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CHAPTER 12

Understanding the mind of a worm: hierarchical network structure underlying nervous system function in *C. elegans*

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Abstract: The nervous system of the nematode *C. elegans* provides a unique opportunity to understand how behavior (‘mind’) emerges from activity in the nervous system (‘brain’) of an organism. The hermaphrodite worm has only 302 neurons, all of whose connections (synaptic and gap junctional) are known. Recently, many of the functional circuits that make up its behavioral repertoire have begun to be identified. In this paper, we investigate the hierarchical structure of the nervous system through k-core decomposition and find it to be intimately related to the set of all known functional circuits. Our analysis also suggests a vital role for the lateral ganglion in processing information, providing an essential connection between the sensory and motor components of the *C. elegans* nervous system.

Keywords: *C. elegans* neural network; nematode behavior; k-core decomposition; brain-mind; hierarchical network; degree correlation; assortativity

Introduction

> Behavior is the result of a complex and ill-understood set of computations performed by nervous systems and it seems essential to decompose the problem into two: one concerned with the question of the genetic specification of the nervous system, and, the other with *the way nervous systems work to produce behavior*.
>
> Brenner (1974)

As suggested by the above quotation, one of the fundamental problems in brain-mind studies is to understand the process by which electrophysiological activity at the level of the neuronal network gives rise to the complete set of stimulus–response behavior characteristic of a particular organism. Ideally, we would like to understand mental phenomena as a direct consequence of activity in the neurons that make up the brain. However, for the human brain having over 10^9 neurons and 10^{12} synaptic connections amongst them, such an undertaking seems impossible with current technology. Even if it had been possible by some means to record the activity of every neuron, it would be extremely hard to make sense of this enormous quantity of data and thereby understand their relation to various mental states.

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For this reason, it seems more fruitful to focus on a much simpler organism having relatively very few neurons, and yet, which has a complex behavioral repertoire capable of surviving successfully in the complex natural environment. The nematode *Caenorhabditis elegans* (*C. elegans*), so far the only organism whose nervous system has been completely mapped, is the perfect system which satisfies these requirements. The hermaphrodite animal, which is ~1 mm in length, has a nervous system comprising 302 neurons, a third of all the somatic cells in its body. The morphology, location and connectivity of each neuron has been completely described and is almost invariant across different individuals (Ward et al., 1975; Hall and Russell, 1991). Approximately 5000 chemical synapses, 600 gap junctions and 2000 neuromuscular junctions have been identified. Moreover, the nematode displays a rich variety of behavioral patterns, including several forms of non-associative learning that persist over several hours, and there is also indication that it is capable of associative learning (Hobert, 2003). In fact, it has already been used extensively as a model system to study the relationship between behavior and genetics (Brenner, 1974). The easy accessibility of the *C. elegans* nervous system to manipulation, has allowed identification of several reflexes which function as the basis of many aspects of organismal behavior (du Lac et al., 1995).

The fact that only ~300 neurons seem to be enough for an organism to survive in the wild has been a particular challenge to scientists involved in modeling the brain/mind, who have struggled to simulate individual aspects of mental activity, e.g., memory, using many thousands of model neurons (Hertz et al., 1991). It seems there is little hope of understanding how the much more complicated human brain works, until we can explain the behavior of *C. elegans* in terms of its neural network dynamics. This is especially so because the complexity of behavior of an organism appears to be related to the complexity of its nervous system. Here *behavior* refers to the set of actions or reactions in relation to the environment, allowing adaptation to various external stimuli. While behavior can be conscious or unconscious, overt or covert, voluntary or involuntary, it requires decision-making on the part of the neural circuits involved.

In *C. elegans*, neuronal circuits have been delineated based on patterns of synaptic connectivity derived from ultrastructural analysis. Individual cellular components of these anatomically defined circuits have previously been characterized on the sensory, motor and interneuron levels (Tsalik and Hobert, 2003). In the present work we have chosen eight functional circuits, namely, (a) touch sensitivity, (b) egg laying, (c) thermotaxis, (d) chemosensory, (e) defecation, and, three types of locomotion: when (f) satiated (feeding), (g) hungry (exploration) and (h) during escape behavior (tap withdrawal). Over the last few decades, in order to gain insight into the neuronal mechanisms regulating these reflexes or behaviors, individual neurons have been selectively and systematically ablated by laser microbeam. For example, laser ablation showed that nine classes of sensory neurons and four classes of interneurons are involved in the basic four steps involved in locomotion: forward and backward movements, omega-shaped turns and resting stages (Wakabayashi et al., 2004). The thermotaxis functional circuit was observed to contain relatively few neurons (Mori and Ohshima, 1995; Mori, 1999). On the contrary, the chemosensory functional circuit involves no less than nine pairs. The interneurons in *C. elegans* receive inputs from many modalities and are often multifunctional. But every functional circuit possesses a few dedicated sensory neurons. For example, the chemosensory circuit not only has chemosensory neurons (Bargmann and Horvitz, 1991; Troemel et al., 1995, 1997; Sambongi et al., 1999; Pierce-Shimomura et al., 2001), but also neurons specific for the olfactory component of chemosensation (Bargmann. et al., 1993; L'Etoile and Bargmann, 2000). Motor neurons in the functional circuits may also be very specific. The egg-laying circuit occurring only in the hermaphrodite animal has specialized motor neurons (Horvitz et al., 1982; White et al., 1986; Desai et al., 1988) some of which direct their synaptic output exclusively to vulval muscles and other motor neurons (Waggoner et al., 1998). With connectivities and composition (Chalfie et al., 1985; White et al., 1986) of several functional circuits identified, the next step is to integrate them to analyze for any patterns that might be emerging.

In this paper we employ a core decomposition method to reveal the fundamental structure underlying the connectivity profile of the *C. elegans* neural network. By using a process that peels away successive layers leaving behind the core of the network, we investigate whether there is correlation between a neuron (a) having a critical functional role and (b) occupying a central position in terms of structure. Our results indicate that there is indeed a structural basis behind the roles played by neurons in the functional circuits.

Materials and methods

Connectivity data

The *C. elegans* nervous system is naturally divided into two parts: the pharyngeal system composed of 20 cells (Albertson and Thomson, 1976) controlling the rhythmic contraction of the pharynx during feeding, which is almost completely isolated from the somatic system consisting of the remaining 282 neurons. In this paper, we focus on the 280 connected neurons of the latter system (2 of the neurons being not connected to any other neurons). The connections among these neurons, both synaptic and gap junctional, have been obtained through reconstructions of electron micrographs of serial sections (White et al., 1986). Further, this data has been collated together and made available in an electronic format (Achacoso and Yamamoto, 1992). In the data set, the neurons have been arranged into 10 ganglia, a classification based on physical proximity of the cell bodies to each other. The actual locations of these ganglia are shown in Fig. 1. In addition, the neurons are specified according to their function type, i.e., sensory, motor, interneuron or combinations thereof.

k-core decomposition

Core decomposition, introduced by Seidman (1983), is a technique to obtain the fundamental structural organization of a complex network through a process of successive pruning. The technique has been used to show core–periphery organization in a large number of biological networks (see Holme, 2005; Wuchty and Almaas, 2005). The *k-core* of a network is defined as the

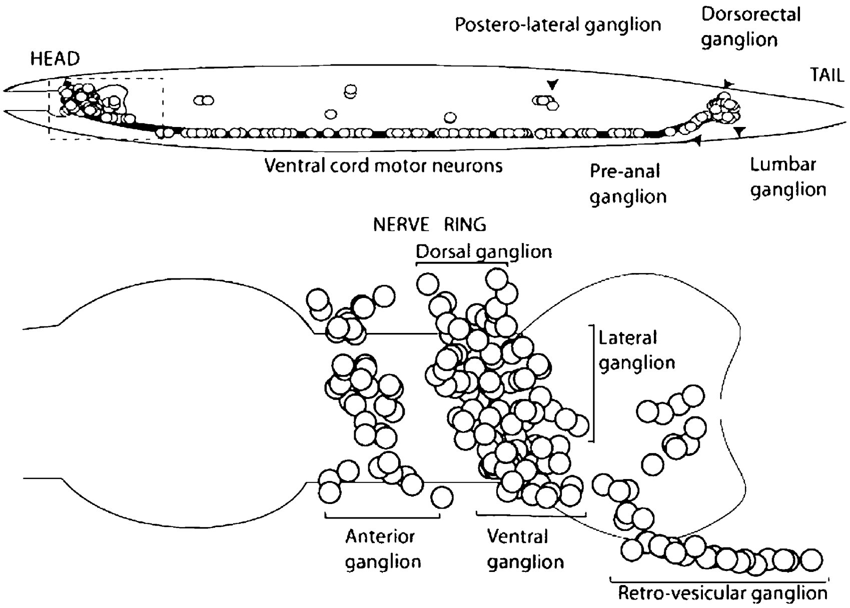

Fig. 1. Diagram indicating the locations of various neural ganglia in *C. elegans*. The bottom figure is a magnified view of the region in the head enclosed with the broken lines in the top figure. (Adapted from Ahn et al., 2006.)

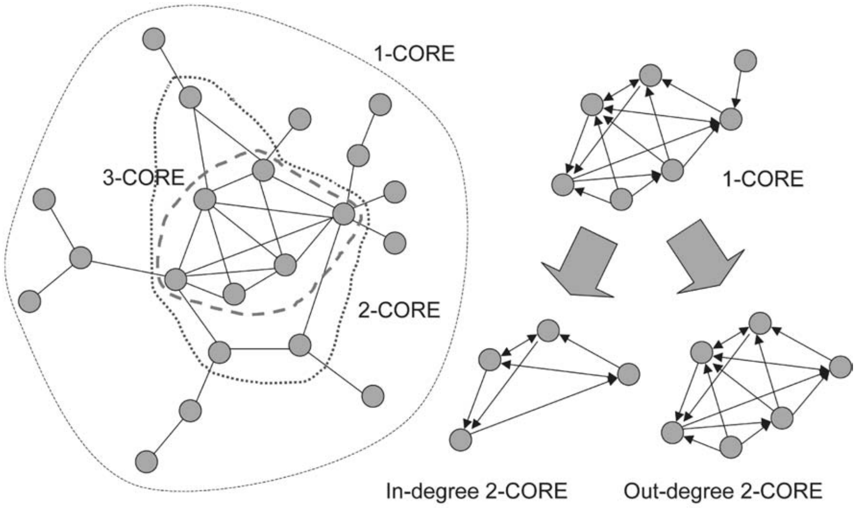

Fig. 2. Schematic diagram showing the k-core decomposition of (left) a undirected network and (right) a directed network, with arrows indicating the direction of connections. For the undirected network, the 1-core is made up of all nodes within the thin broken curve, while the 2-core and 3-core nodes are bounded by the dotted curve and thick broken curve, respectively. For the directed network, the k-core obtained depends on whether one is looking at the in-degree (inward links to a node) or out-degree (outward links from a node).

subnetwork containing all nodes that have degree[1] *at least* equal to *k*. An iterative procedure for determining the k-core is (i) to remove all nodes having degree less than k, (ii) check the resulting network to see if any of the remaining nodes now have degree less than k as a result of (i), and if so (iii) repeat steps (i)–(ii) until all remaining nodes have degree at least equal to k (Fig. 2, left). This resulting network is the k-core of the original network. In particular, the 2-core of a network is obtained by eliminating all nodes that do not form part of a loop (a closed path through a subset of the connected nodes). In fact, there exist at least k paths between any pair of nodes belonging to a k-core.

The procedure indicated above is for an undirected network, i.e., where the links do not have any directionality. However, a synaptic link between two neurons has an inherent direction, and therefore we need to define k-cores for directed networks. By focusing exclusively on either the *in-degree* (number of connections arriving at a neuron from other neurons) or the *out-degree* (number of connections from a neuron to other neurons), one can define k-cores for a directed network. Not surprisingly, one can arrive at different cores for the same network, depending on whether one used the in-degree or out-degree for recursive pruning of the network (Fig. 2, right). It is worth noting here that for a general network, it is possible that the inner k-cores may consist of disconnected parts. However, for *C. elegans*, k-cores for the networks defined in terms of both directed and undirected synaptic connections, as well as for the network defined in terms of gap junctions, remain connected at all orders of k for which the core exists.

Pair-wise degree correlation

While degree is a property associated with a single neuron, one can also look at the relation between degrees of a connected pair of neurons. In particular, one can ask if high-degree neurons connect preferentially to other high-degree neurons, or whether instead, they prefer connecting to low-degree neurons. These two possibilities result in two rather different kinds of network structure, assortative and disassortative, respectively, with

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[1] The *degree* of a node (neuron) is the total number of its links or connections.

most biological networks seeming to be of the latter kind (Newman, 2002). In this paper, we use Pearson's correlation coefficient between the degrees of all pairs of connected nodes as a measure of the pair-wise degree correlation. For directed synaptic networks, the correlation coefficient can be measured in four different ways, as one can focus on either the in-degree or the out-degree of the pre- and post-synaptic neurons. Therefore, one can define correlations among (i) pre-synaptic in-degree and post-synaptic out-degree, (ii) pre-synaptic out-degree and post-synaptic in-degree, (iii) pre-synaptic in-degree and post-synaptic in-degree and (iv) pre-synaptic out-degree and post-synaptic out-degree. Each of these measures has distinct functional implications for the neural group concerned. For example, high positive values for (ii) will mark neuronal groups that are primarily involved in carrying information from sensory to motor neurons in the nervous system.

Results

Our approach to understand the functional role of the specific patterns in connectivity among the *C. elegans* neurons is to extract statistically significant structural features among them, i.e., properties that would not be expected to arise in a randomly assembled network. Let us focus on the property of degree, the number of links of a neuron. The degree distribution, i.e., the relative frequency of neurons having various degrees q, sharply decays with q, in a manner that is indistinguishable from a random network. However, by looking at the actual values of in-degree and out-degree of the different neurons, we notice that while sensory neurons have low in-degree and motor neurons have low out-degree, interneurons with high in-degree also tend to have high out-degree (Fig. 3, left). This would not have been expected had the connections among them been made at random. Moreover, the neurons with high degree also tend to be strongly interconnected, another feature not expected in a random network. In fact, the latter feature suggests the existence of a "core group" of neurons, a notion that we shall explore in detail below.

Examining the matrix of synaptic connectivities, arranged according to ganglia, reveals that most connections occur between neurons belonging to the same ganglion, implying a modular structure. Moreover, when the connections between ganglia

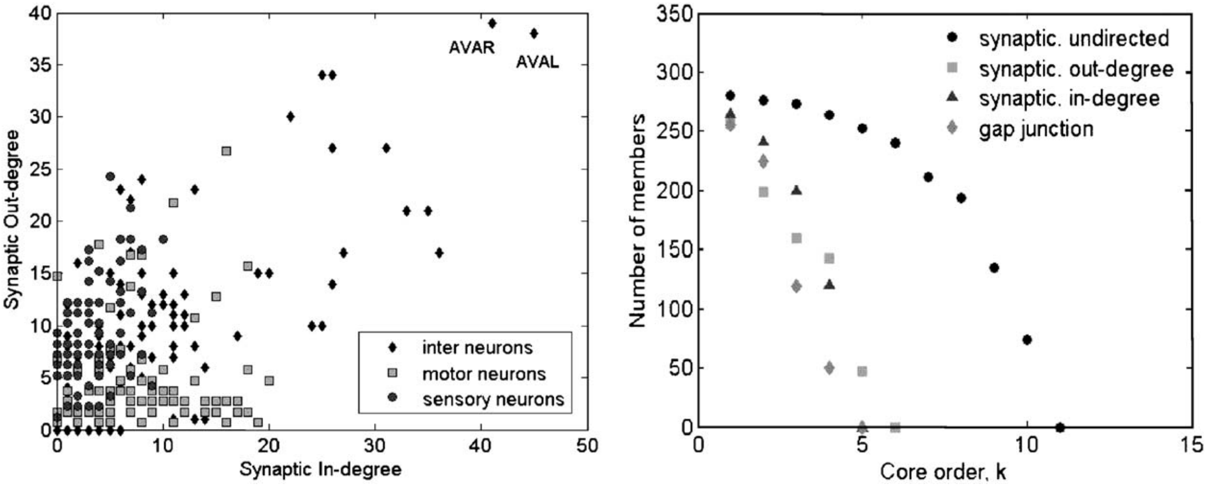

Fig. 3. Left: Relation between synaptic in-degree (number of post-synaptic connections to a neuron) and the synaptic out-degree (number of pre-synaptic connections) for sensory, motor and interneurons. Right: The number of neurons belonging to the k-cores for networks defined according to the type of connection and/or their direction. Except for the undirected synaptic network, the innermost cores are not of very high order.

are examined in detail, we observe that a few receive a significantly higher proportion of the interganglionic connections compared to others. In particular, the lateral ganglion is observed to receive many connections from other ganglia and, in turn, sends out many connections to the ventral cord motor neuron group. This is especially significant as the lateral ganglion hosts the "command" interneurons (White et al., 1986), so-called because they have a prominent role in a large number of functional circuits.

This brings us to the question of whether the *C. elegans* nervous system has a core–periphery structure (i.e., all neurons can be classified as belonging to either a densely connected central core or a sparsely connected periphery), and if so, then what is its functional significance. We shall attempt to answer this question by identifying the neurons belonging to the core group and ascertaining their functional properties, specifically by noting their membership in the different behavioral circuits. Note that, for neural networks there is intuitively a natural division into core and periphery in terms of the function of the neurons, where interneurons that take part in information processing should form the bulk of the core, while the majority of sensory and motor neurons should belong to the periphery.

We use the k-core decomposition technique (explained earlier) to identify the neurons that belong to the inner layers of structural organization for both the synaptic as well as gap-junctional networks. Figure 3 (right) shows how the number of neurons belonging to a k-core decreases with the order of the core, k. It is of interest that for both the directed synaptic networks as well as the undirected gap junctional networks the order of the innermost core is not large, never exceeding five. This is non-trivial, as we show that for the control case of the *undirected* synaptic network (which has no relevance for network function, synapses being essentially directional) the core order can increase to 10.

Investigating the functional types of the neurons making up the inner cores of the synaptic out-degree and in-degree networks, we find that in the former, sensory neurons significantly increase their presence with increasing k, while motor neurons dominate the latter (Fig. 4). This is perhaps not surprising when one recalls that most sensory neurons have out-degree significantly larger than the in-degree, the opposite being true for motor neurons. However, on inspecting the ganglionic membership of the core neurons, we observe over-representation of the lumbar and lateral ganglia neurons in the synaptic out-degree core, whereas in

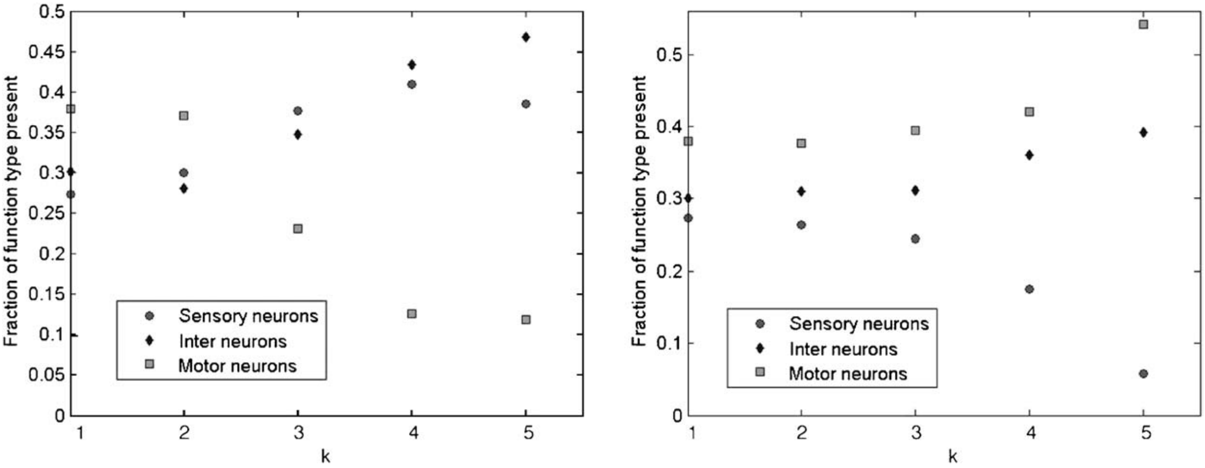

Fig. 4. The fraction of neuron types (sensory, motor or interneuron) in the k-core of the (left) synaptic out-degree network and the (right) synaptic in-degree network. The fraction of motor neurons drops and that of sensory neurons rises as we approach the innermost cores of the out-degree network, while the opposite is true for the in-degree network.

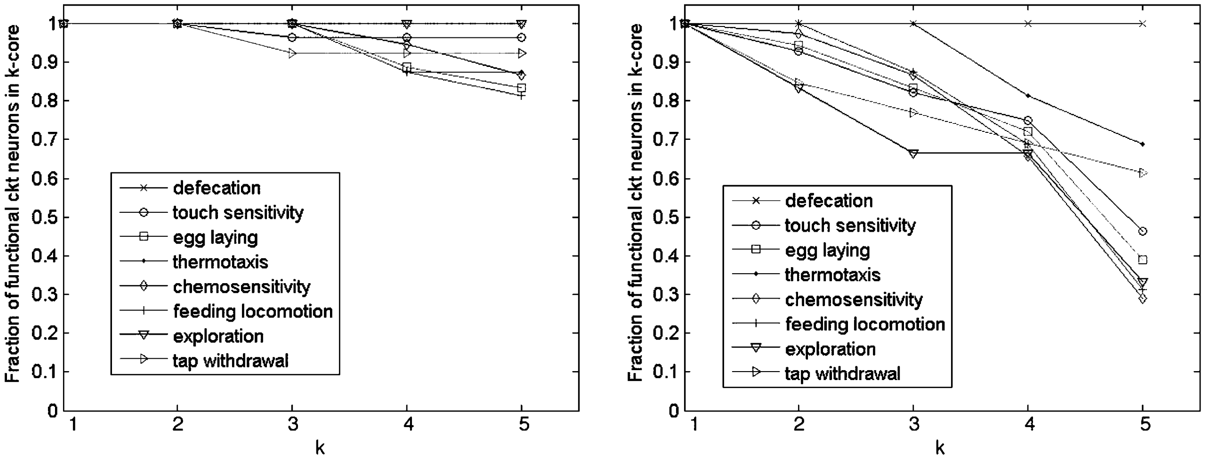

Fig. 5. Fraction of neurons belonging to specific functional circuits in the k-core of the (left) synaptic out-degree and the (right) synaptic in-degree network. Most of the neurons critically involved in various functions are in the innermost cores of the out-degree network.

the in-degree core there is an over-representation of neurons belonging to the lateral ganglion and the ventral cord neural group. This indicates that our earlier observation of the significant position of the lateral ganglion in the synaptic connectivity matrix is not an accident. Indeed, it suggests that the lateral ganglion acts as the "information processing hub" of the *C. elegans* nervous system, the principal bridge between its sensory and motor components.

We now turn to examine the role that core neurons play in the organism's behavior. Figure 5 indicates that a large fraction of the neurons involved in the various functional circuits are present in the inner cores of the directed synaptic networks. Moreover, most neurons belonging to the inner core are involved in the different functional circuits. This is perhaps natural because functional circuit membership is determined by eliminating a neuron and observing its behavioral consequence. It stands to reason that eliminating a neuron that belongs to the inner core will have a larger effect than eliminating one in the periphery, and therefore, more likely to result in behavioral anomaly. The identification of the position of a neuron in the k-core hierarchy with its importance in the functional circuits, shows that one can indeed relate structural features of the neural network with behavioral function.

Finally, we explore the pair-wise degree correlation for the gap junctional and directed synaptic network cores. We find that at the lowest order, the gap junction network shows a preference for connections between high-degree and low-degree neurons (i.e., disassortative) but as one goes higher up the hierarchy to the inner cores, they become *less* disassortative. This can possibly be because the gap-junctional network is star-like, with its hub composed of a densely connected group of high-degree neurons, to each of which several low-degree neurons are linked. For the synaptic network, (Fig. 6) we find that the network, even at the lowest order, is assortative, i.e., high-degree neurons show a significant preference for connecting to other high-degree neurons. Moreover, as one proceeds to higher orders of k, this assortative tendency increases for the synaptic in-degree network cores (comprising mostly motor and interneurons), while it decreases for the synaptic out-degree network cores (consisting of mostly sensory and interneurons). This suggests that the group of sensory and interneurons may have a different core structure (more star-like) than the group of motor and *command* interneurons (more clustered).

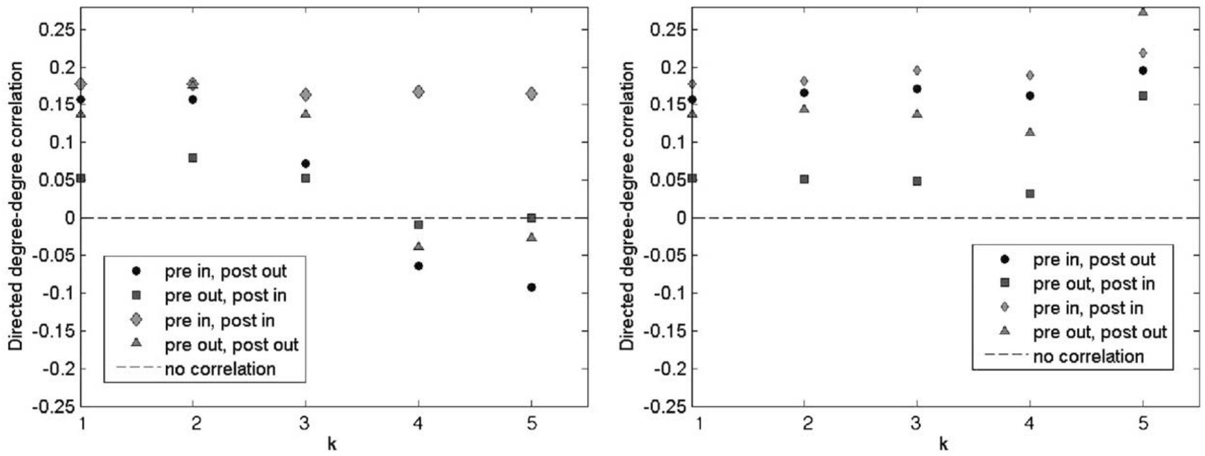

Fig. 6. Pair-wise degree correlation for the synaptic (left) out-degree and the (right) in-degree network cores, defined according to the direction of connections in the pre-synaptic and post-synaptic neurons.

Conclusions

In this paper we have used the k-core decomposition technique to analyze the hierarchical structure of the *C. elegans* nervous system. Our results point towards a key role played by neurons belonging to the lateral ganglion in processing information traveling through the stimulus–response path between the sensory and motor neurons. Comparison of the neurons belonging to the inner cores as defined by network structure with the neurons belonging to the different functional circuits as indicated by their crucial role in behavior, suggests a strong correlation between the two. Almost all neurons identified as belonging to any functional circuit are present in the inner cores. This suggests an intriguing relation between the structural centrality and functional importance of a neuron. In addition, we obtain a glimpse of the possibly different structural principles used in connecting the sensory–interneuron and the motor–interneuron components of the nervous system by investigating the pair-wise degree correlation along the core order hierarchy. The occurrence of assortativity in a biological neural network, in contrast to most other biological networks which are disassortative, is especially intriguing. It may indicate that the nervous system had to face significantly different constraints in its evolutionary path compared to other biological circuits. This may shed light on one of the central questions in evolutionary biology that resonates strongly with the theme of this volume, namely, why did brains or central nervous systems evolve? An alternative could have been a nervous system composed of a set of semi-independent reflex arcs. Structurally, this would have been manifested as a series of parallel pathways that process information independently of each other, rather than the densely connected networks that we are familiar with. This question becomes even more significant in light of the argument that the larger complexity inherent in densely connected networks has led to the emergence of a conscious mind from the simple stimulus–response processing capability of primitive organisms.

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