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Wiring Optimization in Cortical Circuits

Viewpoint

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Summary

Wiring a brain presents a formidable problem because neural circuits require an enormous number of fast and durable connections. We propose that evolution was likely to have optimized neural circuits to minimize conduction delays in axons, passive cable attenuation in dendrites, and the length of "wire" used to construct circuits, and to have maximized the density of synapses. Here we ask the question: "What fraction of the volume should be taken up by axons and dendrites (i.e., wire) when these variables are at their optimal values?" The biophysical properties of axons and dendrites dictate that wire should occupy 3/5 of the volume in an optimally wired gray matter. We have measured the fraction of the volume occupied by each cellular component and find that the volume of wire is close to the predicted optimal value.

Introduction

The problem of wiring a brain can be appreciated by considering the number of components that must be packed into every cortical region: a microliter of cortex contains approximately 10^5 neurons, 10^9 synapses, and 4 km of axons (Braitenberg and Schüz, 1998). Furthermore, each cortical neighborhood must not only pack the cellular components at high density, but must also have just the right balance of components. If too many dendrites were present in a particular mm cube of cortex, for example, insufficient space would remain for the axons and synapses needed to make the required circuit connections.

In considering how neural circuits are constructed, we made the surprising discovery that cortical function is optimal—in ways described below—when the volume of axons and dendrites combine to occupy 3/5 of the neuropil volume; this combined quantity is designated the *wire fraction*. We derived an equation (see below) that describes how the wire fraction depends on four factors: the conduction delay along axons, the cable attenuation of signals in dendrites, the number of synapses, and a "layout" parameter that specifies the length of wire used for a particular arrangement of components. The derivation of this equation makes use of the geometrical properties of axons and dendrites, and of the fact—derived from the cable equation—that dendritic cable attenuation and axon conduction velocity both depend on the square root of "wire" diameter. We find that if we vary the wire fraction while keeping all but one of these quantities fixed, each quantity reaches an extremum when the wire fraction has a value of 3/5. We also have measured the wire fraction from three mouse cortical regions and find that its actual value is not significantly different from the special value of 3/5. Based on these observations, we conclude that cortical circuits are optimally organized in the sense that conduction delays and passive cable attenuations are close to their theoretical minimum values, and the "layout" parameter and number of synapses are close to their theoretical maximum values when the rest of parameters are fixed.

Our reasoning can be described by three thought experiments.

Results

First Thought Experiment

For the initial thought experiment, suppose that we start with a small sample of cortical neuropil that is arranged just like some actual cortical region (same shape, size, and relative positions of all axons, dendrites, and synapses). We shall investigate the effect of changing the volume of "wire": "wire" is made up of axons and dendrites, and "non-wire" consists of boutons (or, more precisely, the portion of the bouton that is larger than the axon segment with the same length), spine heads, glial processes, and extracellular space. We consider here samples of cortex without cell bodies and capillaries. Initially, we give our general argument in a simplified form by supposing that wire consists only of axons and ignore the contribution of dendrites. Later we shall indicate why including dendrites in "wire" does not alter the conclusions we reach through this initial thought experiment. Our first goal is to examine the effect of changing axon diameter (while holding biophysical properties of the membrane constant) on conduction delays from one point in the circuit to another.

Start with a limiting case in which axon diameters in the cortical sample have been reduced to zero (so that axons take up no space) and the other components have been moved closer together to fill up the vacant space but are otherwise unchanged in size and physiological properties; additionally, axons have been shortened to run as directly as possible between locations where they make synapses in the actual circuit. Furthermore, we require that the biophysical properties of axons are kept constant so that the proportionality constant between conduction velocity and the square root of axon diameter (Rushton, 1951) is unchanged. Because the conduction velocity of unmyelinated axons is proportional to the square root of their diameter, such a hypothetical cortex would not, of course, function: conduction delays would be infinite. We ask: "What axon diameter minimizes conduction delays?" To answer this question, now imagine that the axon diameters are all

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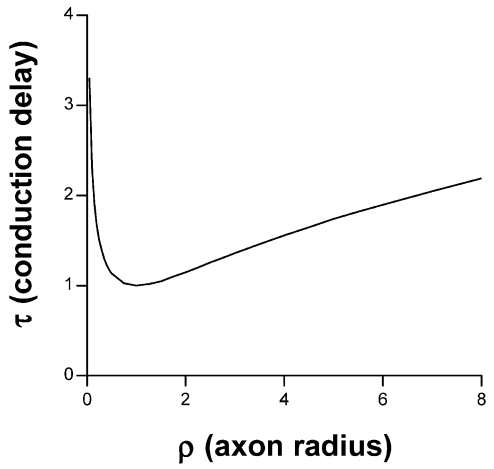

Figure 1. Relative Conduction Delay (τ) as a Function of the Axon Diameter (ρ) for the First Thought Experiment

Relative conduction delay (τ) is defined as the ratio of conduction delay in the perturbed cortex to that in the real cortex. Thus, when $\tau = 1$, the conduction delay would be the actual one in the cortical sample. The unperturbed wire volume for this curve is $\phi_0 = 3/5$, and the minimum occurs at $\rho = 1$ and $\tau = 1$.

increased to reduce conduction delays; the axon size is specified by a parameter ρ, which is defined to be our hypothetical axon diameter divided by the diameter of the corresponding axon in the real cortical region. In our starting hypothetical cortical region, with zero diameter axons, ρ would be zero, and when $\rho = 1$, the axon diameter would be just what it is in the real brain.

As diameters of axons increase, the conduction delay would naturally decrease from its initial infinite value (when axon diameter is zero). As ρ is further increased (while biophysical properties of the axons are kept constant), however, the conduction delay would reach a minimum and then start to increase (see Figure 1), because increasing axon diameter has two opposing effects. When diameter is increased, the conduction velocity increases so delays become shorter, and this effect varies as the square root of the axon diameter. But increasing axon diameter requires that axons be longer: since axons run in all directions, a particular axon must go farther to reach its synaptic target because of the volume added by the larger sizes of all of the other axons whose diameters are also increased. This increased length causes longer conduction delays and opposes the greater conduction speed. As axon volume becomes sufficiently large, the length of axons increases linearly with diameter. The conduction delay is axon length divided by conduction velocity, so conduction delay would increase like the square root of diameter ($\rho^{1/2}$) in the limit of large diameters. Some axon diameter must, then, be optimal in minimizing conduction delays. According to the analysis that follows, the minimum conduction delay occurs when the volume of wire is 3/5.

To determine the conditions for which conduction delays are at a minimum, we must relate the volume of our cortical sample to the axon diameter (measured by ρ). The equation that describes this situation is

$$v = \rho^2 \phi_0 v^{1/3} + (1 - \phi_0),$$

where ρ is the parameter that specifies the axon diameter as described above, v is volume of the hypothetical cortical piece relative to the real one ($v = 1$ if $\rho = 1$), and ϕ_0 is the fraction of volume occupied by "wire" in the real cortical sample. This equation says that the volume of the cortical sample is made up of a wire component and a non-wire component whose volume (we require, for this thought experiment) is unchanged by altering axon diameters. If we define the linear dimension of the region as $x = v^{1/3}$, the preceding equation for v becomes a cubic in linear dimensions of the sample

$$x^3 - \rho^2 \phi_0 x - (1 - \phi_0) = 0,$$

an equation that can be solved to give $x(\rho)$, although the answer is very messy.

Because axon conduction velocity u is proportional to the square root of axon diameter as long as the biophysical properties of the axon are unchanged (Rushton, 1951)—that is, $u \sim \rho^{1/2}$—the conduction delay τ measured from one specified point in the cortical circuit to another is proportional to

$$\tau \sim x/\rho^{1/2} = v^{1/3}/\rho^{1/2};$$

note that linear distances vary as $v^{1/3}$ as the volume of the sample changes. Once $x(\rho)$ is known from solving the cubic equation for x above, this last equation permits the relative conduction delay τ to be plotted (Figure 1). The result is that, as axon diameter is increased from zero, the conduction delay first decreases—as expected from the fact that conduction velocity increases with the square root of the axon diameter—but then increases again after a certain diameter is reached, as described above. The critical point at which increasing axon diameters starts to increase rather than decrease conduction delays comes when wire volume is 3/5 of the total. For this thought experiment, we consider the optimal cortex to be the one with the shortest conduction delays when all cortical properties are fixed except axon diameters. The cortex is optimal (has shortest delays), then, when the wire takes up 3/5 of the total volume.

In the preceding discussion, we based our argument entirely on axons and their properties, but "wire" also includes dendrites. If the diameter of dendrites is increased, their length constant increases and signals propagated passively along the dendrite are attenuated less. In fact, the dendritic length constant is proportional to the square root of dendrite diameter, just as the conduction velocity of axons is proportional to the square root of their diameter (Rall et al., 1992). A more detailed version of the analysis given above including attenuation of signals by dendrites demonstrates that when either the axonal delays are minimized (while dendritic attenuation is fixed) or the dendritic attenuation is minimized (while axonal delays are fixed), the volume fraction of axons and dendrites combined is 3/5. Therefore, any cost function monotonic in axonal delays and dendritic attenuation is minimized when wire fraction is 3/5.

Wire Fraction Estimated by Electron Microscopy

What fraction of the actual brain volume is taken up by wire? We find that neuropil in mouse neocortex (visual

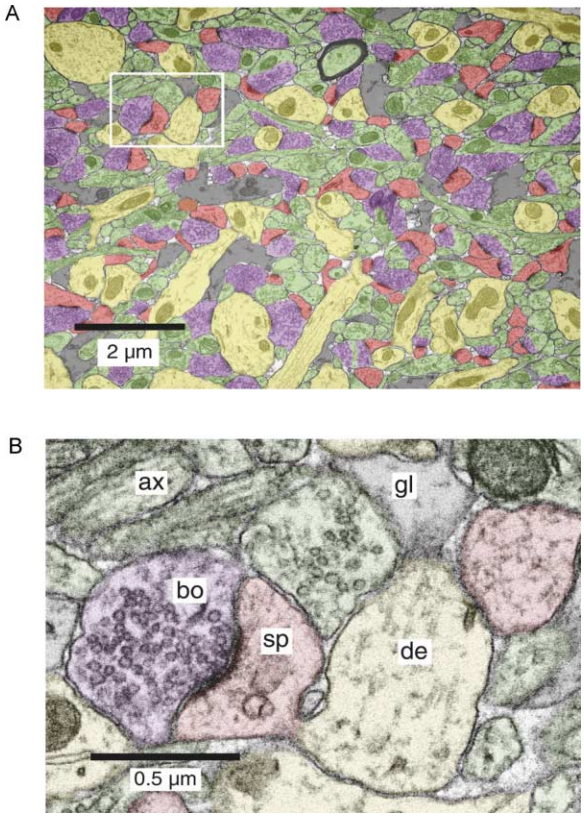

Figure 2. Electron Micrograph of Hippocampus (CA1) to Illustrate the Fraction of Different Components

(A) Low power, with axons (ax, green), dendrites (de, yellow), boutons (bo, purple), spines (sp, red), and glia (gl, gray); unclassified (white) is mostly extracellular space. (B) Higher power view of the boxed region of (A).

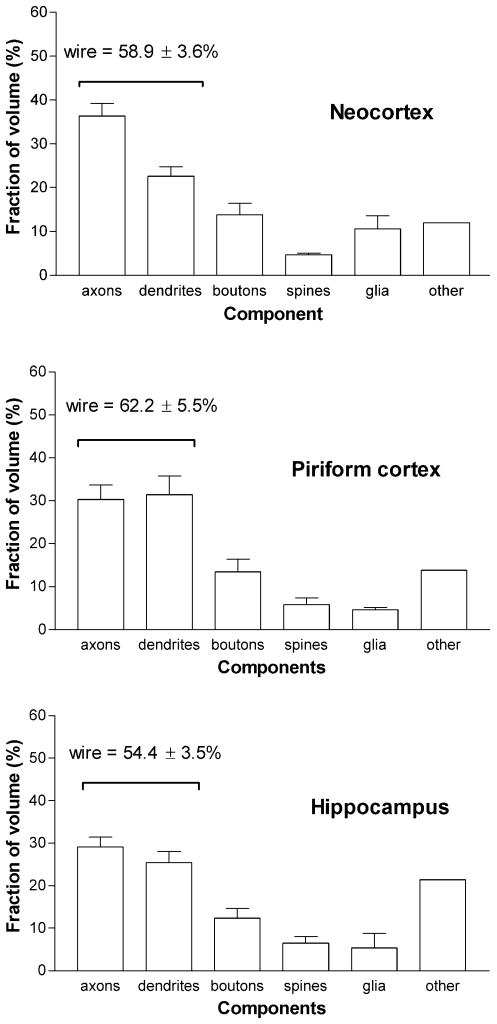

Figure 3. Fraction of Total Neuropil Volume Occupied by Axons, Dendrites, Boutons, Spines, Glia, and Other for Three Cortical Regions: Neocortex (Visual), Piriform Cortex, and Hippocampus

Note that the axons occupy more, and dendrites less, of the volume in neocortex than in the other two cortical regions.

area), olfactory cortex, and hippocampus (stratum radiatum of CA1) has a wire fraction that is not significantly different from the special value of 3/5, an observation that argues in favor of optimal wiring in the sense we use that term here.

In each of the three cortical areas, we have analyzed four independent samples of neuropil (one sample appears in Figure 2A). Profiles in electron micrographs were classified into categories (axon, dendrite, bouton, spine head, glial process, and other, which is mainly extracellular space but includes a small number of unidentified profiles) and we have calculated the relative volume of each category. Altogether we measured the area of 4837 profiles (3129 axons, 381 dendrites, 539 boutons, 422 spines, and 366 glia) in the 12 samples of neuropil. An example electron micrograph from hippocampal cortex, one of the 12 samples we analyzed, is presented in Figure 2, where the various components are colored (yellow for dendrites and green for axons). The histogram of relative proportions of each category is presented in Figure 3; the fraction of wire is 0.59 $\pm$ 0.036 ($N = 4$) for layer IV of visual cortex, 0.62 $\pm$ 0.055 ($N = 4$) for layer Ib of piriform cortex, and 0.54 $\pm$ 0.035 ($N = 4$) for the stratum radiatum of hippocampal field CA1. The overall average is 0.585 $\pm$ 0.043; these values are not statistically different from the optimal 3/5.

Although similar data reported previously (Braitenberg and Schüz, 1998; Ikari and Hayashi, 1981) are not quite comparable to those given here because the earlier authors considered only neocortex and defined components somewhat differently—for example, Braitenberg and Schüz (1998) included boutons in the axonal volume, so part of what we considered "non-wire" they lumped with a "wire" component—these observations are generally consistent with ours and with our conclusion that axons and dendrites occupy about 60% of the neuropil volume.

Second Thought Experiment

The same equation that led to the conclusion that axonal conduction delay and dendritic cable attenuation are minimized when the wire fraction is 3/5 also has other consequences; we consider two additional thought experiments to elucidate these consequences. For the next two thought experiments, we shall start with the

cortical circuit as it actually is—that is, we shall suppose all of the connections and the cortical operation (including axon conduction delays and passive dendritic cable attenuation) are kept constant—and explore the consequences of rearrangements of this circuit.

The goal of the second thought experiment is to determine the relation between wire fraction and synapse density. For this thought experiment, start with a hypothetical cortical region that is identical to a real piece of cortex except all of the synapses (boutons and spines) have been removed. Each bouton is replaced by a length of axon with the same diameter as the corresponding interbouton axon segments. In this thought experiment, we imagine adding synapses (in their real locations) with the parameter η specifying the fraction added relative to the actual number. When $\eta = 0$, we have no synapses in the hypothetical cortex, when $\eta = 1$, we are back to the actual cortex, and when η is greater than one, we would have a hypothetical cortex with a greater synaptic density than the real one (and with some synapses that were not present in the real case). In all cases, components are pushed together (and wires are shortened to travel as directly as possible) to fill vacant space, or separated to make room for the added synapses but are otherwise unchanged in size or biophysical properties.

The fraction of the volume occupied by synapses in the real cortical region is taken to be σ, a value determined by the number of synapses and their average size; synaptic size, in turn, is set by functional considerations such as the number of synaptic vesicles that are required to sustain the desired release rates, and we keep this size constant to examine the effect of changing just synaptic number. For this thought experiment, we suppose that the axonal conduction delays and dendritic cable attenuations are constrained to have their actual values, so that the equation relating the relative volume of the cortex to the starting wire fraction (ϕ_0) and number of synapses (η) is

$$v = \phi_0 v^{5/3} + (1 - \phi_0 - \sigma) + \eta\sigma$$

This equation can be rewritten to relate the wire fraction (ϕ) to the parameter η that specifies the relative number of synapses:

$$\eta = [(\phi/\phi_0)^{3/2}(1 - \phi) + \phi_0 + \sigma - 1]/\sigma$$

The derivative $d\eta/d\phi$ vanishes for $\phi = 3/5$ no matter what values ϕ_0 and σ have, so the maximum possible number of synapses (given the actual brain architecture) occurs when the wire fraction is 3/5 and, in this sense, the cortical circuits are optimal when the wire fraction is actually 3/5; in order for the number of synapses to exceed this maximum, some other characteristic of the brain's structure or function must be altered, such as the conduction delay from one location to another or the size of synapses.

Third Thought Experiment

A third aspect of wiring optimality relates to the efficiency in arrangement of neural components such as cell bodies or synapses. To examine the effects of rearranging components—for example, moving cells from one place to another so that axons would have to travel farther to reach their targets—we consider a third thought experiment: imagine a cortical region in which the circuit diagram, conduction delays, and cable attenuation are kept constant, but we change component positions. The effect of this perturbation is characterized by the "layout" parameter λ that specifies the increase or decrease in average wire length necessary to form the circuit. For example, if the layout of neurons were changed so that the same circuit connections required wires 25% longer than those in the actual brain, then $\lambda = 1.25$. The equation that relates wire fraction ϕ to the layout parameter λ is

$$\lambda^5 = (\phi/\phi_0)[(1 - \phi)/(1 - \phi_0)]^{2/3}$$

Setting the derivative of λ with respect to ϕ equal to zero and solving for ϕ_{max}, the wire fraction that makes this function a maximum, we find that $\phi_{max} = 3/5$. That is, neural components of a brain with wire fraction 3/5 may not be wired less efficiently than they are, unless some compromise is made such as decreasing the number of synapses or increasing conduction delays. In this sense, then, the arrangement of actual components is optimal when the wire fraction is actually 3/5.

Discussion

Our observation of an actual wire fraction close to the predicted 3/5 argues that conduction delay and cable attenuation are close to their minimal values, and that the "layout" parameter and number of synapses (given the actual cortical architecture and the properties of other elements) are close to the maximum. Therefore, we suggest that these parameters play a key role in determining cortical architecture. We must stress, however, that we do not know that cortex was designed to be optimum in these ways. That is, the properties of cortical circuits that have been optimized might be something else, and the fact that the quantities we have found to be at their extrema is a consequence of optimizing some property we did not consider.

Any theoretical treatment of cortical circuits is, of course, an idealization of the actual case, and we must underline what idealizing assumptions we have made in deriving the equations used above. We have supposed that the cortex is homogeneous (that is, that the density of the various components—axons, dendrites, synapses, etc.—is the same everywhere) and that, when the size of one component (axon diameter, for example) is altered, other components can be rearranged or slightly deformed to fill in all of space (without changing that component's size). This assumption means that when the volume of one component is modified, the linear dimensions of the sample vary as the cube root of the changed volume. Our assumptions would be violated if we were to generalize our argument beyond the neuropil by including the glial and neuronal cell bodies (15.9% $\pm$ 0.9% of the cortical volume for our sample of neocortex) and the blood vessels (1.3% $\pm$ 0.4% of the neocortical volume) because their linear size is much greater than that of the neuropillar components we consider.

If we ignore this problem and apply our argument to the gray matter including the cell bodies and blood

vessels, we find that the wire fraction depends on the classification of these components as "wire" or "non-wire." If we assume that the cell bodies and blood vessels do not scale with the axons and dendrites and thus classify them as "non-wire," we find that the wire fraction is 50%. If the cell bodies scale the same way as the size of axons and dendrites, then they should be included in "wire," which would result in wire fraction of 66%. These percentages are hard limits on the empirical value of wire fraction. In reality, the cell bodies and blood vessels are likely to scale weakly with the axons and dendrites, and, therefore, should be classified partly as "wire" and partly as "non-wire" thus yielding wire fraction close to 60%.

Also, we assumed that the wire distribution is isotropic (that is, axons and dendrites run equally in all directions). This assumption would be violated if we were to extend our argument to the cortical white matter. In addition, we assumed that axonal and dendritic diameters can be varied independently of biophysical properties of membrane (such as resistance and capacitance per unit area). Finally, we have assumed that wire—axons and dendrites—can always run directly from one place to another when component size is changed; this assumption appears when the length of axons is calculated to vary as the cube root of sample volume. Although these assumptions cannot be exactly true, they seem to us to be good approximations to the actual case, a view supported by the agreement between theory and experiment.

We have argued that dendritic diameters should scale so that the passive cable length of a dendritic tree is unchanged when the dendrites are made longer. As is well known, dendrites posses active conductances that will alter the cable properties of dendrites as a function of voltage (see Reyes, 2001). Nevertheless, cable attenuation will still occur—although perhaps modified by the voltage profile throughout the dendritic tree—and we argue that the cable lengths should be maintained as the neurons are scaled. If active properties of dendrites dominated their behavior, one should not see linear addition of synaptic responses as one typically does (Reyes, 2001). Hippocampal pyramidal cells scale between brains of different sizes (Bekkers and Stevens, 1990) in a way that maintains the cable length for the entire dendritic tree, an observation that supports the notion that cable length is conserved when dendritic length is changed. A minority class of cells, granule cells in hippocampus, do not maintain a constant cable length, so the analysis given here may not apply to regions of neuropil in which such dendrites constitute the majority.

In the Experimental Procedures section, we consider the consequences of relaxing two of our assumptions, the constancy of axon and dendrite properties, and the variation of all linear dimensions as the cube root of the sample volume. If one were to suppose that, for example, channel density varies with axon diameter, then the conduction velocity would not be proportional to the square root of diameter. Also, if axons or dendrites must make detours around objects, like synapses, of fixed size, then linear distances would not vary exactly as volume to the 1/3 power. In both cases, the results we derived appear as limiting cases as described in Experimental Procedures. We are not aware of any data that permit us to estimate the extent to which these two assumptions are inaccurate.

Our observations have an interesting consequence for notions about memory storage in the brain. Long-term memory is often considered to involve the formation of new synapses (Bailey and Kandel, 1993; Luscher et al., 2000) but, according to our analysis, an increased number of synapses could not be accommodated without degrading performance in some way because the cortex is already optimally wired in the sense that the number of synapses is already maximal. To fit in additional synapses without compromising performance, some synapses would have also to be eliminated. If memories were stored by increasing synapse size, compensatory decreases in synapse size would have to accompany to maintain optimal wiring.

The idea that neural circuit design is under pressure to minimize signal delay and attenuation dates back to Cajal (1995). Our observations suggest that the layout cannot be less efficient than in the real brain without compromising brain function, thus supporting the importance of wire length minimization in brain organization. Such wire length minimization arguments have recently been used to explain why retinotopic maps exist (Allman and Kaas, 1974; Cowey, 1979), why cortical regions are separated (Mitchison, 1992), why ocular dominance (Chklovskii, 2000a; Chklovskii and Koulakov, 2000; Mitchison, 1991, 1992) and orientation preference patterns (Durbin and Mitchison, 1990; Koulakov and Chklovskii, 2001) are present in primary visual cortex, why white and gray matter is partitioned as it is (Ruppin et al., 1993), why axonal and dendritic arbors have particular size (Chklovskii, 2000b) and branching angles (Cherniak, 1992), and why the cortical areas and ganglia in *C. elegans* are arranged as they are (Cherniak, 1994, 1995).

The argument we have presented here supports the use of optimization theory and points out which key factors are likely to determine brain architecture. This provides a powerful general principle that can be used to explain many anatomical features of the brain.

Experimental Procedures

Derivation of Equation for the First Thought Experiment

Consider a reference region of neuropil-1 μl of hippocampal CA1 stratum radiatum, for example—of volume V_0. We perturb this region by reducing the axon radius from its actual value r_0 to r, and compressing the region (and shortening wire lengths accordingly) to eliminate the space created so that the volume becomes V. The non-wire volume of this reference region is defined to be V_n, and is taken as constant. In the following, we shall use a subscript "0" to indicate the unperturbed value of a parameter. For example, if s_0 is the actual axon length of axon segment between synapses, s would specify the length assigned to the same axon segment in a perturbed brain. Our starting equation is

$$\begin{aligned} V &= N\pi r^2 s + V_n \\ &= N\pi\rho^2 r_0^2 s_0 v^{1/3} + V_n \\ &= \rho^2 (N\pi r_0^2 s_0) v^{1/3} + V_n \\ &= \rho^2 (V_0 - V_n) v^{1/3} + V_n \end{aligned}$$

where N is the number of synapses, r is the axon radius in the perturbed brain, r_0 is the average axon radius in the real cortex, s

is the average length of axon per synapse, s_0 is the value of s in the actual brain, $v = V/V_0$, $\rho = r/r_0$, $\sigma = s/s_0$, and $(V_0 - V_n) = (N\pi r_0^2 s_0)$. Notice that when we compressed the region to eliminate the space created by reducing axon radius, we shortened all of the axons so that they run from one synapse to the next in the shortest possible way (that is, s_0 is reduced to s). Now divide the equation by V_0 and use the definitions $v = V/V_0$ and $\phi_0 = (V_0 - V_n)/V_0$; the non-wire fraction, then, is $(1 - \phi_0) = V_n/V_0$) and the equation becomes

$$v = \rho^2\phi_0 v^{1/3} + (1 - \phi_0),$$

the equation used in the text.

Relation of Conduction Delay to Wire Fraction

To find the equation that relates the normalized conduction delay τ to the relative wire fraction to ϕ, start with the last equation above. We need to eliminate $v^{1/3}$ and ρ and replace these variables with τ and ϕ. The first step is to eliminate ρ by using the expression for the conduction delay, $\tau = v^{1/3}/\rho^{1/2}$. This manipulation gives

$$v = \tau^{-4}\phi_0 v^{5/3} + (1 - \phi_0)$$

The next step is to eliminate v in favor of ϕ, the fraction of the volume occupied by wire in the perturbed sample, by using the equation for the non-wire volume $v(1 - \phi) = (1 - \phi_0)$; this equation makes use of the fact that the non-wire volume does not change when we vary ρ. When v is eliminated from the equation above, the result is

$$\tau^4 = (\phi_0/\phi)((1 - \phi_0)/(1 - \phi))^{2/3}$$

The minimum conduction delay occurs when $d\tau/d\phi = 0$, and this condition is met when $\phi = 3/5$. Thus the minimal conduction delay occurs if the wire fraction is 3/5, as claimed in the text.

Equation for the Second Thought Experiment

Consider now a situation, based on the first equation in the preceding section, in which the conduction delays are kept constant (at their actual values in the real brain, so $\tau = 1$) and the number of synapses is changed by removing synapses from or adding them to the existing axons and dendrites. The relative fraction of the real brain volume that is made up of synapses is designated by σ, and the parameter η specifies the number of synapses in our sample. When $\eta = 0$, all of the synapses have been removed; the number of additional synapses increases with increasing η so that $\eta = 1$ for the real brain. The equation that describes this situation is

$$v = \phi_0 v^{5/3} + (1 - \phi_0 - \sigma) + \eta\sigma$$

We wish to examine the result of adding synapses that are supported by the existing wire, so imagine starting with $\eta = 0$ and then increasing the parameter. Because the non-wire components, except for synapses, do not change in volume, the volume of non-wire can be written two ways that appear on the left and right of the equation

$$v(1 - \phi) = (1 - \phi_0) + (\eta - 1)\sigma$$

or

$$v = [(1 - \phi_0) + (\eta - 1)\sigma]/(1 - \phi)$$

Insert this into the equation above for v to give

$$(\phi/\phi_0)\{(1 - \phi)/[1 - \phi_0 + (\eta - 1)\sigma\}^{2/3} = 1$$

This equation can be solved for η to give

$$\eta = [(\phi/\phi_0)^{3/2}(1 - \phi) + \phi_0 + \sigma - 1]/\sigma$$

The derivative $d\eta/d\phi = 0$ for $\phi = 3/5$ for any value of σ or ϕ_0, so the number of synapses cannot be increased beyond the point at which $\phi = 3/5$.

Equation for the Third Thought Experiment

Imagine that we rearrange components so that the average length of wire per synapse is $\lambda = s/s_0$. The normalized volume then is

$$v = \lambda^5 v^{5/3}\phi_0 + (1 - \phi_0)$$

From above, we have that (non-wire volume written two ways)

$$v = (1 - \phi_0)/(1 - \phi)$$

which can be substituted into the equation for v above to give

$$\phi/\phi_0 = \lambda^5[(1 - \phi_0)/(1 - \phi)]^{2/3}$$

When solved for λ^5, this equation yields

$$\lambda^5 = (\phi/\phi_0)\,[(1 - \phi)/(1 - \phi_0)]^{2/3},$$

the equation used in the text.

General Equation

The general equation that relates the wire fraction ϕ to the axon conduction delay τ, the dendritic cable attenuation α, the density of synapse η, and the "layout parameter" λ is

$$\phi(1 - \phi)^{2/3} = (\phi_a/\tau^4 + (1 - \phi_a)/\alpha^4)\lambda^5\phi_0(1 - \phi_0 + (\eta - 1)\sigma)^{2/3},$$

where ϕ_0 is the wire fraction in the actual neuropil, σ is the fraction of volume that is occupied by synapses in the actual neuropil, and ϕ_a is the proportion of the wire fraction that is taken by axons. The parameters τ, α, λ, and η are all normalized so that they equal 1 for the actual neuropil; for example, the average conduction delay from one synapse to the next along an axon is $\tau = 1$ for an actual neuropil. This equation incorporates the three equations that were derived in the previous sections. They can be obtained by setting all parameters (τ, α, λ, η) except one to unity.

We assumed that both axonal conduction velocity and dendritic attenuation^{-1} scale with the square root of fiber diameter. If the scaling exponent, β, is different from 1/2, then the optimal wire fraction is given by the expression $3\beta/(2+\beta)$. For example, our argument applied to isotropically organized white matter would predict wire fraction approaching 1 because the speed of signal propagation in myelinated axons scales linearly with diameter.

We further assumed that the length of a wire path between synapses varies as the cube root of volume, but sometimes an axon or dendrite might have to make a detour around a component, such as a synapse, whose size is fixed. The effect of this would be to increase the tortuosity of the wire and cause the wire segment to be longer than predicted by $v^{1/3}$ scaling. If we assume that the wire path can be divided into a portion α of the length that does not scale with $v^{1/3}$ and the remaining wire $(1 - \alpha)$ whose length does scale with the cube root of the volume, then the wire fraction is not 3/5 but rather $(3/5)/(1 - \alpha)$); thus, the maximum wire fraction is 3/5, but the actual value could be somewhat less than this. We assume in the text that $\alpha = 0$.

Electron Microscopy

Since the identification of profiles in single sections is often impossible, serial electronmicrographs were used throughout this analysis. Most relatively large objects are easy to classify. Profiles were identified as axons when they contained microtubules and/or synaptic vesicles, and were continuous with boutons. Profiles that contained microtubules but lacked synaptic vesicles and contained postsynaptic structures (postsynaptic densities and spines) were classed as dendrites.

Many small fibers cannot be classified with these criteria. Because dendrites rarely have diameters less than 0.2 μm, all microtubule-containing profiles smaller than 0.2 μm in diameter were classified as axons. A small percentage of microtubule-containing fibers, ranging from 0.2 to 0.5 μm in diameter, could not be classified using the above criteria. These fibers were considered "wire" (since they contain parallel running microtubules) and half were classed "dendrite" with the other half assigned to "axon."

Boutons are easily identified by the presence of large numbers of synaptic vesicles. Often some portion of a bouton contains microtubules: this part was considered to be the axon running through the bouton and was marked as "axon," with the remainder of the profile counted as "bouton." Spines were classified as postsynaptic structures which contained no microtubules. Spine necks, when cut transversely, are very difficult to classify as they appear as small round profiles that are very similar to small axons without microtubules. In this study, we classed such profiles as "axon." The resulting

error in determining wire volume—the focus of this study—is minimal (less than 1%).

The profiles described above typically are rather circular (round); glia, however, often display very irregular outlines that appear to be following the boundaries of the other structures and seem to fill what would otherwise be extracellular space. Furthermore glial profiles do not contain parallel running microtubules, are not postsynaptic, and do not contain clusters of small translucent vesicles; they do, however, contain endoplasmatic reticulum and are rich in ribosomes. The proportion of dendrites, axons, spines, boutons, and glia was measured in four areas of neuropil in each mouse in the following layers: layer 1b of piriform cortex, stratum radiatum of the hippocampal CA 1 region, and layer IV of the occipital cortex; the neuropil areas analyzed contained neither blood vessels nor cell bodies. The total volume of each cortical sample was very close to 16.4 μm^3 (four sections, each with an area of 71 μm^2).

Two mice were perfused with oxygenized Ringer solution containing 1 μM tetrodotoxin followed by a fixative containing 2.5% glutaraldehyde (Fluka) and 0.5% acrolein (EM Sciences) in HEPES-bufferd saline (HBS, 20 mM HEPES, 143 mM NaCl., and 0.2 mM $CaCl_2$, pH 7.2). After perfusion, brains were dissected and immersed in a glutaraldehyde fixative, without acrolein, at 4°C overnight. Three hundred micrometers vibratome sections were cut in HBS and post-fixed in 1% OsO_4 and 1.5% K-ferrocyanide in s-collidine buffer at 4°C for 1 hr. After washing in Millipore filtered water, the sections were block contrasted in 2% aqueous uranyl acetate at 4°C for 1 hr, washed, dehydrated in an ascending acetone series, and flat embedded in Epon.

Serial ultrathin sections were cut (at silver, approximately 60 nm thick) from specific brain regions (piriform cortex, hippocampal CA1 region, and the occipital cortex). Sections were stained with Sato lead (Hanaichi et al., 1986) and photographed at 10,000× with a Jeol 100CX II electron microscope.

The electronmicrographs were digitized at 600 dpi. Using Photoshop (Adobe), the different profiles (for identification criteria see (6)) were colored in separate layers. Binarized images of those layers were measured using MetaMorph (Universal Imaging).

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