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Inhibitory synapses are central to structural balance in *C.elegans* neuronal network

Real world networks display emergent phenomena arising out of interplay of positive and negative relationships among its constituent nodes. The functionality of networks is often due to the balance they achieve in the presence of competing relationships. Beyond studying systems level topological properties such as network traversal, transitivity (clustering), modularity, this calls for enumeration of such a balance. The harmony in networks of people, societies and countries has been investigated using the notion of 'structural balance' and this abstract concept could also be meaningfully applied in other networked systems.

7.1 STRUCTURAL BALANCE

Cartwright-Harary have proposed structural balance theorem to suggest combinatorial interactions among nodes under which 'balance' could be achieved. Here, 'structural balance' refers to harmonious coexistence in the presence of conflicting relationships. Any network is said to be balanced if every vertex is associated with its neighbors with all positive or all negative relationships. But, realistically networks tend to have mixed relationships. Balance in such networks can be adjudged with the help of the balance theorem [Cartwright and Harary, 1956; Harary, 1953]: "A labeled complete graph is balanced, if all pairs of nodes are friends or else nodes can be divided into two groups such that, each pair in both groups are in positive relationship with each other, but have negative relation with nodes of the other group." This theorem can be generalized as: 'a graph is balanced if it does not contain any cycle with odd number of negative edges' [Cartwright and Harary, 1956; Easley and Kleinberg, 2010; Harary, 1953; Heider, 1946] (Figure 7.1).

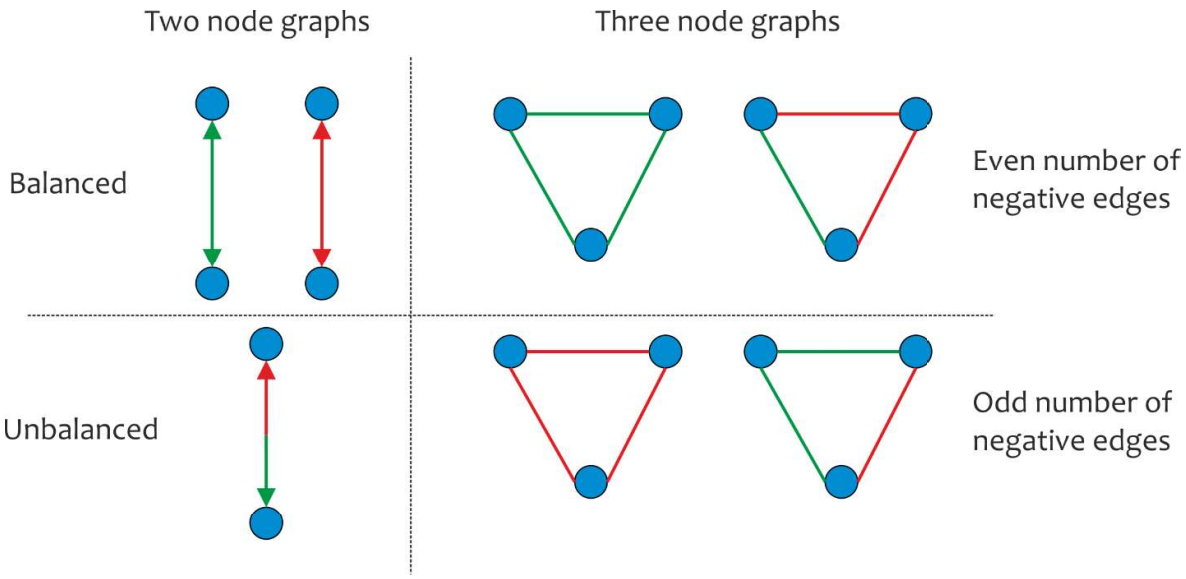

Figure 7.1 : Visualization of structural balance on the basis of positive and negative edges. Green edges represent a positive relationship, red edges represent a negative relationship between two nodes.

7.2 STRUCTURAL BALANCE IN NEURONAL NETWORKS

Neurons, the building blocks of nervous system, connect with each other via synapses for transfer of impulse. The structure of neuron is such that, information can flow in one direction from dendrites to axon. Hence the neuronal systems can be modeled as directed graphs, where neurons are nodes and synapse between them form the edges of a graph. As the most comprehensively mapped neuronal network, *C. elegans* presents an interesting system for study of structural connectivity and balance [White et al., 1986]. Despite rudimentary nervous system, this model organism displays an array of sensory, motor and cognitive functions [Ardiel and Rankin, 2010]. The crosstalk between neurons is generally based on chemical neurotransmitters which are released at synapse for impulse conduction. These biochemical messengers can be of excitatory or inhibitory types and hence can promote or constrain the transmission of information. Similar to many any other biochemicals in the cell, neurotransmitters are typically coded by genes located in the DNA. These genes can express in a neuron-specific manner to send signals to the next neuron. These excitatory/inhibitory signals are interpreted as positive/negative relationships in this neuronal network.

7.3 CONSTRUCTION OF SIGNED GRAPH OF CeNN

Starting from *C. elegans* neuronal connectivity graph, we found out the information of genes uniquely expressed in each neuron (Annexure B). Neurons were divided into 116 groups on the basis of gene expression. These neurons are connected with each other via chemical junctions that are classified as either excitatory or inhibitory synapses, depending upon the type of neurotransmitter released in the synapse. The information about inhibitory and excitatory neurotransmitters which are released in *C. elegans* neuronal network was obtained from Wormatlas [Altun and Hall, 2002]. We found that there were primarily six neurotransmitters which are responsible for impulse propagation in *C. elegans* neuronal network. Within these three were of excitatory nature and other three were of inhibitory type. The genes responsible for synthesis of these neurotransmitters in *C. elegans* were also collected (Table 7.1). The signed graph was constructed by assigning positive signs to synapses releasing excitatory neurotransmitters and negative signs to those releasing inhibitory neurotransmitters (Figure 7.2).

Table 7.1 : Neurotransmitter list and their actions performed in *C. elegans* neuronal system. Data collected from WormAtlas [Altun, Z.F., Herndon, L.A., Crocker, C., Wolkow, C.A., Lints, R. and Hall, 2016].

Neurotransmitter	Action	Genes
Acetylcholine	Excitatory [Bessou, Giugia, Franks, Holden-Dye, and Ségalat, 1998]	cha-1, unc-17, cho-1, snf-6 [Altun-Gultekin et al., 2001; Janet S. Duerr, Han, Fields, and Rand, 2008; Kim, Rogers, Richmond, and McIntire, 2004; Matthies, Fleming, Wilkes, and Blakely, 2006; Rand and Russell, 1985].
Serotonin	Excitatory (anterior)/inhibitory (posterior) [Horvitz, Chalfie, Trent, Sulston, and Evans, 1982; Rogers, Franks, Walker, Burke, and Holden-Dye, 2001]	tph-1, cat-4, bas-1, cat-1, mod-5 [J S Duerr et al., 1999; Flames and Hobert, 2009; Hare and Loer, 2004; Nurrish, Ségalat, and Kaplan, 1999; Ranganathan, Sawin, Trent, and Horvitz, 2001; Sze, Victor, Loer, Shi, and Ruvkun, 2000; Sze, Zhang, Li, and Ruvkun, 2002].
Dopamine	Indirect excitatory [Holden-Dye, Krogsgaard-Larsen, Nielsen, and Walker, 1989]	cat-2, cat-4, bas-1, cat-1, dat-1 [J S Duerr et al., 1999; Flames and Hobert, 2009; Hare and Loer, 2004; Jayanthi et al., 1998; Lints and

		Emmons, 1999; McDonald et al., 2007; Nass, Miller, and Blakely, 2001; Nurrish et al., 1999; Sze et al., 2002].
Gamma aminobutyric acid (GABA)	Inhibitory [Holden-Dye et al., 1989]	unc-25, unc-47, snf-11 [Jiang et al., 2005; Jin, Jorgensen, Hartwieg, and Horvitz, 1999; McIntire, Reimer, Schuske, Edwards, and Jorgensen, 1997; Mullen et al., 2006].
Glutamate	Inhibitory [Avery, 1993; Pemberton, Franks, Walker, and Holden-Dye, 2001]	eat-4, glt-1, glt-3, glt-4, glt-5, glt-6, glt-7 [Lee, Sawin, Chalfie, Horvitz, and Avery, 1999; Mano, Straud, and Driscoll, 2007; Ohnishi, Kuhara, Nakamura, Okochi, and Mori, 2011].
Octopamine	Inhibitory [Horvitz et al., 1982; Rogers et al., 2001]	tdc-1, tbh-1 [Alkema, Hunter-Ensor, Ringstad, and Horvitz, 2005].

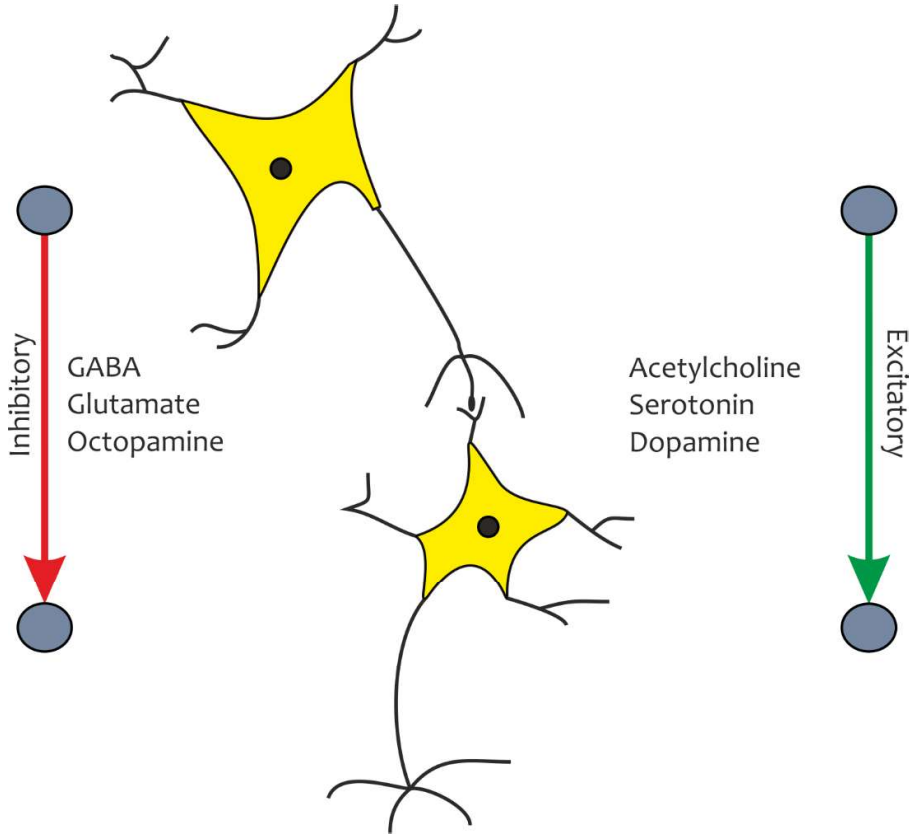

Figure 7.2 : Diagrammatic depiction of signed graph creation.

The nature of synapses were ascertained based on genes expressed in presynaptic neurons (listed in Table 7.1), which are responsible for synthesis and transport of respective neurotransmitter barring those associated with catabolism of the same.

To define a particular edge to be excitatory or inhibitory, from the differential list of genes expressed in neurons we found out how many were responsible for synthesis of neurotransmitters. Genes from each of these two categories viz. excitatory (G_e) and inhibitory (G_i) were counted. The potential of synapse (S_p) was judged on the basis of relative strength of these genes using Eq.(7.1).

$$S_p = \frac{k_1 G_e}{k_2 G_i} \tag{7.1}$$

Where k_1 and k_2 are the potential of each neurotransmitter to excite or inhibit the next neuron, which was considered as equal for this purpose i.e. $k_1 = k_2$. If $S_p \geq 1$, then the synapse and hence the edge was tagged as excitatory else it was considered as an inhibitory edge. The excitatory edges were marked as positive and inhibitory edges were marked as negative signs. This signed graph was then fragmented into two subgraphs on the basis of positive or negative signs.

7.4 BALANCE INDEX

In accordance with Cartwright-Harary theorem on structural balance, a graph is balanced only if there are no cycle of odd number of negative edge is present [Cartwright and Harary, 1956; Harary, 1953]. For application of this notion in signed neuronal network we need to consider this network as an assembly of interconnected cycles. We computed all cycles using depth first search and backtracking [Sedgewick, Robert, Wayne, and Kevin, 2011]. We find that CeNN is not a balanced graph, since it holds cycles with odd number of negative edges. Hence, we computed the extent of balance in the network with balance index (B_i). B_i is defined as the ratio of number of balanced cycles (C_b) with that of number of unbalanced cycles (C_{ub}) in a graph where a balanced cycle is defined as a cycle devoid of odd number of negative edges (Eq.(7.2)).

$$B_i = \frac{C_b}{C_{ub}} \tag{7.2}$$

For achieving absolute balance, all cycles in the network must be balanced with $C_{ub} = 0$.

7.5 RESULTS

7.5.1 Signed graph

The signed graph obtained contains positive and negative labeled edges. The graph is visualized using Cytoscape [Smoot et al., 2011]. We fragmented this signed graph to study graph theoretical properties of positive subgraph and negative subgraph. The positive subgraph have higher edge density by a factor of 1.76 than negative subgraph. On the basis of topological properties like clustering coefficient and characteristic path-length, positive subgraph shows more likeliness to the original complete network, whereas negative subgraph is more or less random in nature (Table 7.2 and Figure 7.3).

Table 7.2 : Graph theoretical properties of CeNN and signed subgraphs.

	No. of nodes	No. of edges	Clustering Coefficient	Characteristic path-length
CeNN	277	2105	0.172	4.018
Positive subgraph	264	1359	0.191	4.315
Negative subgraph	255	748	0.061	4.863

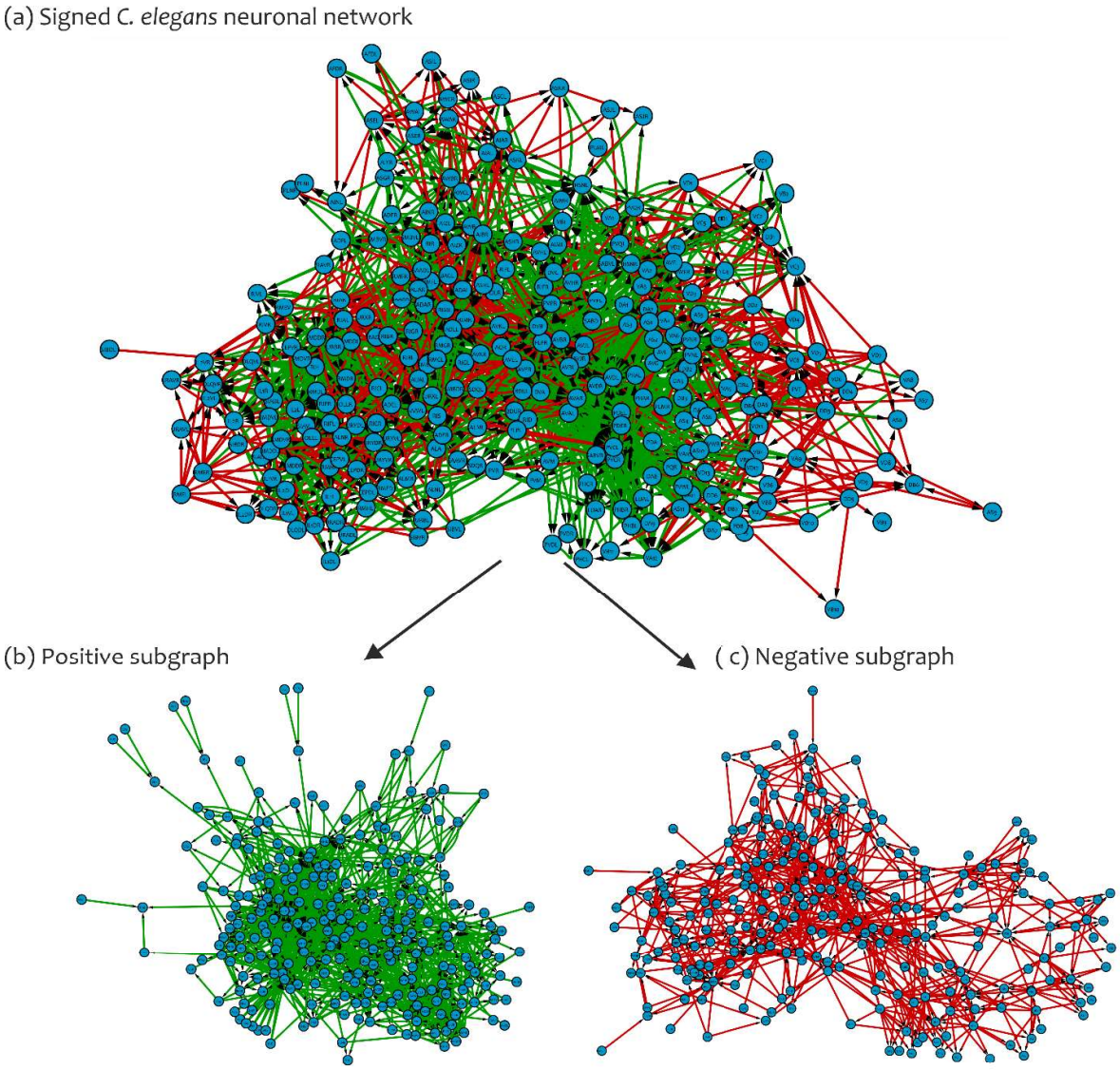

Figure 7.3 : Visualization of signed graph. (a) *C. elegans* neuronal network signed graph; green edges represents positive (excitatory) edge red edge represents negative (inhibitory) edge. (b) Positively signed subgraph. (c) Negatively signed subgraph.

7.5.2 Balance index in CeNN

In brain networks, neurons interact with each other to control the behavior of the organism. The neuronal network of *C. elegans*, comprises of 277 neurons interconnected with 2105 excitatory or inhibitory synapses. We divided this graph to study topological properties of the excitatory (positive) and inhibitory (negative) subgraphs. We found that, while the negative subgraph is akin to a random network, the positive component has higher clustering, closer to the original network. By the notion of Cartwright-Harary theorem [Cartwright and Harary, 1956], this neuronal network is unbalanced. We propose quantification of structural balance to enumerate the 'frustration' in a network with the balance index B_i, which quantifies the extent of balance in the network. Network with increasing/decreasing extent of balance would have higher/lower balance index compared to a random network which would have balance index of 1. We observe that *C. elegans* neuronal network has a B_i of 1.19, higher than its Erdős–Rényi control ($\overline{B}_i = 1.01$) and degree distribution preserved control ($\overline{B}_i = 1.00$). $\overline{B}_i$ in these random controls was computed over 100 instances. Given the relevance of structural balance in the presence of conflicting relationships, our results suggest importance of excitatory synapses vis-à-vis inhibitory synapses in this neuronal system.

7.6 DISCUSSION

Balance is an interesting concept reflecting emergent property of the network that is assessed based on interplay of harmonious and discordant relationships. While this concept is intuitive when applied to networks of people and communities, its utility in other systems has been unclear. In this study, we borrowed this idea to explain harmonious co-existence of neurons in the presence of excitatory and inhibitory synaptic communications. Towards this, we implemented a strategy to label neurons based on gene expression data, and also proposed a metric (Balance Index) that quantifies the proportion of balanced cycles vis-à-vis unbalanced cycles. Balance index reflects the extent of balance in the network, which is expected to be 1 in a comparable random graph matched for number of neurons and synapses of each type. A network with higher proportion of balanced cycles will have an index more than 1 and vice versa. Presumably, saturation with balanced cycles is an indicator of harmonious relationships that could of functional significance for the network.

The CeNN is characterized with a balance index of 1.19, which was higher compared to its random counterpart (1.01). Interestingly when the network is divided into excitatory (positive) and inhibitory (negative) sub-graphs, the inhibitory circuit was found to be have balance characteristics similar to that of a random graph. On the other hand, excitatory sub-graph was closer to the original CeNN. This is potentially indicative of role of excitatory synapses in achieving balance, and hence of appropriate functional significance.

Earlier studies have indicated that oscillatory integration window can be formed in the absence of inhibition. At the same time, the role of inhibitory synapses cannot be ignored as it is suggested to be important for biological control of cognitive processes [Aron, 2007; Bari and Robbins, 2013; Gupta, Singh, and Stopfer, 2016]. Hint of randomness in inhibitory neuronal circuit of *C. elegans* may points towards the scope for development during the course of evolution [Bonner, 2013]. Thus our study, while proposing a new measure for capturing synergetic communications among neurons, opens up avenues for its biological interpretations and studies to probe its potential evolutionary significance.

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