Analysis of neuronal networks in *Caenorhabditis elegans*

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Certificate of Examination

This is to certify that the dissertation titled "Analysis of neuronal networks in *Caenorhabditis elegans*" submitted by Mr. Varun Sanjay Birari (Reg. No. MS14132) for the partial fulfilment of BS-MS dual degree program of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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(Supervisor)

Dated: April 26^{th}, 2019

Declaration

The work in this dissertation has been carried out by me under the guidance of Prof. Somdatta Sinha at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Varun Sanjay Birari

(Candidate)

Dated: April 26^{th}, 2019

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Prof. Somdatta Sinha

(Supervisor)

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Abstract

The complete set of neurons and their synaptic connectivity in the worm *Caenorhabditis elegans* has been worked out experimentally. These neurons, with their chemical and electrical synapses, form a network through which informational cues are transferred for the worm to generate appropriate behavioral responses. Thus, the *C. elegans* neuronal connectivity presents itself as an ideal system to understand if/how connectivity pattern can explain different behaviors observed in a worm. I have used the network theory to analyze the neuronal connectivity pattern in *C. elegans*, and looked for functional correlations. Here, first I have experimentally analyzed the role of EXP-1 protein in AWC neuron mediated chemotaxis behavior, using chemotaxis assay. Then, I have analyzed both undirected and directed neuronal networks based on types of synaptic connections, and compared their properties to the ones reported in literature. Using modularity and shortest path analysis, I have shown how connectivity pattern underlies the chemotaxis behavior in worms, and propose alternate neuronal circuits for navigation in *C. elegans*.

Chapter 1

Introduction

1.1 *Caenorhabditis elegans*

Caenorhabditis elegans is a tiny (about 1mm) free-living nematode with a transparent body. Naturally it exists as self-fertilizing hermaphrodites but can give rise to males in a population with 0.2% frequency. It has a short life-cycle of about 3 days from the time when embryos are hatched to the time when the adult hermaphrodite starts laying eggs[4]. One of the advantages of using *C. elegans* to study development and neurobiology is because it has invariant number of somatic cells in all individuals. Be-

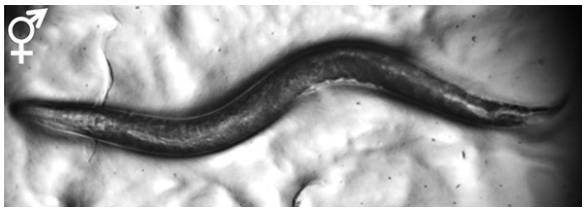


Figure 1.1: Hermaphrodite *C. elegans*

source:www.wormbook.org

cause of this property of the *C. elegans* researchers were able to reconstruct shape of each and every cell using electron micrograph images. This included its 302 neurons and their more than 7000 synapses [26] (this data is only for adult hermaphrodites, males have additional sex-specific neurons [14]) .This makes *C. elegans* the only organism whose complete neuronal wiring diagram is known.

1.1.1 Nervous system of *C. elegans*

Nervous system of adult hermaphrodite consists of 302 neurons which consists of two independently functioning nervous systems, one consisting of 280 neurons is called somatic nervous system and other consisting of 22 neurons is called pharyngeal nervous system, both of which are connected by a single pair of neurons. Based on where the neurons occur in circuitry, they are divided into four functional types, **sensory neurons** which sense the environment through specialized structures, **motor neurons** make synaptic contacts with muscle cells, **interneurons** receive and send synaptic contacts to other neurons and **polymodal neurons** can carry out more than one above mentioned functions.

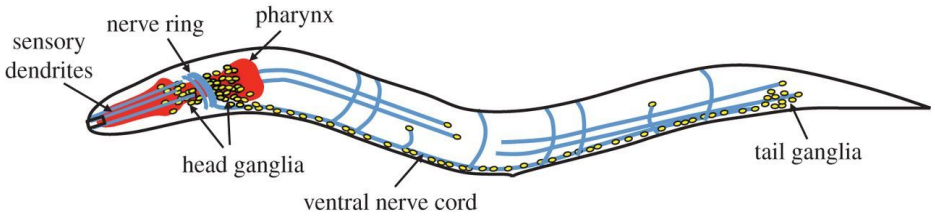


Figure 1.2: Representation of *C. elegans* nervous system[9]

Synapses are junctions through which neurons communicate with each other. Unlike mammalian nervous system where synapses are axodendritic (axon to dendrite), in *C. elegans* synapses are formed *en passant*[26]. Based on the nature of message being exchanged, the synapses in *C. elegans* are divided into three types, first one is chemical synapse where chemical message is transferred from one pre-synaptic neuron to other post-synaptic neuron(s), second is electrical synapse where electrical ions flow between the two neurons through gap junctions, and the third is neuromuscular junction (NMJ) where neurons make contact with muscle cells.

1.1.2 Chemotaxis

Worms move on food containing agar plate by carrying out sinusoidal body bends. These movements are generally in forward direction but occasionally it can carry out certain movements that allow it to change its direction. One of such movements is called gentle head turning (steering), where worm biases its movement towards one

particular direction during reversal movement. Chemotaxis is movement of worm along or against a chemical gradient through steering motions. This helps the worm to locate bacteria (food of *C. elegans*), which is constantly releasing chemicals, while avoiding harmful bacteria by detecting it early[21].

Chemotaxis in *C. elegans* can occur in response to either perception of water-soluble chemicals (taste) or volatile chemicals (odor). There are 26 sensory neurons which are known to be involved in sensation of taste or odor in *C. elegans*. These neurons are generally referred to as chemosensory neurons. Each of this neurons is involved in sensation of different chemical and can sometimes sense multiple chemicals.

1.1.3 Navigation Circuit

Navigation circuit of *C. elegans* refers to a set of neurons and their connectivity pattern that are involved in behaviors seen during navigation of *C. elegans* while in search of food (bacteria). This circuit, shown in figure 1.3, was proposed by Gray *et al*[11] based on experimental studies. The head motor neurons responsible for behaviors (such as reversals and omega turns) which allow it to change its direction were known. To determine the possible circuits involved in this behavior, authors performed shortest path analysis on the the undirected network of *C. elegans* neuronal connectivity. Using criteria, mentioned in the study, number of possible interneurons

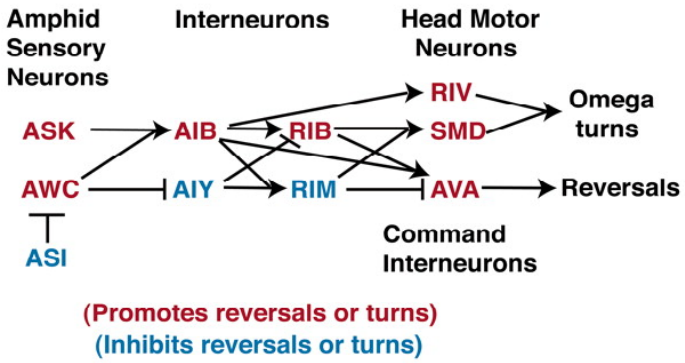


Figure 1.3: Navigation Circuit

and motor neurons were reduced. To check for the role of remaining neurons, either genetic mutants, with particular a neuron dysfunctional, were used or laser ablation of neurons was done. Then navigation behavior of the mutant worms was recorded and

compared with wild-type worms. Based on the behavioral studies, role of different neurons in behaviors (such as reversals and omega turns) was ascertained and the circuit in 1.3 was proposed.

1.2 Networks

1.2.1 Introduction to Networks

A network, also called as graph in mathematical literature, is a collection of vertices (also called nodes) joined by edges (also called links)[20]. Networks are studied using graph theory from mathematics. Depending on system to be studied, nodes can be people (in Friendship Network[22]), webpages (internet[8]), protein (protein-protein interactions[15]), neurons (neuronal connectivity[27]), etc linked by relations, products, internet, hyperlink, synapses, etc respectively. Such diverse systems can be studied using graph theory from mathematics. In mathematical terms a network can be represented as a matrix called Adjacency Matrix (A), where the rows and columns of the matrix are the vertices of the network and elements of matrix A_{ij} are given by,

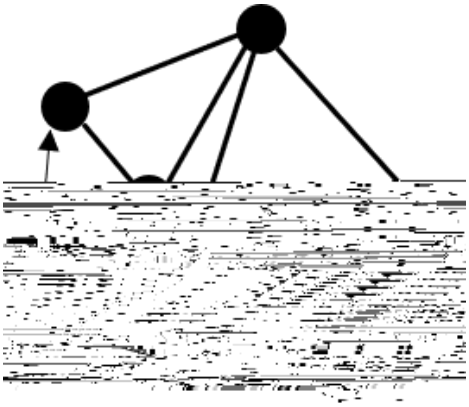


Figure 1.4: A simple network

$$A_{ij} = \begin{cases} 1 & \text{if there exists edge between vertices i and j,} \\ 0 & \text{otherwise.} \end{cases}$$

For a network with no self-edges, the diagonal elements of this matrix are all zero. If there are multiple edges between two vertices they can also be represented in above matrix be assigning the value of corresponding element A_{ij} equal to multiplicity of the edge.

single node can be calculated in following way,

$$C_v = \frac{2n}{k_v(k_v - 1)}$$

where n is number of links between k_v neighbours of node v.

Shortest Path Length

The smallest number of links connecting a pair of nodes is called the shortest path between them and length of such a path is called shortest path length. The average of all the shortest path lengths, between all node pairs in a network, is called characteristic path length (L) of a network. It can be calculated as follows,

$$L = \frac{2\sum_{i=1}^{n_r-1}\sum_{j=j+1}^{n_r} L_{ij}}{n_r(n_r - 1)}$$

where n_r is number of nodes and L_{ij} is shortest path length between nodes i and j.

1.2.4 Small-World Networks

When the nodes in a network are connected randomly it gives a Random Network. If p is the probability of randomness, then connections with p=0 will give a Regular Network and p=1 would give rise to a Random Network. Regular Networks have high

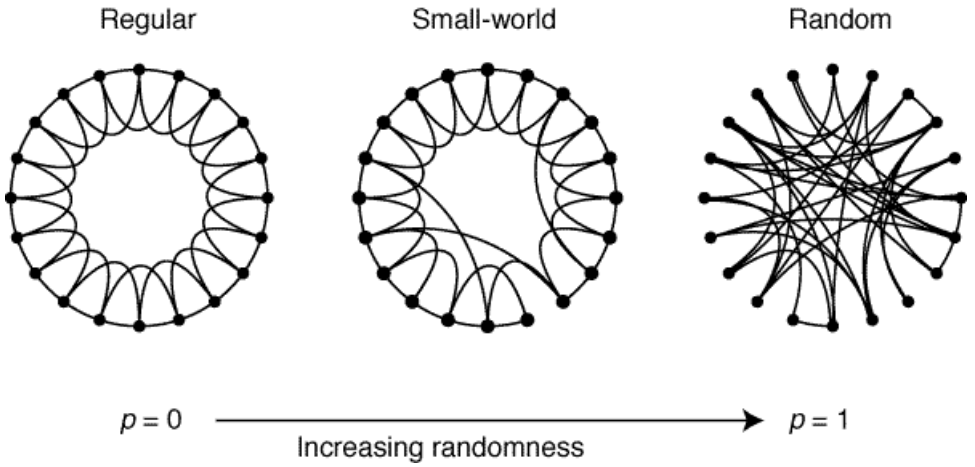


Figure 1.6: Small-world Networks[25]

clustering coefficient and characteristic path length, while random networks have low

clustering coefficient and characteristic path length. For certain values of p between 0 and 1, there are networks which have high clustering coefficients and low characteristic path lengths. Such networks are called Small-World Networks.

Most real-world networks have been seen to show small-world properties.

1.2.5 Power-law Distributions

Degree distribution, which is frequency ($P(k)$) of neurons with a particular degree k, plotted as a histogram, is one of the fundamental properties of network. One of the ways of visualizing degree distribution is to plot it on a log-log plot. It has been often found that the degree distribution of real-world networks is seen as straight line on a log-log plot, whose slope is equal to the power-law exponent α. If a degree distribution follows power-law then,

$$\ln P(k) = \alpha \ln (k) + C,$$

taking log on both sides gives us,

$$P(k) = Ck^{-\alpha}$$

where the constant α is called exponent of the power-law. For most real-world networks the values of α are generally between 2 - 3 and can even be little bit more or less.

1.2.6 Community Analysis

In networks, often group of nodes have more connections among themselves than other groups. Such groups are said to be forming communities or modules. Various methods have been proposed to place nodes in communities based on different measures of networks. One such method is based on calculation of modularity quotient (Q) [19], which is a measure of the extent to which similar nodes are connected to each other

in a network. It is defined as follows,

$$Q = \frac{1}{2m} \sum_{ij} (A_{ij} - \frac{k_i k_j}{2m}) \delta(c_i, c_j)$$

where m is number of edges in the network, k is degree and $\delta(c_i, c_j)$ is kronecker delta function and c_i, c_j are classes of the nodes.

The thesis reports work, done during the year 2018-19, in two parts- experimental and theoretical. The experimental part focuses on carrying out behavioral assays to understand the chemotaxis behavior of the *C. elegans* and the underlying neuronal circuitry. The theoretical part focuses on studying neuronal connectivity in *C. elegans* using graph theory to see if analysis of connectivity pattern can explain the observed behavior in worms by doing community analysis and shortest path analysis. The behavior of interest here is chemotaxis, which is mediated by chemosensory neurons.

Chapter 2

Material & Methods

2.1 Experimental

2.1.1 Strains Used

The *C. elegans* N2 strain was used as control for all assays performed. The mutant strains were, *exp-1*, *rb2302* (daf-7), *rb1667* (tax-6), *rb2464* (tax-2), *vc2413* (str-2), *vc3113* (tax-4), *lin4* (development defective mutant). The double mutants were, *rb2302*exp-1, rb1667*exp-1, rb2464*exp-1, vc2413*exp-1, vc3113*exp-1, lin4*exp-1.* *tax-2* and *tax-4* mutants are known to affect siganl transduction in chemotaxis of *C .elegans*, while tax-6 is known to regulate the tax-4 and tax-2. STR-2 is GPCR involved in volatile chemotaxis behavior of *C. elegans*[1].

2.1.2 Egg Synchronization

Egg synchronization was done to obtain *C. elegans* population of same developmental stage, to perform behavioral assays.

1. M9 buffer was poured onto Nematode Growth Media (NGM) food (OP50 strain of *Escherichia coli*) plates containing particular strain of *C. elegans* and plates were kept at an angle for buffer, containing worms, to collect at bottom edge of the plate.

2. Using 1 ml micropipette, the buffer was collected and transferred into 1.5ml MCT (microcentrifuge tube).

3. Into the MCT, 400μl of bleach solution (Alkaline Hypochlorite Solution) was added and MCT was vortexed for about 10 minutes.

4. The MCT was then centrifuged at approximately 4600 rpm for 60 seconds.

5. Supernatant from the MCT was discarded and 1 ml M9 buffer was added. The MCT was then centrifuged at 4600 rpm for 60 seconds.

6. Step 5 was repeated one more time.

7. Supernatant was then discarded and the pellet was mixed properly with 100μl of M9 buffer.

8. Inside a laminar hood the pellet was the transferred onto a NGM food plate, at the edge of the plate and liquid was allowed to dry of the plate.

9. The plate was then transferred into incubator and kept at 20^0Celsius.

2.1.3 Chemotaxis Assay

All the assays were done at the temperature of 20^0-24^0 C. NGM agar plates without food were used for assays. The plates were allowed to dry in laminar hood for about an hour. Plates were then marked at bottom as per assay design. The assay design was as shown in figure 2.1[17]. Here T represents Test Chemical, for which response of the worms will be assayed and C represents Control Chemical, which was the chemical in which test chemical was diluted. Test chemicals used were, 2-Butanone, Isoamyl alcohol, Benzaldehyde, Diacetyl, Nonanone. The Control chemical used for all the cases was Ethanol. Each assay for every strain was carried out with multiple Test chemicals and was done in triplicate. For every strain tested, N2 strain was used

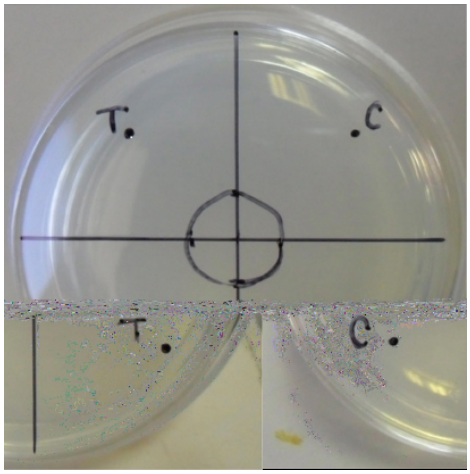


Figure 2.1: Chemotaxis Assay design

as control. The the assay was conducted in following way:

1. Worms were collected from a NGM food plate, using M9 buffer, into a MCT.
2. Once the worms were settled at bottom of the MCT, the supernatant was aspirated out and again 1ml M9 buffer was added (washing).
3. Step 2 was repeated two more times.
4. After the 3rd wash as the worms were settling down, on the assay plate, 4μl of sodium azide (to paralyze the worms) was added in four spots marked on the plate.
5. Then the supernatant from the MCT was aspirated out and worms were collected in micropipette tip and put at the center of the assay plate, inside the marked circle.
6. As liquid dried off the plates and worms started to show movement, Test and Control chemical 2μl each were added to the respective spots.
7. The plates were then covered and left undisturbed for 90 minutes. After 90 minutes, plates were oberved under light microscope and number of worms in each quadrant were calculated. Then chemotatic index (CI) for each assay was calculated as follows,

$$CI = \frac{\text{\# worms in Test quadrants} - \text{\# worms in Control quadrants}}{\text{Total number of worms}}$$

2.2 Theoretical and Computational

2.2.1 Neuronal Connectivity Data

C. elegans neuronal connectivity data was obtained from `http://www.wormatlas.org/neuronalwiring.html`, provided by Varsheny *et al*[24]. The data was in csv (comma separated values) format. Data was arranged in four columns, Neuron 1, Neuron 2, Type and Nbr. Columns Neuron 1 and Neuron 2 consisted of names of neurons between which there exists a synapse. Type column described the type

of synapse that existed between Neuron 1 and Neuron 2 and consisted of following entries, R, Rp, S, Sp, EJ, NMJ[28], as described below-

- Send or output (Neuron 1 pre-synaptic to Neuron 2)
- Sp: Send-poly (Neuron 1 is pre-synaptic to more than one postsynaptic partner. Neuron 2 is just one of these post-synaptic neurons.
- R: Receive or input (Neuron 1 is post-synaptic to Neuron 2)
- Rp: Receive-poly (Neuron 1 is one of post-synaptic partners of Neuron 2)
- EJ: Electric junction
- NMJ: Neuromuscular junction. The Nbr column represented number of synapses of particular type between the neurons in Neuron 1 and Neuron2

2.2.2 Network Construction and Analysis

For network construction and analysis, NetworkX[12] library in Python was used. The data was processed for network construction using Pandas[18] library available in Python (programming language). The network was constructed in following way, each neuron was considered a node and the synapse between two neurons was considered as edge/link connecting the nodes. From the data, each row represented the edge between the nodes in two columns (specified while importing the data). All of the analysis was done without considering the number of synaptic connections between two neurons.

Construction of Networks

- Undirected Network
    1. Data was imported in Pandas dataframe (DF).
    2. The column Type was dropped from the DF.
    3. Duplicate rows were removed from DF.
    4. Rows with same Neuron 1 and Neuron 2 values were deleted.

5. DF was then used for undirected network construction using NetworkX.

6. Multiple edges were ignored while constructing the network.

7. *For network without NMJ, all rows with NMJ as neuron were deleted from the DF and same protocol as above was followed.

- Chemical and Electrical Synapse Network

1. Data was imported in Pandas dataframe (DF).

2. For chemical Synapse Network, only those rows for which Type was R, Rp were kept and rest were deleted from DF. Similarly, for Electrical Synapse Network, only rows where Type was EJ were kept and others were deleted.

3. Rows with same Neuron 1 and Neuron 2 values were deleted.

4. For chemical Synapse Network, graph to be constructed was explicitly specified as Directed in NetworkX, with Neuron 2 kept as source and Neuron 1 as target. For Electrical Synapse Network the undirected graph in NetworkX was used.

5. Multiple edges were ignored while constructing the network.

- Network of Chemosensory Neurons

1. Step 1 to 4 of Undirected Network construction.

2. From each column all rows were deleted except for those containing chemosenosry neurons in both of the columns.

3. Step 5 and 6 of Undirected Network construction.

Network Parameters

Parameters such as Degree, Clustering Coefficient and Shortest Path Lengths were calculated for all the nodes and for whole networks, both undirected and directed, using algorithms provided in NetworkX.

Community Analysis

Community analysis was done using Fast-Greedy algorithm[5] as implemented in NetworkX. For Chemical Synapse Network, directionality of edges was ignored while doing community analysis. For each network modularity score was calculated.

Shortest Path Analysis for Navigation Circuit

All possible shortest paths were calculated for navigation circuit of C. elegans, with AWC(L/R) as starting nodes and SMD(v/D)(L/R), RIV(L/R), AVA(L/R) as ending nodes using NetworkX.

2.2.3 Visualization

All visualizations were done in Cytoscape[23] and Gephi[3] in following way:

1. Nodes of networks in cytoscape were given attributes function (type of neuron) , community (which community the neuron belonged) and chemosenosry (whether neuron was chemosensory or not).
2. Network was then exported in Graphml format from NetworkX.
3. Graphml was then imported either in Cytoscape or Gephi.
4. Circular layout was chosen to arrange nodes as ring. The nodes were colored depending upon the attributes.

Distribution Plots

The plots were made using matplotlib[13] library in Python. For histograms of degree distributions, each bar represented one degree. In case of histograms of clustering coefficient distributions, bar width was calculated based on Freedman-Diaconis rule[10].

Based on eye-estimation, only those nodes which appeared to follow power-law (on log-log plot) were selected. Then linear regression method, using SciPy library in

Python, was used to fit the data to a straight line, slope of which gave the value of α (power-law coefficent).

Small-World Networks

Random and regular graphs were generated using the graph generators available in NetworkX.

To generate regular network, p was kept 0 and average degree per node (k) was chosen such that number of edges for resulting network was similar to neuronal networks of *C. elegans*.

To generate random network, p was taken 1 and number of edges for resulting network were same as test-network. Then average clustering coefficient and average shortest path length for 100 such networks was recorded and then averaged to compare with the values of neuronal networks of *C. elegans*.

Shortest Path Graphs

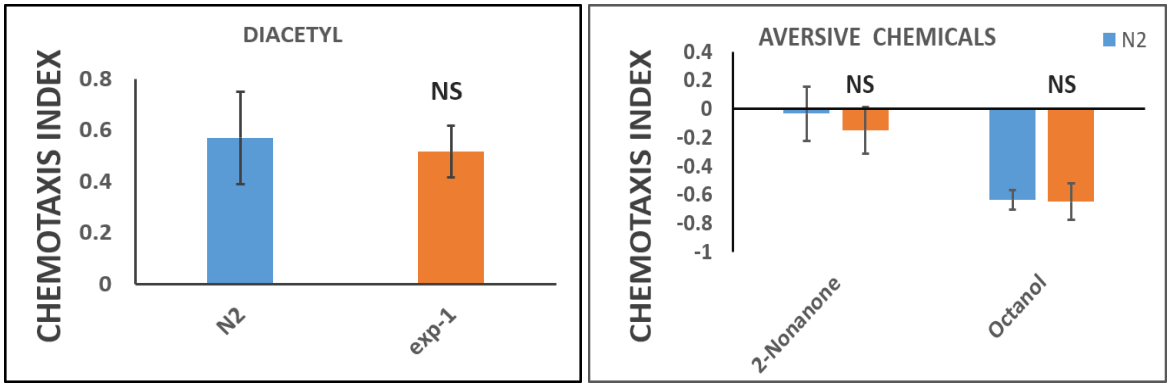
The shortest path graphs were made in Cytoscape. Neurons of same class were manually put together from the shortest path analysis data. Then each neuron class was arranged based on its function, from sensory neuron to interneuron to motor neuron and the edges were connected if neurons in those classes shared a shortest path.

Chapter 3

Results & Discussions

3.1 Experimental - Behavioral assays

Chemotaxis assays, as shown in figure 2.1 were performed to check the role of EXP-1 protein in AWC dependant signalling. To test for the role of EXP-1 in AWC dependant chemotaxis, single and double mutants of exp-1, ceh-36, tax-4 and tax-2 were used. *tax-4, tax-2* and *ceh-36* have known to affect the chemosensory ability of *C. elegans* [16][6]. The positive chemotaxis index (CI) indicates that worms were

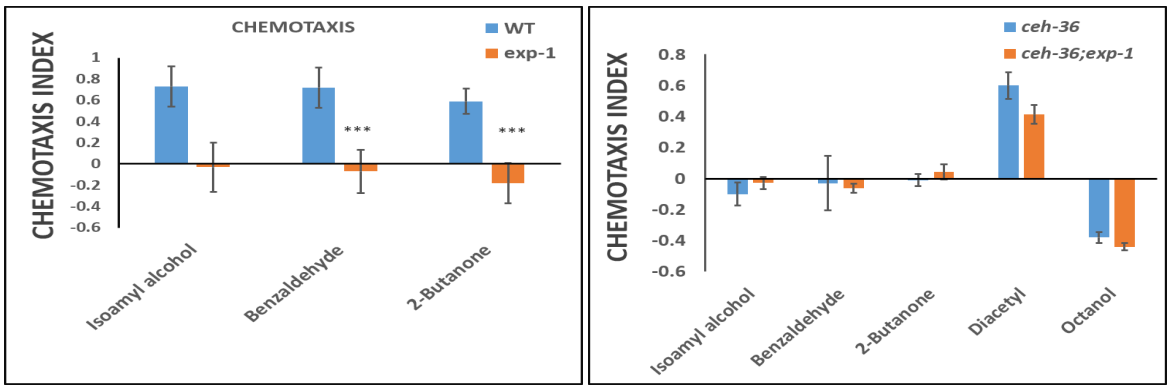


(a) AWA dependant chemotaxis (b) AWB dependant chemotaxis

Figure 3.1: EXP-1 in AWA and AWB

attracted by the chemical, while negative CI indicates that worms were repelled by chemicals. Generally, When effect of *exp-1* on AWA (figure 3.1a) and AWB (figure 3.1b) dependant chemotaxis was studied, there was no significant difference between N2 and *exp-1*. On the other hand, can be seen in figure 3.2a, *exp-1* shows significant

repulsion to the chemicals which are otherwise very good attractants for N2. Since these chemicals are known to be sensed by AWC[2], it suggests that mutation in EXP-1 does affect AWC mediated chemotaxis behavior but not AWA or AWB neuron (which are also chemosensory neurons) mediated chemotaxis behavior. To check for the



(a) WT vs *exp-1* (b) *ceh-36* vs *exp-1;ceh-36*

Figure 3.2: Chemotaxis Assay

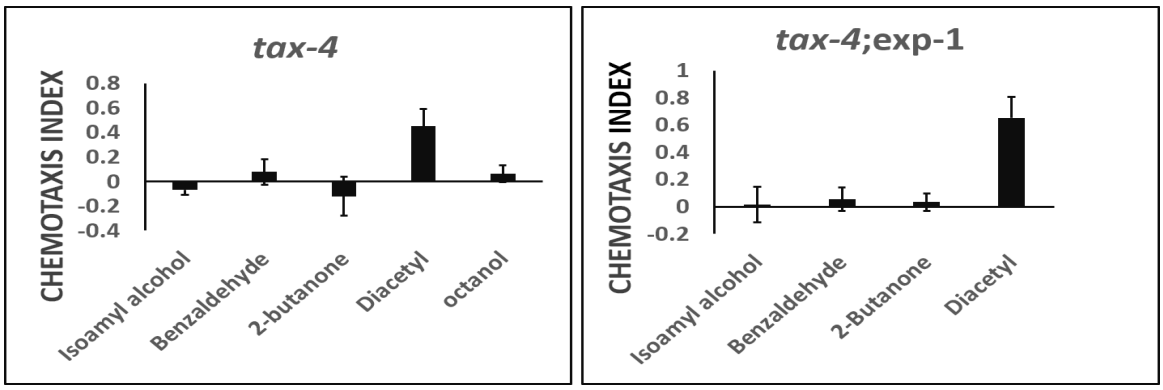


Figure 3.3: *tax-4* vs *exp-1;tax-4*

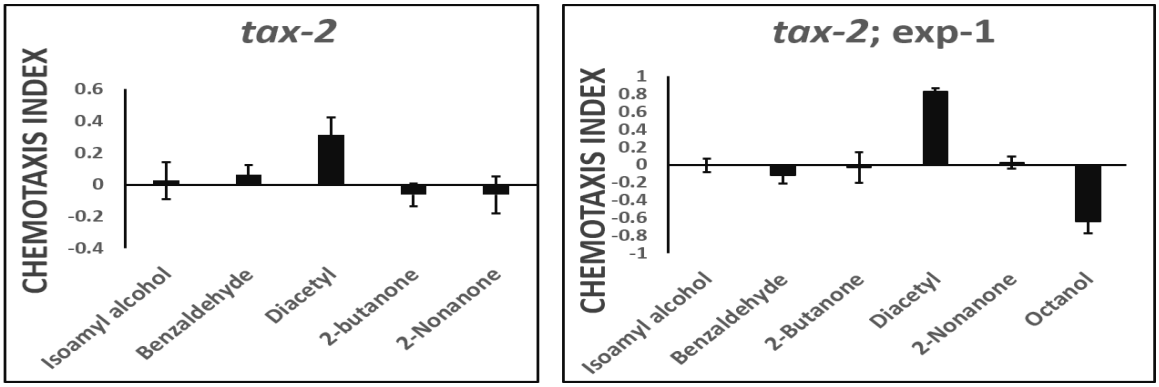


Figure 3.4: *tax-2* vs *exp-1;tax-2*

role of EXP-1 in signal transduction of AWC neurons, assays were done for *tax-4,*

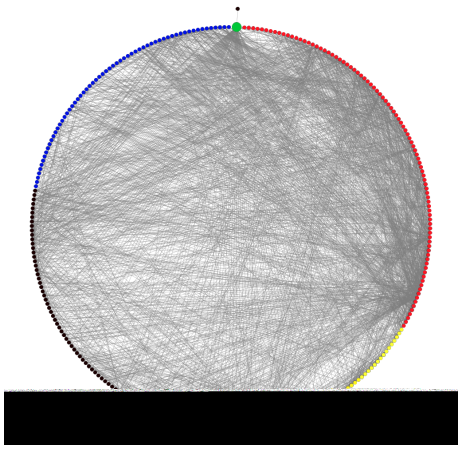
tax-2 and *ceh-36* single mutants and their double mutants with *exp-1*. As can be seen from 3.2b, there is not much difference between chemotaxis index of *ceh-36* and *exp-1;ceh-36*. Similarly, no significant difference was observed between single mutant of *exp-1* and double mutants of *tax-4* (figure 3.3) and *tax-2* (figure 3.4).

Therefore it can be predicted that EXP-1 might be involved in the signal transduction of AWC neurons, in response to volatile chemicals, to generate chemotaxis behavior.

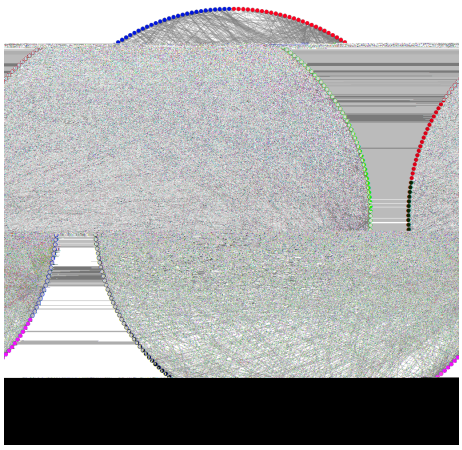
3.2 Analysis of Networks

3.2.1 Neuronal Networks of *C. elegans*

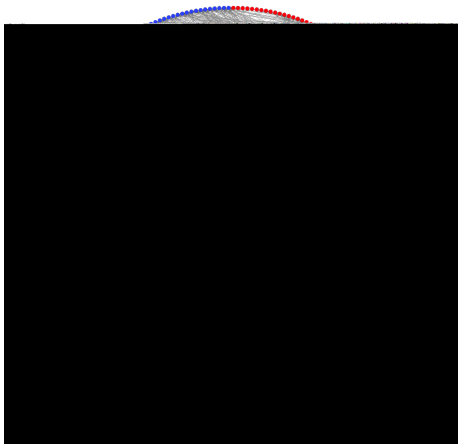
Figure 3.5 shows the networks constructed from *C. elegans* neuronal connectivity data. The networks are visualized as ring graphs, where neurons (nodes) are placed at the periphery of the ring and their connectivity indicated with black edges and color of nodes indicate their functions. Figures 3.5a and 3.5b are undirected networks with all neurons and all types of connections shown, 3.5c is directed network comprising only of chemical synapses while, figure 3.5d is undirected network comprising only of electrical synapses.



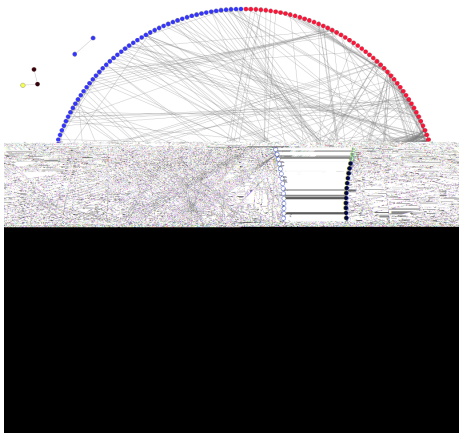
(a) Undirected Network with NMJ



(b) Undirected Network without NMJ



(c) Chemical Synapse Network



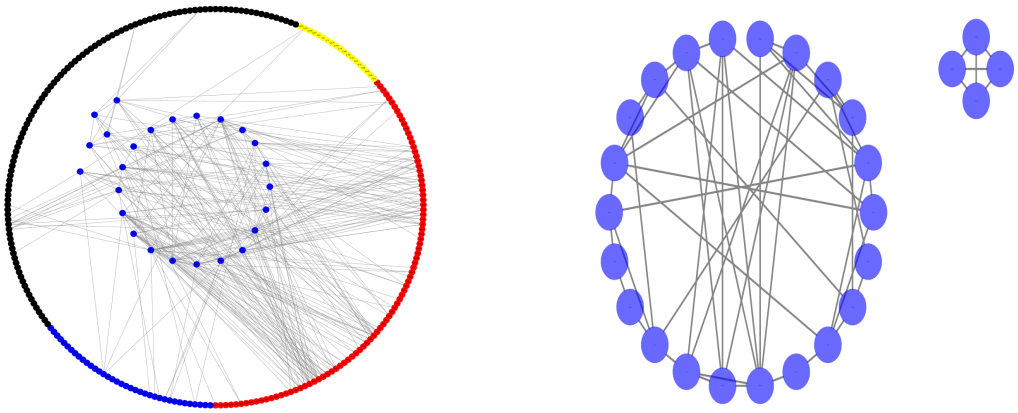
(d) Electrical Synapse Network

Figure 3.5: Networks as ring graph

Color indicates function of the neuron. Blue is sensory neuron, Red is interneuron, Black is motor neuron and Yellow is polymodal neuron. Green node is NMJ.

In undirected networks, figure 3.5a and 3.5b, when NMJ (Green node in 3.5a) is removed, lots of edges are removed in 3.5b. Removal of NMJ also removes one neuron from all the other networks, as that neuron only makes NMJ synapse. In Chemical Synapse Network (3.5c), the synapses allow flow of information (chemical messages) only in one direction, from pre-synaptic to post-synaptic neuron, making the edges directed. The electrical synapse allow bi-directional flow of information (electrical ions) thus making Electrical Synapse Network (f3.5d) undirected. From the graphs it can be seen that the nodes have a large number of connections among them, and among the types of neurons, the interneurons (in Red) are densely connected within and also with motor neurons. This indicates that interneurons communicate not only among each other but also with motor neurons for efficient functioning.

Network of Chemosensory Neurons



(a) CSN in Undirected Network (b) Networks of CSN neurons

Figure 3.6: Networks of Chemosensory Neurons

Figure 3.6a shows how chemosensory neurons (CSN) are connected among themselves and to other neurons in the undirected network. Most of the edges from chemosensory neurons are are to other chemosensory neurons or to interneurons. Figure 3.6b shows the connectivity of chemosensory neurons among themselves. These neurons form two separate networks based on within CSN connections. The network consists of two components, large component consists of 22 neurons, while smaller one consists of only 4 neurons. Even though they carry out same function, the neurons in smaller component are physically located in the tail region of the worm, while the other 22

neurons are located in the head region and thus have no direct connection among them.

3.2.2 Network Parameters

Figure 3.7 is a comparative table of size (number of nodes and edges) and network parameters for all the four networks 3.5. The parameters studied were Degree, Shortest Path and Clustering Coefficient. Data mentions NMJ both as a neuron and as a

	Undirected with NMJ	Undirected without NMJ	Chemical Synapse (Directed)	Electrical Network (Undirected)
Nodes	281	279 + 1	279	245 + 3 + 2
Edges	2404	2287	2194	512
Average Degree	17.09	16.39	**In** : 7.86 **Out** : 7.86	4.09
Average Clustering Coefficient	0.346	0.337	0.212	0.204
Average Shortest Path Length	2.32	2.43	2.95	4.5

Figure 3.7: Parameters

synapse. But NMJ is common name given to all the different neuron-muscle contacts. NMJ, as a node, is collection of all different muscle cells interacting with neurons through NMJ synapse. Removal of NMJ, as node, removes all neuromuscular contacts and also, one other neuron from the network which only had NMJ synapse. This tells why Undirected Network without NMJ has only 279 nodes and 2287 edges only. For Chemical Synapse Network, the average in-degree and out-degree is half of the average degree for Undirected Network, even when the total number of edges are nearly the same. The Electrical Synapse Network has very low average degree because the number of nodes in not much different compared to Chemical Synapse Network, but number of edges is very low, making it a very sparse network. Comparison of Chemical and Electrical Synapse Networks reveals that chemical synapse is more prominent in neuronal communication of *C. elegans* compared to electrical

synapse. The average clustering coefficient (C) is nearly same for undirected networks but it is lower for Chemical Synapse Network and Electrical Synapse Network. C is similar for Electrical and Chemical Synapse Networks, even though the number of edges is very different for both.

Small-World Properties

The average clustering coefficients (C) and average shortest path lengths (L) were compared for all the four networks 3.5a, 3.5b, 3.5c, 3.5d, against equivalent random and regular networks. As can be seen figure 3.8 the all regular networks (Blue) have very high L and C while, all random networks (Red) have very low L and C. For all the *C. elegans* neuronal networks (Green) L is lower than that of regular network while C is greater than that of random networks. This suggest that pattern in the

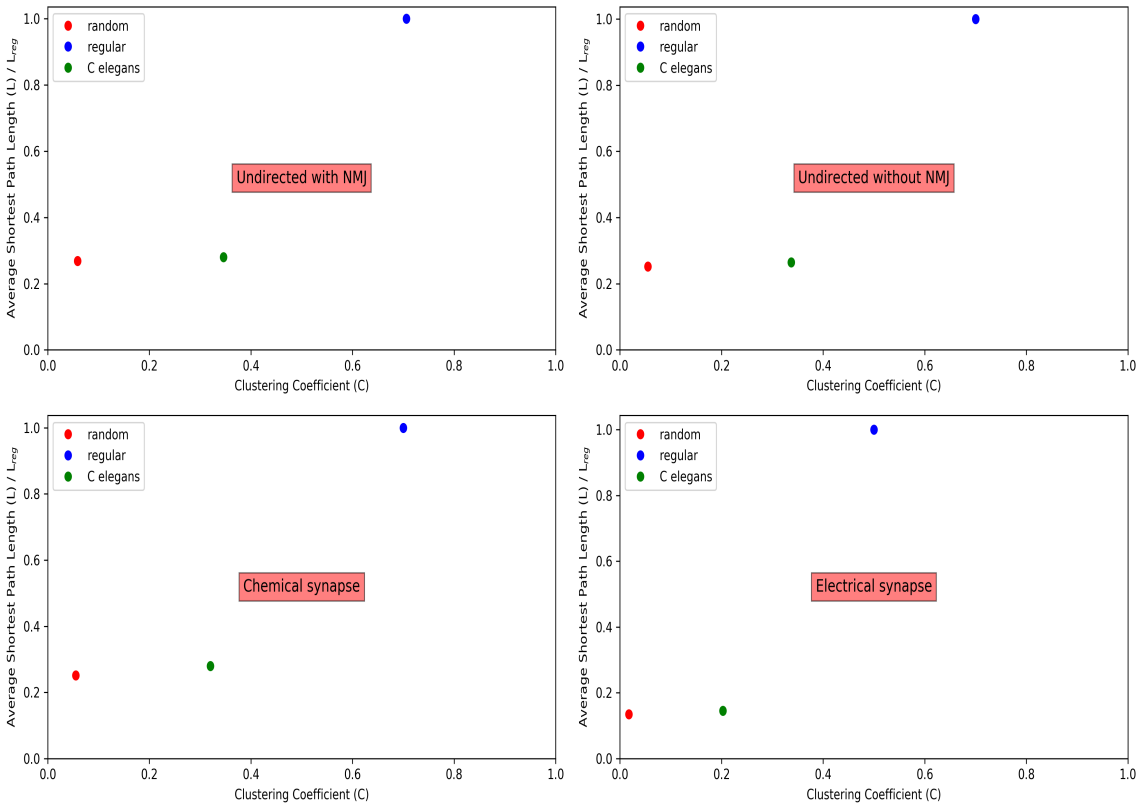


Figure 3.8: L-C plot

worm is distributed such that the shortest paths in all four are quite small, but the neurons are more clustered than the Random but certainly much less than the Regular networks. This allows fast information transfer among the neurons, and that makes

the networks "Small-World' (figure 1.6). (X-axis is C in range of 0-1, Y-axis is L/L_{reg} (where L_{reg} is L for regular network) in range of 0-1.

3.2.3 Distributions of Network Parameters

Degree Distribution

One of the most important signatures of a the pattern of connectivity in a network is its degree distribution, which is a histogram of number of nodes with a particular degree, where X-axis represents degree k and Y-axis denoted fraction of nodes with degree k. Degree distribution was plotted for all the four networks shown in figure 3.5 for all four networks and shown in Figures 3.9 (a-e), where the in and out-degree distributions are plotted separately for the chemical synapse network. As can be seen in figure 3.9, none of the distributions are normal (bell-shaped) distributions. All of them have a long tail towards right. This indicates the presence of hubs (nodes with very high

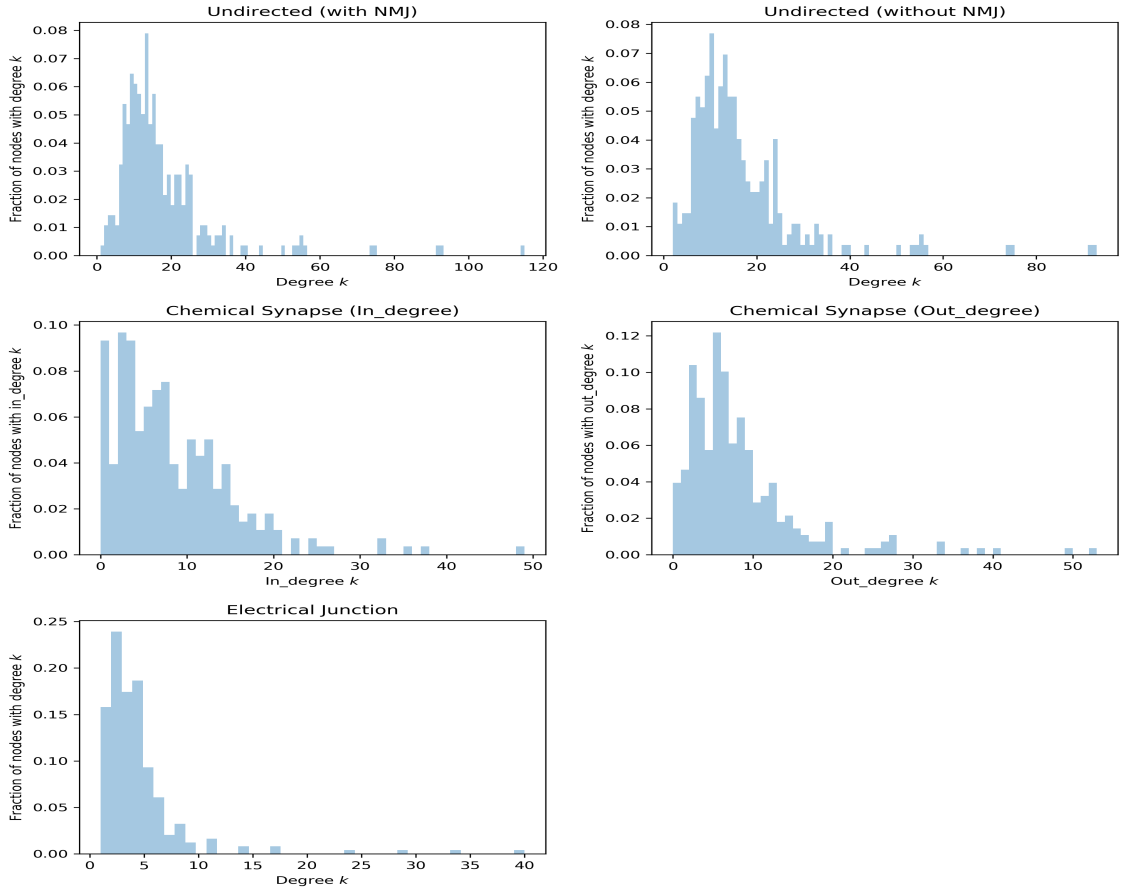
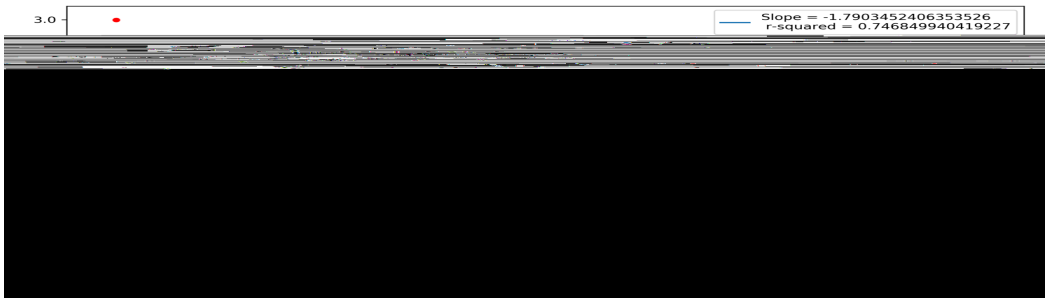


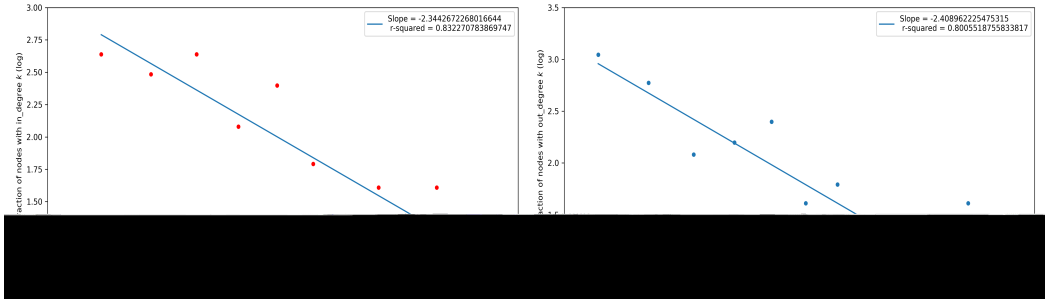
Figure 3.9: Degree Distributions

degree). Hubs are very few in all the networks and most nodes have degree around the average degree of the network. Presence of hubs has been suggested to provide resilience against random attacks on a network, because in such random attacks the chances of hubs being affected is very low as they are very small in numbers, thus preventing large damage to the network [7]. But, if a targeted attack is made on the hubs then network will become dysfunctional very quickly. Hubs also allow effective transfer of the information in the network. In all the four networks the major hubs are interneurons, which function in processing of the information received from sensory inputs.

Power-law fit

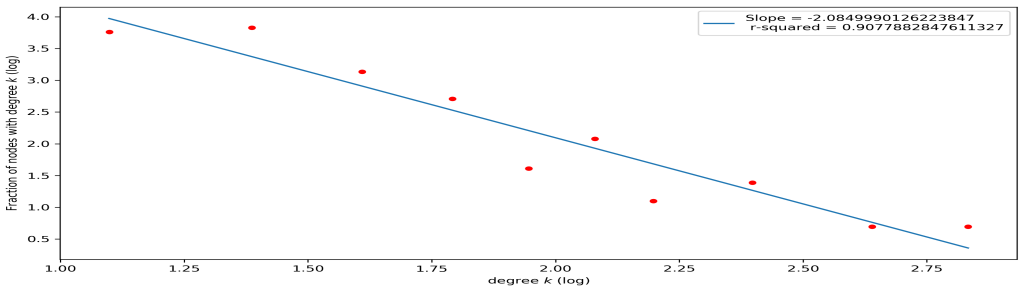


(a) Undirected Network (no NMJ)



(b) Chemical Synapse Network

left:In-degree Distribution, right:Out-degree Distribution



(c) Electrical Synapse Network

Figure 3.10: Power-law fit for degree distributions

Generally, for real-world networks, power-law fit is done for only the tail region of the distribution, starting from the values for which power-law holds [24]. Power-law fit was done using the linear regression method for Degree Distributions of Undirected Network (without NMJ), Chemical Synapse Network and Electrical Synapse Network, where data was plotted on the log-log plot. The results of which are shown in figure 3.10. Value of α for 3.10a is 1.7, for In-degree distribution and Out-degree distribution in 3.10b it is 2.4 and 2.3 respectively and for 3.10c it is 2.1. Except for Undirected network without NMJ, the values of α lies in $2 \leq \alpha \leq 3$.

Clustering Coefficient Distribution

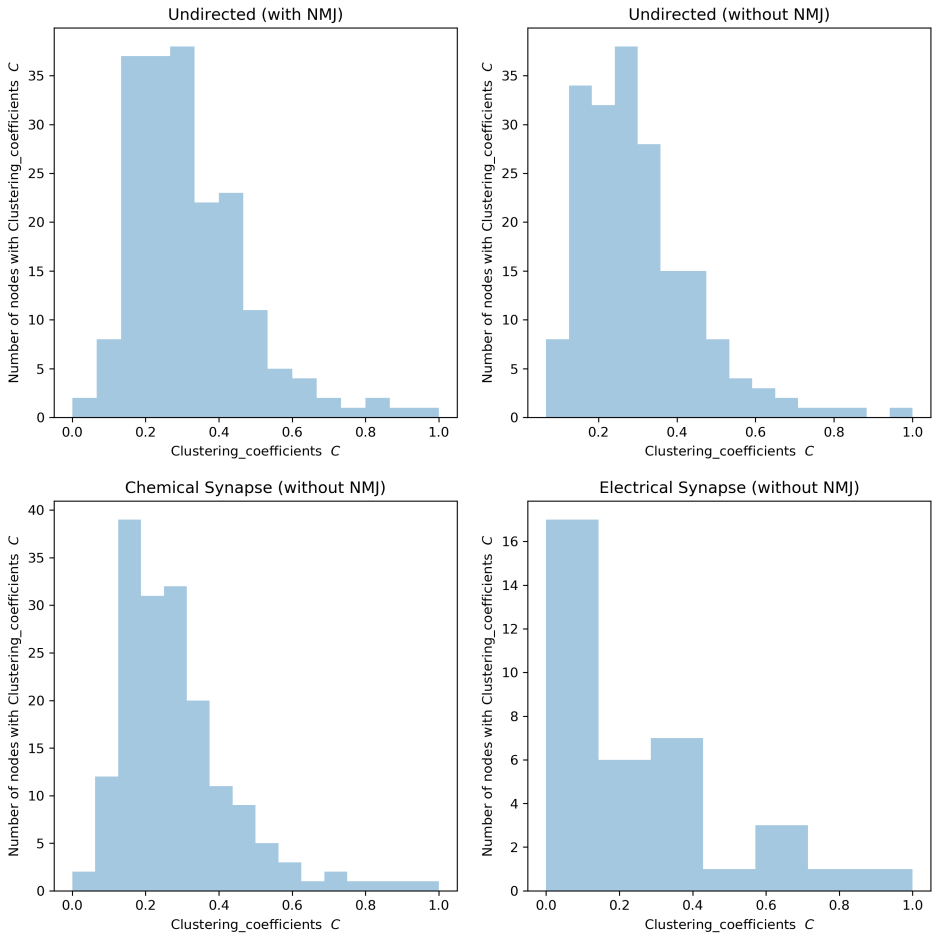


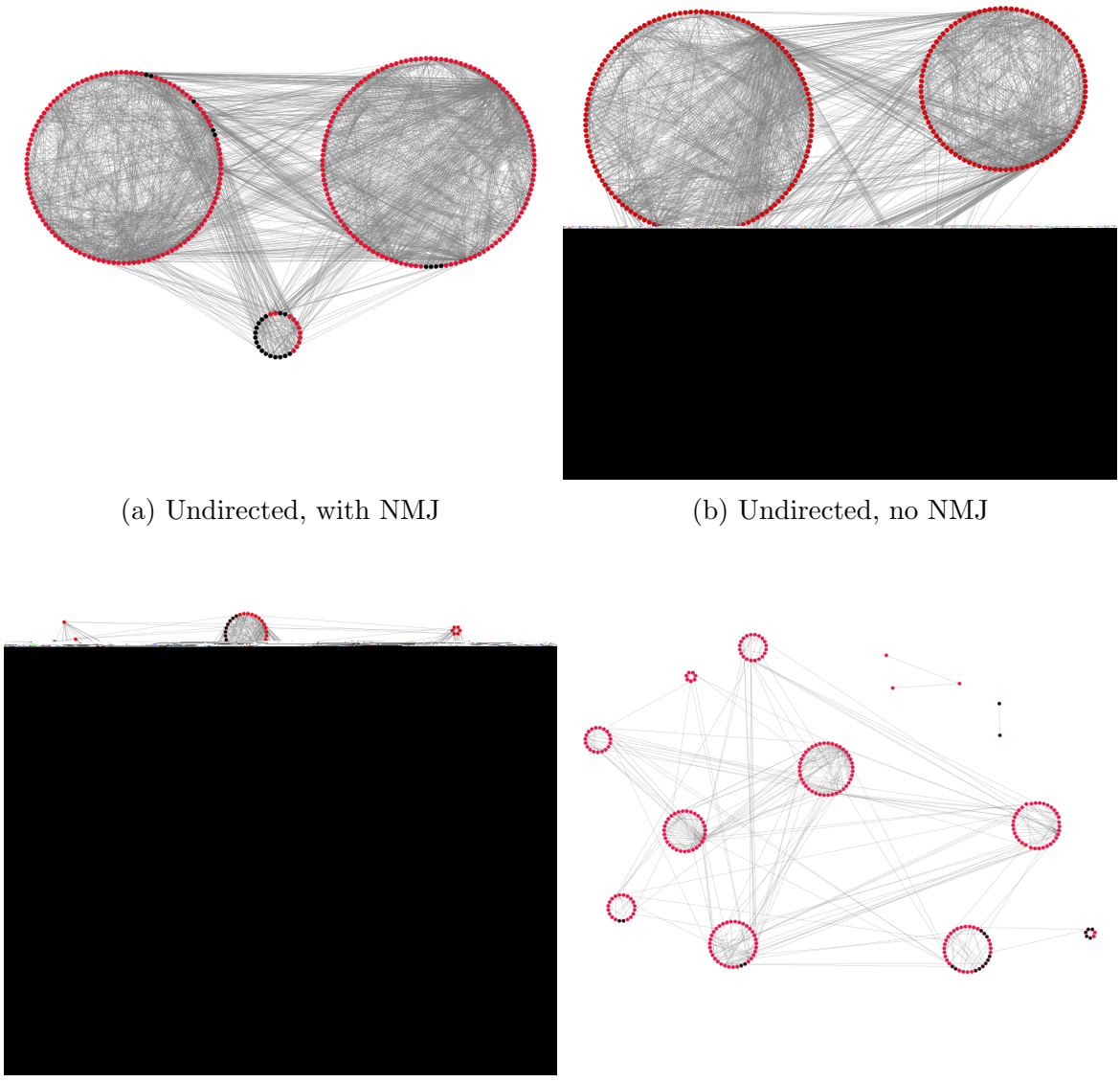
Figure 3.11: Clustering Coefficient Distribution

X-axis: Clustering Coefficients(cc), Y-axis: Fraction of nodes with cc

The Clustering Coefficient distributions, similar like degree distributions, were not normal distributions (bell-shaped) and had long tail on right side. It means there

are few nodes with very high clustering coefficients while most of the nodes have clustering coefficients around average value for the whole graph.

3.2.4 Community Analysis



(a) Undirected, with NMJ

(b) Undirected, no NMJ

(c) Chemical Synapse

(d) Electrical Synapse

Figure 3.12: Community View of Networks

Black:Chemosensory neurons, Red:All other neurons in the network. Q for (a) is 0.35, (b) is 0.38, (c) is0.37, (d) is 0.63

When community analysis was done on all the four networks, each network was divided into certain number of communities, as shown in the figure 3.12. All four networks

formed different number of communities with varying modularity values. But when analyzed for distribution of chemosensory neurons (CSNs), in all of the four networks CSNs were mostly found in a single community. In 3.12a only 3 communities are formed and out of the three, the smaller community predominantly consists of CSN. When NMJ is removed in 3.12b, the network now has 4 communities and here also out of four communities, in one community nearly half of the neurons are CSN and only 4 CSNs are present in other community. The reason for this is the physical separation of 4 neurons from other 22 neurons in tail and head regions respectively, as mentioned earlier. Similarly, in Chemical Synapse and Electrical Synapse network, the CSNs were predominantly present in a single community. This could be because the CSNs are all involved in same function, that is, sensation of chemicals. Each of the CSN senses a different chemical and can also sense multiple chemicals. This might be the reason why connectivity of CSNs is greater among themselves than with other neurons, to allow efficient transfer of information of chemical cues received, so as to generate appropriate behavioral response to the cues. Thus, connectivity pattern of chemosensory neurons is indicative of the functions they carry out.

3.2.5 Navigation Circuit

To check if analysis of network parameters in neuronal networks of *C. elegans* can predict neuronal circuits that are experimentally derived, shortest path analysis was done of Undirected Network without NMJ and Chemical Synapse Network.

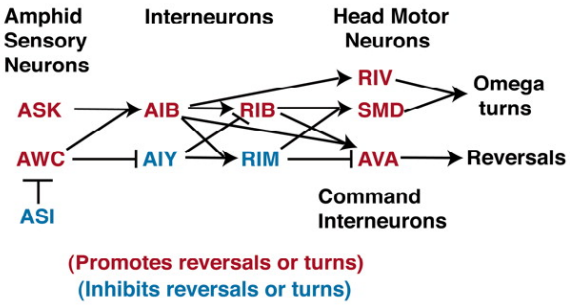
Undirected Network without NMJ

The experimental navigation circuit as described in figure 1.3 and shown in figure 3.13a, connects the starting node AWC to the ending nodes RIV, SMD, AVA in two paths of 2 links each and one path of 3 links, with intermediate neurons being interneurons. To see if analysis of undirected neuronal network can reveal additional possible connectivity patterns as in the experimentally derived navigation circuit, shortest path analysis was done. Figure 3.13b shows all the shortest paths between AWC and RIV, SMD, AVA, with general scheme of information transfer from sensory

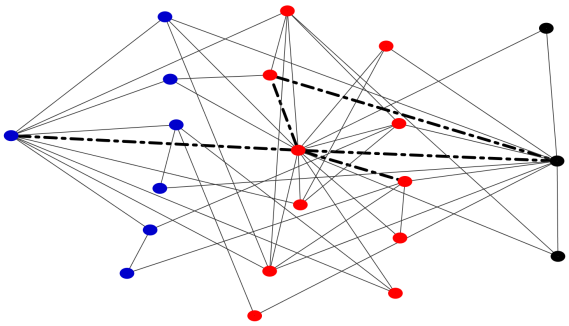
neuron to interneuron to motor neuron. The dotted path in figure 3.13b indicates the experimental navigation circuit present as shortest path. Thus, shortest path analysis does reveal the experimental circuits. In figure 3.13c alternate circuits from AWC to RIV, SMD, AVA, with interneurons in between and with 2 or 3 links are predicted.

Chemical Synapse Network

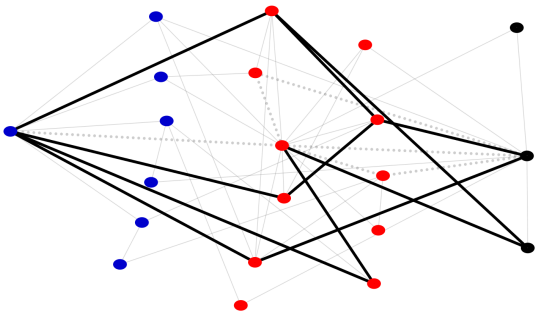
In shortest path analysis of directed network (figure 3.13c), the navigation circuit is not present as shortest paths. Even first link from AWC neuron to AIB neuron from navigation circuit is absent in shortest paths of directed network. The possible explanation for absence of AWC-AIB link here could be that this connection is mediated by electrical synapse. But that possibility is also not valid because AWC has no electrical synapse. This indicates that there might be other neurons, connected with more than 2 or 3 links, that regulate the behaviors in the navigation of the worm.



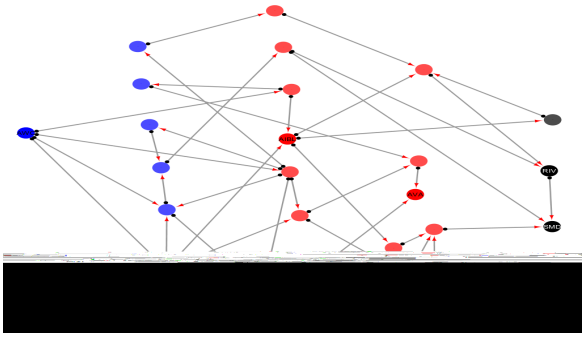
(a) Navigation Circuit



(b) Navigation Circuit in Shortest Paths



(c) Alternate Circuits



(d) Shortest Paths in Directed Network

Figure 3.13: Shortest Path Analysis

Blue:Sensory Neuron, Red:Interneuron, Black:Motor Neuron

3.3 Discussions

The thesis work focused on analyzing neuronal connectivity of *C. elegans* using experimental and theoretical approaches. The experimental approach was used to discern the neuronal circuitry underlying chemotaxis behavior of *C. elegans* by analyzing the role of EXP-1 protein in chemosensory ability of AWC neurons. Chemotaxis behavioral assays were carried out on N2 strain and mutants of exp-1 along with mutants of proteins known to be essential for chemosensory signal transduction in AWC. Based on preliminary analysis it was found that exp-1 affects only AWC mediated chemotaxis but not the chemotaxis mediated by other chemosensory neurons. Also, it was found that exp-1 is involved in signal transduction of AWC in response to volatile chemicals.

In theoretical approach, graph theory was used to study neuronal connectivity of *C. elegans* to see if the connectivity pattern can explain observed behavior in *C. elegans*. First, two undirected networks were constructed (with and without NMJ) to understand the general properties of neuronal connectivity. Then networks based on types of synapses, chemical and electrical, were constructed and analyzed. Visualizations of the networks revealed that interneurons had higher connectivity among themselves compared to sensory or motor neurons. To understand the global properties degree, clustering coefficient and shortest path length were calculated for whole network. Analysis of these parameters revealed that all the four networks showed Small-World Network properties. On analyzing the degree distribution, it was found that the distributions were not normal and showed presence of hubs in network. When power-law fit was done for the tail region of the distributions, the power-law exponent obtained ranged in between 2 - 3, except for Undirected Network without NMJ, which indicates that they show the properties of real-world networks.

To further understand the nature of connectivity in *C. elegans* community analysis was done for all the four networks. Each network formed different number of communities, but when checked for the distribution of chemosensory neurons, most of the chemosensory neurons always occurred in one single community. Each of the chemosensory neuron senses different chemical and can even sense multiple chemicals. This requires the chemosensory neurons to have greater communication among

themselves than with other type of neurons. Therefore, the connectivity pattern of chemosensory neurons in *C. elegans* is seen to be indicative of their function in this case.

Once it was seen that community structure is related to chemosensation function, the next question was to see if analysis of network could reveal or/and predict some experimental data. To answer this question, experimentally derived navigation circuit involved in food search behavior of *C. elegans* was tested against shortest path analysis results. It was found that, for undirected network, the shortest path analysis could reveal experimental navigation circuit and also predict alternative circuits that could affect the same behavior. The shortest path analysis of directed network (Chemical Synapse), which has same number of neurons, did not show the navigation circuit as shortest path. It indicates that there might be other neurons that also might be affecting the same behavior.

These preliminary experimental and theoretical studies lay the basis for further detailed study of the roles of specific neurons that turn out as hubs in both degree and clustering co-efficient distributions. Their nature and connectivity to other other types of neurons can offer means to understand functional connectivity pattern underlying different functions in *C. elegans*. Such information can offer specific experiments and/or mutations underlying different functional circuits.

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