>Signal power ( Variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Laser power = 9.13 mW</td>
<td>Laser power = 32.28 mW</td>
<td>Laser power = 51.47 mW</td>
<td>Laser power = 71.17 mW</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>2.5273</td>
<td>0.00904</td>
<td>0.105</td>
<td>0.09688</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>3.5614</td>
<td>0.023</td>
<td>0.105</td>
<td>0.02995</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>3.4619</td>
<td>0.02998</td>
<td>0.1063</td>
<td>0.02882</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>3.3546</td>
<td>0.6336</td>
<td>0.1068</td>
<td>0.4754</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>3.2382</td>
<td>0.02876</td>
<td>0.09865</td>
<td>0.4103</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>3.1463</td>
<td>0.03003</td>
<td>0.0841</td>
<td>0.4059</td>
</tr>
</tbody>
</table>

Table 5.6: Statistical parameters of neuronal signals recorded from cockroach leg nerve after Nd: YAG laser irradiation (λ = 1064 nm) for different exposure time and power.
Figure 5.20: Neuronal signal entropy from cockroach leg nerve is plotted against laser power for constant exposure time of 10 sec.

<table>
<thead>
<tr>
<th>File no.</th>
<th>Condition</th>
<th>Signal Entropy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Control</td>
<td>5.6977</td>
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<tr>
<td>A2</td>
<td>Irradiated power 9.13mW</td>
<td>5.8278</td>
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<tr>
<td>A3</td>
<td>Irradiated power 32.28 mW</td>
<td>5.5930</td>
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<td>A4</td>
<td>Irradiated power 51.47mW</td>
<td>5.5518</td>
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<tr>
<td>A5</td>
<td>Irradiated power 71.17mW</td>
<td>5.3457</td>
</tr>
</tbody>
</table>

Table 5.7: Statistical parameters of neuronal signals recorded from cockroach leg nerve after Nd: YAG laser irradiation ($\lambda=1064$ nm) for different power at constant exposure time (10sec)
Figure 5.21: Neuronal Signal entropy from cockroach leg nerve is plotted against the exposure time for different laser power.

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Time in sec</th>
<th>Signal Entropy Laser power = 9.13 mW</th>
<th>Signal Entropy Laser power = 32.28 mW</th>
<th>Signal Entropy Laser power = 51.47 mW</th>
<th>Signal Entropy Laser power = 71.17 mW</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5.6205</td>
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<tr>
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<td>10</td>
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<tr>
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<tr>
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<td>5.4802</td>
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<td>5.981</td>
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<td>5.5029</td>
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<tr>
<td>6</td>
<td>90</td>
<td>6.0237</td>
<td>5.2099</td>
<td>5.4559</td>
<td>5.4771</td>
</tr>
</tbody>
</table>

Table 5.8: Neuronal Signal entropy from cockroach leg nerve with different exposure time (Nd: YAG $\lambda=1064$ nm)
Figure 5.22: Neuronal Signal power from cockroach leg nerve is plotted against exposure time for constant power 2mW of HeNe laser ($\lambda$= 632 nm).

Figure 5.23: Neuronal Signal entropy from cockroach leg nerve is plotted against exposure time with constant power 2 mW of HeNe laser ($\lambda$= 632 nm).
<table>
<thead>
<tr>
<th>File no</th>
<th>condition</th>
<th>Time in sec</th>
<th>Signal power</th>
<th>Mean signal power</th>
<th>Entropy</th>
<th>Mean Entropy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Control</td>
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<td>0.223</td>
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<td></td>
<td>5.3240</td>
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<tr>
<td>A2</td>
<td>Irradiated</td>
<td>10</td>
<td>0.243</td>
<td>0.24</td>
<td>5.3561</td>
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<td>0.199</td>
<td>0.199</td>
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<tr>
<td>A4</td>
<td>Irradiated</td>
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<td>0.176</td>
<td>0.176</td>
<td>5.2120</td>
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<tr>
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<td>0.175</td>
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<td>5.281</td>
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<tr>
<td>A6</td>
<td>Irradiated</td>
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<td>0.168</td>
<td>0.168</td>
<td>5.4763</td>
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<td>5.9631</td>
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<td>0.168</td>
<td>5.2062</td>
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<td>0.166</td>
<td></td>
<td>5.1521</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.9: Statistical parameters of neuronal signals from cockroach leg nerve after irradiating with HeNe laser ($\lambda = 632$ nm, Power= $2$ mW).
5.5.2 Results & Conclusions

Figure 5.18 shows signal power of neuronal spike train recorded from cockroach leg nerve plotted against Nd:YAG laser power for constant exposure time. Neuronal signal or neuronal spike train power for control sample is lower than the radiation exposed leg nerve. However as the laser power increases from 9.13 mW to 71.17 mW gradually neuronal signal power decreases, which is also reflected in the gradual decrease of spike train amplitude (figure 5.19).

Entropy of neuronal signals recorded from cockroach leg nerves shows oscillatory nature as Nd:YAG laser power is varied from 9.13 mW to 71.17 mW gradually for 10 sec exposure time (figure 5.20). Entropy of neuronal signals recorded from cockroach leg nerve is plotted against exposure time for various power values of Nd:YAG laser (figure 5.21). As Nd:YAG laser power increases entropy decreases. Also as time of laser exposure increases entropy value at a particular value of laser power, 9.13 mW remains almost same. However as laser power increases from 9.13 mW to 71.17 mW entropy drops down as a time of exposure is increased.

Figure 5.22 shows signal power of neuronal spike train recorded from cockroach leg nerve plotted against exposure time of HeNe laser ($\lambda = 632$ nm, power = 2 mW). As exposure time of HeNe laser irradiation increases signal power decreases significantly. Figure 5.23 shows signal entropy of neuronal spike train recorded from cockroach leg nerve. HeNe laser ($\lambda = 632$ nm, power = 2 mW) is used to stimulate the leg nerve from 10 sec to 90 sec time duration. There is a deviation in entropy value, however the trend is decreasing. Entropy is a quantity which gives average amount of information per source. The nerve fibers generate time dependent signals called as action potentials or spikes which have a sequence and are discrete. Cockroach leg nerve fibers (mainly sensory) transmit information from mechanoreceptors to thoracic ganglia and then up to the brain in modified codes. The neuronal signals (action potentials) recorded in the present work show changes in amplitude distribution and entropy.

Entropy changes due to laser stimulation of cockroach nerve fibers. Entropy measures the variability of spike train due to laser stimulation. Entropy information in spike train depends on laser stimulus condition (power and exposure time), which is a significant result. This approach forms a basic to study laser neuron interactions using spike trains.
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