**Wiring optimization can relate neuronal structure and function**

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We pursue the hypothesis that neuronal placement in animals minimizes wiring costs for given functional constraints, as specified by synaptic connectivity. Using a newly compiled version of the *Caenorhabditis elegans* wiring diagram, we solve for the optimal layout of 279 nonpharyngeal neurons. In the optimal layout, most neurons are located close to their actual positions, suggesting that wiring minimization is an important factor. Yet some neurons exhibit strong deviations from “optimal” position. We propose that biological factors relating to axonal guidance and command neuron functions contribute to these deviations. We capture these factors by proposing a modified wiring cost function.

*Caenorhabditis elegans* | optimal placement

Because brain structure is intimately related to its function, understanding structure should provide important clues to brain function. Traditionally, structural features of the brain are explained from the perspective of development, a complex process including such events as cell migration (1, 2), axonal guidance (3–5), cellular signaling (6), and synaptogenesis (7–10). Although much progress has been made in understanding the mechanisms of neural development, many unanswered questions remain. In particular, it is not known what determines the placement of neurons and synapses in the body, a question to be addressed in this paper.

Our approach for understanding neuronal structures complements neural development and relies on the existence of general principles governing the architecture of a mature brain. Specifically, we exploit the wiring economy principle proposed by Ramón y Cajal more than 100 years ago (11). This principle postulates that, for a given wiring diagram, neurons are arranged in an animal to minimize the wiring cost. The evolutionary “cost” can be attributed to factors such as wire volume (12–14) and signal delay and attenuation (15–17), as well as metabolic expenditures associated with signal propagation and maintenance (18, 19). Although the exact origin of the wiring cost is not known, the farther apart two neurons are, the more costly is the connection between them. The wiring cost can therefore be expressed as a function of distance between neurons and consequently minimized (12, 20–25).

Despite many successful applications of the wiring minimization principle (refs. 12–14 and 20–27, but see ref. 28), it has never been tested on the level of individual neurons for an entire nervous system. Such testing was precluded by the lack of wiring diagrams and by the computational complexity of the optimization problem. Previous works have shown that wire length minimization can explain the layout of small systems by tabulating the amount of wire required for every possible permutation of components in the network. The actual ordering of ganglia in *Caenorhabditis elegans* (20) and the arrangement of areas in the prefrontal cortex in the macaque (27) were found in this manner to have the shortest total wiring. Unfortunately, this brute force method is impractical for all but the smallest networks (number of components of order 10), because the number of permutations increases exponentially with the number of components. In addition, the results provide only the relative ordering of components and not their exact positions in an actual animal.

In this paper, we solve for the neuronal layout of an entire nervous system of the nematode *C. elegans* using the updated wiring diagram and powerful placement algorithms borrowed from computer engineering (29–33). We consider 279 neurons (pharyngeal and unconnected neurons excluded) of the hermaphrodite worm, whose identity, locations of cell bodies, sensory endings, and neuromuscular junctions, as well as the wiring diagram, have been well studied and found to be largely reproducible from animal to animal (34, 35). The length of the worm is >10 times greater than its diameter, allowing us to reduce the problem into one dimension.

By minimizing the cost of connecting the nervous system, our solution predicts the position of most neurons along the anterior–posterior (AP) body axis of the nematode worm. This result suggests that wiring minimization is a good general description of the relationship between connectivity and neuron placement. A comparison of the cost-minimized layout with actual neuron positions revealed groups of outlier neurons with distinct structural characteristics. Interestingly, neurons within each group have been shown in experiments to play similar roles in the worm nervous system: developmental pioneering and signal integration for motor control. We suggest that the results obtained from cost minimization can be used in a number of ways to infer neuron function.

**Wiring Cost Minimization in the Dedicated-Wire Model**

We start by modeling the nervous system (see Fig. 1B Inset for example) as a network of nodes that correspond to neuronal cell bodies, connected by wires that represent synapses (Fig. 1C Inset). We call such model “dedicated wire,” because each synapse has its own wire (similar to point-to-point axon design in ref. 14). Additional wires connect neurons to sensory endings and muscles. Assuming that the placement of these structures is subject to constraints independent of neuronal organization, their positions are fixed.

The total wiring cost ($C^\text{tot}$) can be expressed as the sum of an internal cost to connect neurons to each other ($C^\text{int}$) and an external cost to attach neurons to the fixed structures ($C^\text{ext}$):

$$C^\text{tot} = C^\text{int} + C^\text{ext}. \quad [1]$$

We assume that the cost of wiring the $i$th and $j$th neurons is proportional to some power, $\zeta$, of the distance between them. Then the total internal wiring cost is:

$$C^\text{int} = \frac{1}{2\alpha} \sum_i \sum_j A_{ij} |x_i - x_j|^\zeta, \quad [2]$$

where $x_i$ is the neuron position, and $\alpha$ is an unknown coefficient. $A_{ij}$ is an element of the adjacency matrix $A$, representing the total number of synapses between neurons $i$ and $j$ in both directions. Because the wiring cost is assumed to be independent of the directionality of synapse (i.e., signal propagation from neuron $i$ to

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wires (colored lines), which must overlap if a synaptic connection exists. Cell model. (Inset) Actual placement of neuronal cell bodies projected onto anterior–posterior axis. Circles of the same color represent cell bodies belonging to the same ganglion. Plots for each ganglion are offset vertically to aid the eye. (Inset) Schematic example of biological network of three neurons and two fixed points: sensory ending; m, muscle. The blue neuron is bipolar, with one neurite attaching to the sensory ending and the other making two excitatory synapses onto the red neuron and one excitatory synapse onto the green neuron (circle represents the cell body). The red neuron makes an inhibitory synapse onto the blue neuron (line ending in circle) and a gap junction (bar) with the green neuron. The green neuron has a neuromuscular junction. (C) Neuronal layout predicted from minimization of quadratic wiring cost in dedicated-wire model. (Inset) Weighted dedicated-wire model. Each black line or wire corresponds to one synapse independent of polarity (excitatory vs. inhibitory), directionality, or modality (chemical vs. gap). (D) Neuronal layout predicted from the binary dedicated-wire model. (Inset) Binary dedicated-wire model. Each wire corresponds to a synaptic connection neglecting a multiplicity of synapses. (E) Neuronal layout predicted from the shared-wire model. (Inset) Shared-wire model. Neurons are represented as nonbranching wires (colored lines), which must overlap if a synaptic connection exists. Cell body location on the wire can be calculated by using different rules.

Yet, in the actual worm, the majority of neurons are nonbranching and bipolar, making an average of 58.6 en passant synapses and neuromuscular junctions with only two neurites (or two wires). This morphology can be taken into account by normalizing each neuron-to-neuron and neuron-to-muscle connection by the average number of synapses per neurite ($\alpha = 29.3$ or 58.6 synapses per neuron divided between two neurites). Sensory neurons, on the other hand, typically send one specialized neurite to the sensory organ (34), which, with a few exceptions, does not make synapses with other neurons or muscles. Thus each sensory fixed point, by construction, connects to a neuron through a dedicated wire and needs not be normalized. An alternative way to incorporate this neuronal morphology is by using a “shared-wire” model (Fig. 1E Inset), which will be introduced later.

We find the optimal neuronal placement that minimizes the wiring cost function defined by Eqs. 1–3. Initially, we assume that the cost of connecting two neurons increases as the square of the distance between them ($\zeta = 2$ in Eqs. 2 and 3). The quadratic cost function can be minimized analytically and the position of neuronal cell bodies is given by (26, 29, 30):

$$x = Q^{-1} \left[ S_1 + \frac{1}{\alpha} M_m \right]$$

$$Q_{ij} = \delta_{ij} \left( \frac{1}{\alpha} \sum_k A_{ik} + \sum_l M_{il} \right) - \frac{1}{\alpha} A_{ij}. \quad [5]$$

Minimization of the quadratic cost function is mathematically identical to finding the equilibrium placement of objects connected with elastic rubber bands (minimum elastic energy of rubber bands with zero length at rest).

Comparison of the Minimum-Wiring Placement with Actual Layout

Using the complete connectivity diagram of the C. elegans nervous system, we calculate neuron positions that minimize the quadratic cost function ($\zeta = 2$ in Eqs. 2 and 3, $1 < \zeta < 4$ to be considered later). Data sets are available at http://www.wormatlas.org/handbook/nshandbook.htm/ns wiring.htm. Fig. 1C shows optimal neuronal layout in the one-dimensional worm, where neurons from the same ganglion are represented by the same color, offset vertically for clarity.

We compare this result to actual locations of neuronal cell bodies projected into one dimension along the anterior–posterior axis of the worm (Fig. 1B). Neurons belonging to the same ganglia are clustered (positioned near each other) in the actual layout. Wiring-cost minimization predicts somewhat more dispersed clusters of neurons located in the anterior two-thirds of the worm and no clustering for neurons in the tail ganglia (see Ganglia Distribution in Supporting Text and Fig. 5, which are published as supporting information on the PNAS web site). Later we will discuss possible causes for such discrepancies. Because a large number of the sensory organs are located in the tip of the head (34), aggregation of neurons in the anterior region of the animal is consistent with minimization of cost required to connect these sensors (20). The predicted anterior–posterior order of the first five ganglia, as defined by the median of neuron positions, agrees with the actual order. The actual ganglia ordering was previously obtained by Cherniak via brute force enumeration of all possible permutations (20). However, as mentioned previously, the method used to obtain Cherniak’s result cannot be applied at the level of individual neurons.

Next, we plot predicted positions of individual neurons as a function of actual positions in the worm (Fig. 2). Neuron locations in the animals are scaled between 0 and 1, where 0 is the head and 1 is the tail. The majority of neurons in the network lie along the diagonal of the plot, where predicted position equals actual posi-
The actual system is not fully optimized. (ii) The wiring diagram is still somewhat incomplete. (iii) The wiring cost function does not fully represent costs associated with neuronal placement, or constraints other than connectivity need to be taken into consideration. Although reason (i) remains a possibility, its exploration lies beyond the framework of the optimization approach (38). Reason (ii) can be
Fig. 3. Analysis of cost-minimization outliers. (A) Histogram of absolute value of predicted-actual positions. (Top) Neurons with cell bodies in the head of the worm. (Middle) Neurons with soma in the midbody. (Bottom) Neurons with soma in the tail. Red vertical line in each plot marks the first standard deviation from the mean. Asterisks indicate neurons with ambiguous wiring (see text and Supporting Text for definitions). (B) Asymmetry of synapse position relative to the soma (1 = all synapses in the head and tail are located on opposite end of the worm as the cell body; 0 = all synapses in the head and tail are close to the cell body) vs. prediction error of wiring cost minimization. Bolded neurons above blue line (asymmetry > 75%) are pioneer neurons. (C) Synaptic inputs near the cell body vs. prediction error of wiring cost minimization. Bolded neurons above the blue line (percent inputs > 75%) are command interneurons for locomotion. The vertical red line is first standard deviation of wiring-cost model deviation.

addressed by future reconstructions. Here, we explore the merit of reason iii.

By taking a closer look at neurons with the greatest deviation between predicted and actual positions, we find that these “outliers” have common morphological features. Fig. 3A shows the histogram of differences between predicted and actual positions for neurons with cell bodies in the head, midbody, and tail of the animal. We define the head region by positions along the body axis <25% from the anterior of the worm, midbody is between 25% and 75%, and tail is >75% (see Neuron Position in Supporting Text). The top 10 outliers in the network are in the neuron classes PVQ, PVT, DVC, PVN, PVP, PVW, and PVC, all located in the tail of the worm. The biggest outliers in the head are AVA, AVG, and RID. In the midbody, SDQL, HSNL, and DA06 have the largest deviations. All of these neurons, except DA06, have long processes that span >25% of the worm body.

**Distribution of Synapse Locations Along a Neuron May Not Predict Cell Body Placement**

Because most outliers have long processes spanning the worm body, could the constraints for cell body placement along the process be different from the dedicated wire model? Using the quadratic wiring cost, the dedicated wire model places the neuronal cell body at the weighted center of mass of the positions of its synaptic partners and fixed structures. Then, the cell bodies should not deviate too far from the center of mass location of their synapses.

We test whether actual cell body locations are consistent with synapse distribution along a neurite as expected from the dedicated-wire model. Because the position of synapses can be approximated only to within one-third of the worm body (see Synapse Position in Supporting Text), we consider long-reaching (>25% body length) neurons with cell body located in either the head or tail (109 neurons). Using an asymmetry factor defined by the percentage of head and tail synapses located on the same end of the worm as the cell body, we study how synapses distribute between head and tail. The asymmetry factor is 0 if all synapses are at the same end of the worm as the cell body. For neurons with 100% of head and tail synapses on the opposite end of the worm as the cell body, the asymmetry factor is 1.

We find all neurons with asymmetry factor >75% (above the blue line in Fig. 3B) are outliers in the wiring-minimized layout (right of the red line in Fig. 3B). This group of neurons includes all developmental pioneers of the ventral cord currently known in *C. elegans*: AVG, PVPL/R, and PVQL/R. By comparing the positional deviations of known pioneers with the deviations of the rest of the neurons in the system, we find that all pioneers are outliers in the wire-minimized layout (significant for pioneers as a group, P = 0.002 from Student’s t test). The most prominent anterior outlier, AVG, is born in the head (39). During development, the neuron sends the first posterior-directed projection into what eventually becomes the right ventral cord, pioneering a path for other anterior neurons to follow (Fig. 2). Along the way, AVG makes synapses with neurons in the midbody and the tail. Neurons PVP and PVQ, the biggest outliers in the tail, behave similarly but in the reverse direction: they are born in the tail and send pioneering processes forward. Ablation of these pioneer neurons results in disorganization of ventral cord fascicles, although a nerve cord is still formed (39). All of these pioneer neurons are characterized by long processes that span the entire length of the worm with the majority of synapses situated outside of the soma region.

Another key player in neural development, PVT, also has synapses mostly on the opposite end of the worm from the soma. The previously published wiring of PVT (34) was later amended (O. Hobert and D.H.H., unpublished work). Interestingly, only after these changes are incorporated does PVT emerge from this outlier analysis. Functionally, PVT acts as a guidepost cell for neurons located in the posterior region of the worm to grow forward (40, 41) and maintains the organization of ventral cord fascicles (42).

Without PVT, axons in the lumbar ganglia fail to enter the ventral cord in a single bundle, and axons already in the ventral cord cross the ventral midline in an aberrant manner.

The remaining neurons with asymmetry factor >75%, DVC and PVR, are also outliers in the wire-minimized solution and, based on their structural characteristics, we propose that DVC and PVR may also play pioneering or developmental roles. PVR, an interneuron located in the lumbar ganglion, is a putative tail sensory neuron, with some animals displaying microtubule bundles in the posterior process (34, 35). The pioneering role of DVC has been previously postulated by Durbin (39) by using independent data. However, this hypothesis was not fully verified by experiments (39).

**Directionality of Synapses Along the Neuron May Bias the Location of Cell Bodies**

Because analysis of synapse position relative to the cell body does not account for all outliers, such as AVA and PVC where synapses...
When subject to a diffused mechanical stimulus, such as a disturbance (e.g., tap) of the substrate on which the worm is resting, the worm responds by moving either forward or backward. Without DVA, the acceleration of such movement is diminished. AVL, acting in conjunction with neuron DVB, is critical for activating muscle contraction for defecation (47). RID has unknown function although both AVL and RID make neuromuscular connections to body muscles.

Wiring Optimization Using the Shared-Wire Model

To incorporate the importance of synapse location and directionality into theory, we propose an anatomically more accurate shared-wire model (Fig. 1E Inset). In this model, each neuron is represented by a wire with multiple synapses. If a pair of neurons is synaptically connected, the corresponding wires must overlap. Similarly, if a neuron makes an external connection, the corresponding wire must include the location of that fixed point. Given these constraints, minimization of total wiring length (31, 33) yields the optimal placement of each synapse as well as the front and back ends of each neuron.

Because the actual locations of most synapses in the worm are not currently known, comparison with data requires predicting cell body positions. One possibility is to assign the cell body position to the center of mass of synaptic locations for each neuron. If connections are treated equally (analogous to the binary dedicated-wire model), the mean deviation of the predicted cell body location is 10.6% from actual. If connections are weighted by their multiplicity (number of synapses per connection analogous to the weighted dedicated-wire model), the mean deviation is 10.7%. In either case, the accuracy of the shared-wire model is no better than the dedicated-wire model.

However, the shared-wire model allows us to apply the results from outlier analysis by adopting different rules for the placement of cell bodies in neurons with specialized functions. First, we incorporate the observation that cell bodies of command interneurons gravitate toward postsynaptic terminals. For these neurons, the cell body is placed at the end of the neuron closest to the center of mass of postsynaptic terminals. Second, we incorporate the observation that cell bodies of neurons important in developmental pioneer are located on the opposite end of the neuron from the majority of synapses. For these neurons, we consider only the synapse-containing region (excluding connections to external structures). The cell body is placed at the end of this region most distant from the synaptic center of mass. Applying these rules for specialized neurons to the distribution of synapses obtained in the shared-wire model, we obtain a placement (Fig. 1E, Shared-Wire Model in Supporting Text and Fig. 7, which are published as supporting information on the PNAS web site) with mean deviation of 9.41%, better than predictions from the quadratic dedicated-wire model.

Wiring optimization using the shared-wire model makes an interesting prediction where a large fraction of all synapses congregates in a single anterior location along the worm (Fig. 7 Lower). It is natural to associate this location with the nerve ring. Of course, because our model is 1D, the actual 3D structure of the nerve ring could not emerge. Yet, this congregation of synapses is an unexpected demonstration of the predictive power of wiring optimization.

Discussion

Here we show that wiring minimization can establish a relationship between neuronal structure and function. We found that, for given connectivity, wiring optimization predicts the layout of many neurons in the animal despite some uncertainty about the exact form of the wiring cost. Thus, wiring optimization is a constructive approach for relating wiring diagram and neuron placement. Detailed comparison of the wiring optimization prediction and actual layout reveals neurons with special structural properties that have
and in vitro are responsible for such optimization in functional constraints, what underlying biological mechanisms in incomplete or ambiguous wiring data for posterior neurons (34).

Finally, absence of clustering and relative forward placement of the tail ganglia may be due to optimal positions (14). Given that positions of neurons are optimized for specified functional constraints, what underlying biological mechanisms are responsible for such optimization in C. elegans? Experimental evidence suggests that wiring minimization may be driven by genetics as well as forces generated during embryonic and postembryonic development. Studies that support evolutionary mechanisms show that the position of synapses can be perturbed without affecting cell body position and vice versa (48). The identification of pioneers in the outlier analysis also demonstrates the importance of genetics in neural layout. Furthermore, a few neurons in the worm migrate long distances during development to positions where connection costs are lower than their initial positions (data not shown) (49). However, mutant worms with miswired neurons demonstrate both wild-type as well as displaced cell body positions (50, 51). This displacement could result from tension in neurites demonstrated in vivo (52) and in vitro (53, 54). Such tension may pull connected cells closer together and optimize the layout during development (55). We hope that future research will contribute to the field of evolutionary developmental biology by shedding light on the interplay between developmental mechanisms and genetic information in specifying neuronal position (56).

In conclusion, we show that neuronal layout can be largely predicted by minimizing the wiring cost for given synaptic connectivity. The discrepancy between optimized and actual placement is mainly due to neurons with stereotypical roles in the network, such as developmental pioneers and command interneurons. This discrepancy may be due to the specialized requirements on synapse placement relative to cell body. Although wiring optimization may not be the only factor in neuronal placement, it is the only one that has been quantified and has predictive power to relate neuronal structure and function.

Note added in proof. After completion of this work, we became aware of two related studies (57, 58).

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