Cortical rewiring and information storage

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Current thinking about long-term memory in the cortex is focused on changes in the strengths of connections between neurons. But ongoing structural plasticity in the adult brain, including synapse formation/elimination and remodelling of axons and dendrites, suggests that memory could also depend on learning-induced changes in the cortical 'wiring diagram'. Given that the cortex is sparsely connected, wiring plasticity could provide a substantial boost in storage capacity, although at a cost of more elaborate biological machinery and slower learning.

The human brain consists of 10^{11} neurons connected by 10^{15} synapses. This awesome network has a remarkable capacity to translate experiences into vast numbers of memories, some of which can last an entire lifetime. These long-term memories survive surgical anaesthesia and epileptic episodes, and thus must involve modifications of neural circuits[1], most likely at synapses[2,3].

What changes in synapses underlie memory storage? The focus of neural learning research has been on activity-dependent 'weight' changes between previously connected neurons. This mode of plasticity could involve either changes in the efficacies of existing synapses, or structural changes that lead to the addition or subtraction of synapses between previously connected pre- and postsynaptic units (Fig. 1). In either case, the network's connectivity matrix, or wiring diagram, is left unchanged. (The term 'unit' could correspond to an individual neuron, although other assignments are possible; see below.) In the weight–plasticity scenario, the storage capacity lies in the system's ability to increase and decrease the weights on existing connections as a means of encoding learned information[4–6] (Box 1).

In addition to weight changes, learning could involve alterations to the wiring diagram, whereby previously unconnected units become connected and vice versa (Fig. 1). Unlike weight changes, wiring changes require structural plasticity. In this learning mode, the storage capacity lies in the system's flexibility to choose which presynaptic units provide input to each postsynaptic unit (Box 1).

Weight and wiring changes are not mutually exclusive (wiring plasticity can even be viewed as a special case of weight plasticity; Box 1), and experimental evidence suggests that neurons and their synapses might be engaged in both forms of learning. It is well accepted that synaptic efficacy can be modulated in a use-dependent manner to produce weight changes[7]. Similarly, structural changes that would be required to achieve wiring changes, including synaptogenesis and outgrowth of axons and dendrites, can occur in the adult brain[8–14].

Despite the likely coexistence of these two forms of plasticity in the adult brain, biological[8–19] and computational[20–24] considerations demand that weight and wiring changes be distinguished from each other. In most areas of the brain, including the mammalian cerebral cortex, only a small fraction of all possible connections between neurons physically exist, even within a local area[25,26,40]. In such sparse networks, a capacity to rewire could dramatically increase the number of functionally distinct circuits available to encode learned information. On the other hand, the task of finding appropriate partnerships between pre- and post-synaptic units in a sparsely connected network is a hard combinatorial search problem, and could require a large number of slow, 'generate and test' operations[21,22]. Whether the brain has evolved the machinery to cope with these 'algorithmic' challenges remains an open question.

In this review, we discuss the possible role of wiring changes in the encoding of learned information in the adult

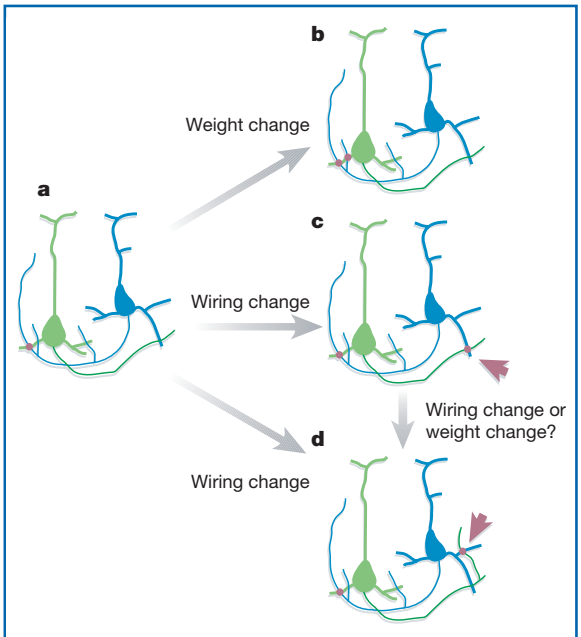
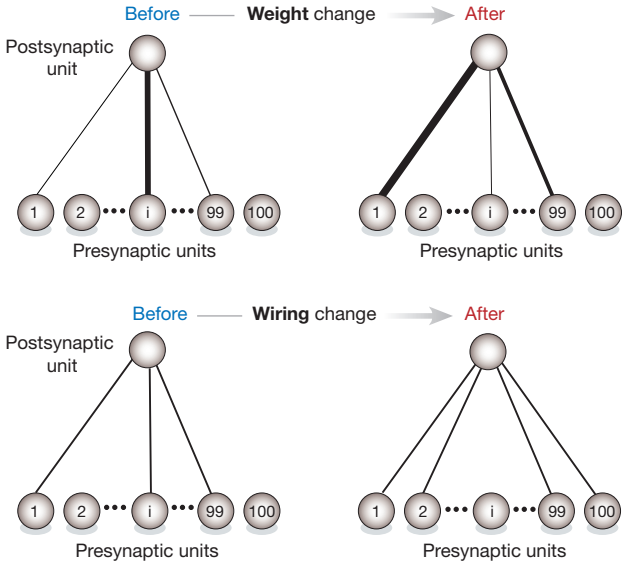


Figure 1 Structural circuit plasticity and the wiring diagram. The schematic shows two neurons (green, blue), dendrites (thick lines), axons (thin lines) and synapses (red circles). **a**, In the initial wiring diagram, signalling is from the blue neuron to the green one. **b–d**, Synapse formation and elimination can result in weight changes alone (**b**) or can include changes in the wiring diagram (**c**, **d**; red arrowheads point to changes). Wiring changes can occur with (**c**) or without (**d**) axon or dendrite growth. In the new wiring diagram, signalling occurs from blue to green and from green to blue. The transition between **c** and **d** might represent a wiring change, depending on the definition of the postsynaptic unit: the transition is a weight change if the postsynaptic unit is the whole neuron, and is a wiring change if the postsynaptic unit is a single dendritic branch.

Box 1

Contrasting weight versus wiring plasticity



The storage capacity of a neural network is a measure of the total learning-related flexibility of the circuit. If a network is represented as a graph of abstract units with weighted interconnections[4–6], then wiring changes are a special case of weight changes as they can be modelled by setting some non-zero weights to zero and vice versa. However, if the network is sparsely connected, it becomes useful to distinguish the two forms of plasticity.

Consider a postsynaptic unit with ten physical input connections ($s = 10$; for simplicity only three are shown above), and a population of $a = 100$ functionally distinct potential presynaptic partners. Each synaptic connection is assumed to have four possible long-term stable values (0, 1, 2 and 3; denoted by line thickness), giving $w = \log_2(4) = 2$ bits of storage capacity per connection. Assuming weight changes only, this system will have $s^*w = 20$ bits of total storage capacity, or two bits per synapse. However, if the cohort of axons connected to the postsynaptic unit can change over the course of a learning episode, the capacity associated with this partnership choice is $\log_2(100 \text{ Choose } 10) = 46$ bits of storage, or 4.6 bits per synapse even with all weights held fixed. The larger the ratio a/s; that is, the more axons that can serve as presynaptic partners for each postsynaptic site, the greater the in-principle advantage to a learning system that can choose its presynaptic partners.

The capacity advantage of wiring flexibility is greatest if/when individual synaptic weights can take on only a limited number of distinguishable values because of noise or other biological limitations. Some experiments hint at the possibility that synapses can have only a very limited range of long-term stable states[57,58,89], although this issue remains controversial and would benefit from further empirical study. Regardless of the value of w in any given neural circuit, it is reasonable to expect that the total learning-related storage capacity will benefit from both weight and wiring flexibility[22,90].

cortex. We discuss evidence and open questions relating to: (1) the identification of the presynaptic and postsynaptic units involved in learning; (2) geometric factors bearing on the inter-accessibility of axons and dendrites in the cortical microcircuit; (3) the existence of structural plasticity in the adult brain, including synapse formation and elimination, and outgrowth and retraction of dendrites and axons; (4) the stability of the neural circuit, that is, how long synaptic connections can be physically maintained; (5) the biological machinery that putatively manages learning-related cortical rewiring; and (6) interactions between weight plasticity and wiring plasticity.

What is a neural unit?

Identifying the neural substrate for learning and memory requires understanding which physical changes observed during learning lead to functionally distinct neural circuits. To do this, it is necessary to establish the proper mapping between the units and weights of the abstract network (Box 1), and the physical components of the biological neural circuit.

A unit is a node of the network whose state can be described by a single variable, such as a membrane potential, spike time or firing rate. In the cortex, one possibility is that individual neurons function as units, but this need not hold in general, and the mapping of presynaptic and postsynaptic units onto the neural hardware might be different.

A presynaptic unit might consist of the axon of a single neuron, or a group of functionally equivalent axons whose firing is strongly correlated. It is not known quantitatively how much overlap exists in the response properties of neurons within any given area of cortex, although there is evidence for substantial redundancy. For example, moving vertically through the layers of sensory cortex, neurons have heavily overlapping receptive fields, and even moving in the tangential direction, receptive field properties change gradually from neuron to neuron[27,28]. This redundancy reduces the number of modifiable parameters available for learning, and thus works against capacity (although it might aid robustness). Estimates of the cell-to-cell redundancy for specific areas of cortex could be made using calcium or voltage imaging methods in behaving animals.

The issues involved in defining the postsynaptic unit are different. The goal is to identify the largest integrative unit whose modifiable parameters during learning consist of only the weights on each of its input connections. For example, the largest subdomain of a neuron whose overall integrative operation is linear would qualify as a postsynaptic unit. In contrast, any significant nonlinear spatial interactions between the inputs to a postsynaptic neuron would violate the above definition, and would force the adoption of a finer-grained mapping of units onto single neurons. Pyramidal neurons have most often been conceptualized as single integrative units, although over the past few decades, the idea that individual neurons could be

divided into functional subunits has had a steady presence in the modelling literature[21,29–32]. Recent *in vitro* and modelling studies suggest that the integrative subunit of a cortical pyramidal cell might be as small as a single dendritic branch or less[33,34]. Within certain limits, this reduction in the 'grain size' of the cortical network implies a larger number of postsynaptic units, and a greater overall storage capacity[22].

Key questions remain unanswered, however. At present, we have no direct experimental evidence bearing on the number and size of integrative subunits within a pyramidal neuron *in vivo*. Subcellular functional imaging *in vivo*, perhaps using two-photon microscopy[35], could be used to map the receptive fields of individual dendritic branches and to help pin down the physical instantiations of presynaptic and postsynaptic units in the behaving brain.

How many wiring diagrams are within 'immediate' reach?

The storage capacity of a neural network depends in part on its ability to rewire, that is, on each postsynaptic unit's flexibility to choose presynaptic partners from a larger set of candidates. This relates to the issue of sparseness as discussed in Box 1, and leads to two questions. First, how many axons representing different presynaptic units can connect to a given postsynaptic unit through spine/dendrite/axon outgrowth? Second, of those units that can potentially connect, how many actually do?

In answering these questions, it is convenient to distinguish two populations of possible synaptic partners, beginning with the population of synapses that can be formed without significant growth of axonal or dendritic arbors[23]. This requires that an axon pass sufficiently close to a dendrite (~2 μm or less) so that a newly formed dendritic spine or terminal bouton can bridge the gap between them (Fig. 2). Such points of apposition between dendrite and axon are called potential synapses[23]. The number of potential synapses can be calculated from anatomical data using two different approaches. One is to calculate the expected number of axons passing within a spine's length of a dendrite. Such a calculation shows that potential synapses outnumber actual synapses by a factor of three to nine depending on the cortical area[23] (Fig. 3). However, this does not by itself imply short-range wiring flexibility; it must also be determined whether the population of axons within a spine's length of the postsynaptic unit includes new potential partners, that is, presynaptic units that do not already form synapses elsewhere on the postsynaptic unit.

To help resolve this uncertainty, a second approach is to use reconstructions of axonal and dendritic arbors from a pair of neurons to calculate the expected number of potential synapses between them[36–39]. Following this approach, it was determined that most neurons located within a few hundred micrometres of each other have at least one potential synapse between them. In other words, potential connectivity between neurons in a cortical column a few hundred micrometres in size is nearly all-to-all. This means that a connection between any two neurons belonging to the same cortical column can be realized by extending a spine or a terminal bouton. So, assuming that each axon carries a unique signal, and that each neuron is a single integrative unit, the storage capacity attributable to wiring plasticity within a cortical column can be substantial — $\log_2([\text{number of neurons in column}]^2/\text{number of synapses in column}) = \log_2([10^5]^2/10^9) = 3$–4 bits per synapse — even if structural changes are limited to spines and synaptic terminals.

This estimate of capacity assumes that connected and unconnected local neurons contribute potential synapses proportionately, that is, the number of potential synapses between two neurons does not depend on the presence of an actual synapse between them[36]. Electrophysiological measurements of synaptic connectivity between pairs of neurons, coupled with reconstructions of their axonal and dendritic arbors[36,40], could test this assumption. If the assumption is validated, many of the potential synapses considered above could belong to previously unconnected neurons, meaning that bona fide wiring changes could take place in cortical tissue with only minimal structural adjustments[23].

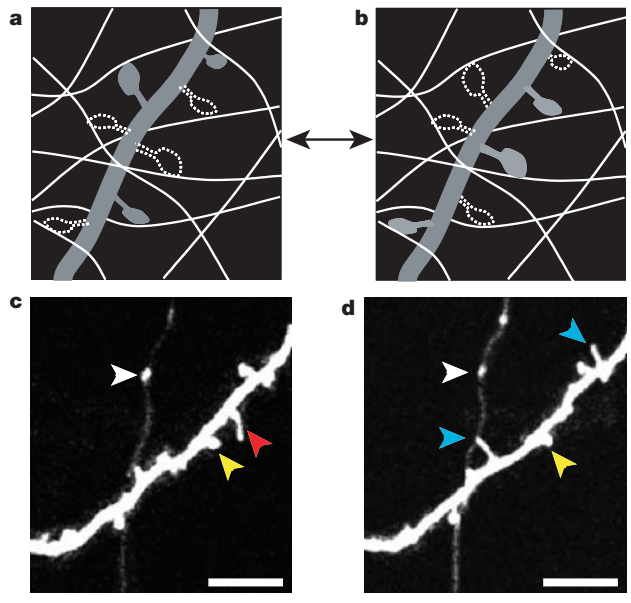


Figure 2 Structural plasticity. **a**, **b**, Schematic of structural plasticity with fixed potential connectivity. Only two of many possible configurations are shown. Dendrites and existing spines are grey. White lines denote axons, dashed white lines are potential synapses. **c**, **d**, *In vivo* microscopy of structural plasticity (A. Holtmaat, unpublished), showing a dendritic branch (thick line) and an axon (thin line). The picture in **d** was taken 16 days after the one in **c**. Note the appearance (blue arrow) and disappearance (red arrow) of dendritic spines. Some spines (for example, yellow arrow) and axonal terminals (for example, white arrow) are stable. Scale bar is 10 μm.

Evidence for synapse formation and elimination

As previously noted, synapse formation and elimination could contribute to changes in either weights or wiring. As such, simply observing synapse addition and subtraction does not help to distinguish between the two basic modes of plasticity, but would imply that wiring plasticity is at least mechanistically possible. Several types of experiments have provided evidence that synapse formation and elimination occurs in the adult brain. Electron microscopic analysis has provided evidence for new synapses in sensory cortex after behavioural enrichment[8] and sensory stimulation[9]. Similarly, long-term, high-resolution imaging experiments in the somatosensory cortex have shown that some dendritic spines appear and disappear, and that the rate of turnover is modulated by sensory experience[10]. Subsequent electron microscopic analysis revealed that at least some of these new spines make synapses. Together these experiments provide convincing evidence that the adult brain maintains the capacity for synapse formation and elimination. *In vivo* imaging experiments have also revealed that a fraction of dendritic spines is stable over months, and this fraction might be higher in the visual than in the somatosensory cortex[10,18]. It is even possible that a subpopulation of synapses persists for most of the life of the animal and that the fraction of stable synapses differs between different cortical areas.

How quickly can new spines form and how long do they, and their synapses, live under diverse experiential conditions? Is the cortical circuit structurally plastic at the level of spine changes, but built on a skeleton of stable dendrites and axons? Answers to these questions could come from time-lapse *in vivo* imaging to track the fates of synaptic structures, such as spines, axonal varicosities and labelled vesicle clusters. However, optical microscopy has certain limitations. High-resolution optical measurements are mostly limited to the superficial layers of the neocortex[41] (but see ref. 42). Furthermore, optical techniques alone do not inform unambiguously about

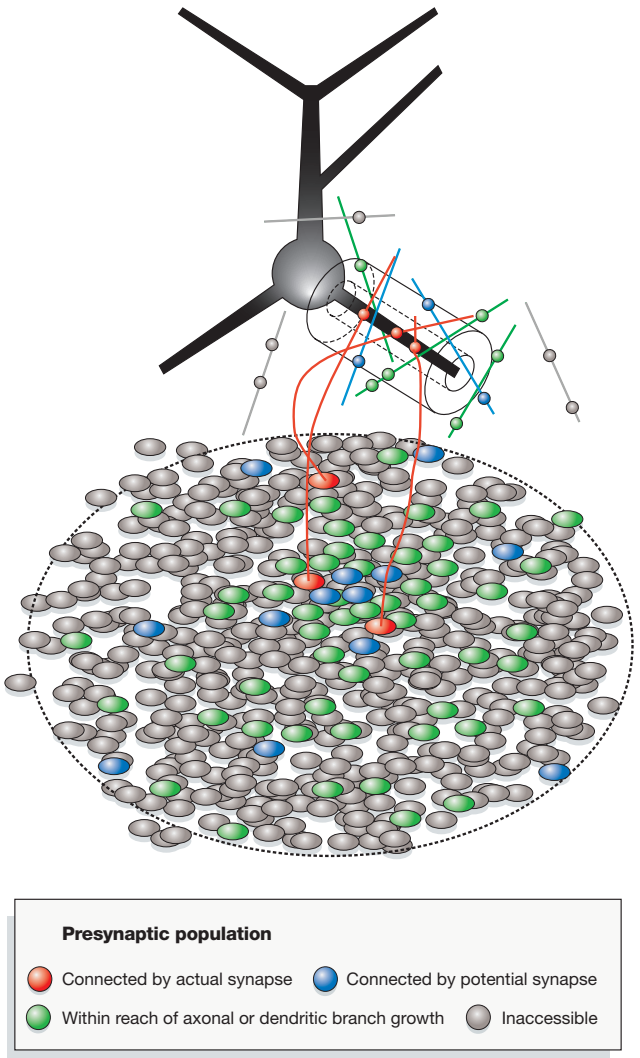


Figure 3 Actual and potential connectivity from a presynaptic population onto a postsynaptic unit. Concentric cylinders surrounding the postsynaptic dendrite show the volume accessible by the spine (inner cylinder), and the volume accessible by remodelling of an axon or dendrite (outer cylinder). Among those presynaptic axons that cross through the inner cylinder (blue), only a small fraction form actual connections (red). Green denotes the population of presynaptic candidates that cross through the outer cylinder. The much larger population of inaccessible axons is shown in grey.

synapse formation and elimination. Overlap of a dendrite and axon, or fluorescent labelling of presynaptic and postsynaptic components within an optical resolution element, do not necessarily imply the presence of a synapse there. Proof requires retrospective analysis using electronmicroscopy[10], or perhaps physiological recordings with single synapse sensitivity[43].

Longer-range wiring connections

The second population of potential presynaptic partners consists of those that can be accessed only through growth of new axonal or dendritic branches. Their number depends on the maximum spatial extent of axonal and dendritic arbors, and can be estimated geometrically. Hypothetically, if axons and/or dendrites could grow without bound, all connections would be realizable. Then each synapse could encode $\log_2([\text{number of neurons}]^2/\text{number of synapses}) = \log_2([10^{11}]^2/10^{15}) = 23$ bits per synapse. Because physical constraints restrict the amount of biological wiring[44], the actual number is certainly far smaller (Fig. 3).

Evidence for dendritic growth in the adult brain

Do dendrites retain their ability to grow in the adult brain, and is such growth related to learning? Studies of dendritic plasticity make the reasonable assumption that synapses are formed and eliminated when dendrites grow and retract. Dendritic remodelling could therefore underlie rewiring of cortical circuits. The dendrites of cortical pyramidal cells can be visualized conveniently using the classic Golgi technique[45]. Studies of dendritic plasticity have relied mostly on static measurements at a single time point and comparisons between groups of animals. A variety of experiential conditions have been tested, including the effects of environmental enrichment and behavioural training[45]. Early studies focused on the effects of the complexity of the environment (for example, impoverished versus complex). With experimental manipulations beginning immediately after weaning, the structural differences are profound, on the order of 50% for higher-order dendrites[11]. The effects of differential rearing on dendritic branching occur selectively in particular cortical areas (for example, the visual cortex, hippocampus), but not in other areas (frontal and motor cortex)[46,47]. Dendrites have also been analysed after training in specific tasks in adult animals. For example, in one experiment rats were trained in a monocular task. Comparing dendritic arbors in the trained and untrained hemispheres revealed relatively subtle changes in the density of the most-distal branches of layer 4 and 5 neurons[12].

The static experimental design used in these studies of dendritic plasticity has obvious limitations: it is only sensitive to robust changes in the averages of morphometric parameters, and thus underestimates the dynamics and maximum spatial extent of the dendritic changes that have taken place in the course of learning. Furthermore, the use of the Golgi method complicates the interpretation of these studies. The method is capricious, and it is not known what determines which neurons are labelled or whether labelling of individual neurons is complete. Without this information, such experiments cannot be viewed as definitive. Recently, long-term, high-resolution *in vivo* imaging has become possible. Such longitudinal measurements are exquisitely sensitive, as they can detect dynamics without changes in averages. These experiments point to remarkable dendritic stability for periods of months in rodent primary sensory areas, including visual and somatosensory cortices and the olfactory bulb[10,18,19].

How plastic are dendritic arbors in the rest of the adult cortex? Is plasticity limited to particular parts of the dendritic arbor, to particular cell types, or to particular (for example, memory-related) cortical areas? Does it occur in response to learning, or only under conditions of chronic enrichment or deprivation? Long-term, time-lapse imaging *in vivo* could help to provide answers to these questions.

Evidence for axon remodelling in the adult brain

Cortical axons span many millimetres of cortical territory and target diverse areas. Long-range growth of cortical axons in the adult would therefore have profound implications for circuit plasticity and would probably imply rewiring. As for dendritic growth, axonal growth would imply changes in the complement of potential synapses.

Evidence for axonal growth comes from experiments involving lesions of the sensory periphery. For example, amputation of digits[48] or limbs[49] leads to massive reorganization of cortical circuits. In monkeys, physiological rewiring has been detected across long distances (>10 mm), suggesting large-scale cortical rewiring that could only be explained by axonal growth[49]. Subsequent anatomical studies directly demonstrated growth of intracortical axons across several millimetres in the adult brain[14]. This process is of clinical importance because the extent of the rewiring correlates with the perception of phantom-limb pain[50].

Similar rewiring is observed in the primary visual cortex after focal retinal lesions[13,51,52]. After several months, the cortical area corresponding to the retinal lesion becomes sensitive to surrounding regions of the visual world. This reorganization might be of value to

the animal because it could lead to perceptual fill-in and completion of visual contours. Direct anatomical analysis reveals that growth of horizontal axons could explain the functional changes triggered by retinal lesions.

These experiments reveal that cortical axons maintain the capacity to grow and elaborate in the adult brain. However, axonal remodelling has only been observed in response to prolonged (months to years) injury. In addition, such lesions are at least in some cases associated with massive subcortical changes, including transneuronal atrophy[53]. Such pathological subcortical changes might release mechanisms of cortical rewiring that are not normally observed in the brain.

Clearly, our understanding of axonal plasticity in the adult brain remains in its infancy. How plastic are axonal arbors in the adult brain and what is the spatial range of growth? Do axons grow in response to learning, or only with injury? Just as for the question of dendrite outgrowth and remodelling, dynamic approaches using *in vivo* time-lapse imaging might help provide answers to these questions.

Finding good partnerships: an expensive proposition

It is clear that the adult cortex retains a substantial capacity for structural remodelling. However, a trade-off exists between the additional storage capacity made possible by long-range growth potential, in principle, and the additional space, time and biological machinery required to take advantage of it. First, the much larger presynaptic candidate pool accessible to a postsynaptic unit through long-range structural plasticity makes the search for groups of correlated afferents far more difficult. Second, longer-range connections presumably take longer to grow, forcing a slower learning rate. Third, longer 'wires' consume more space[54]. As such, the spatial and temporal scales across which axons and dendrites can test and stabilize new connections could be important determinants of the learning rate and storage capacity of the adult cortex.

Setting aside the practical limitations on axonal and dendritic growth rates and tissue volume, the 'algorithmic' challenge faced by a structural learning rule is daunting in and of itself. To illustrate, we return to the example in Box 1, where the task facing the postsynaptic unit is to develop input connections from a particular set of ten axons, chosen from the 100 accessible axons in the neighbourhood. (In this example, we assume each axon represents a distinct presynaptic unit.) The basis for the postsynaptic unit's choice of presynaptic partners might be that the firing pattern of the to-be-selected group of ten axons expresses a statistically significant higher-order correlation; that is, the axons fire together more often than chance after normalizing for individual firing rates. Given that the postsynaptic unit has 17 trillion different combinations of ten axons to choose from, even in this small example, an efficient search scheme must be in place to pull out the special, correlated cohort of axons during the structural learning process. If there were no guidance mechanisms in place to support 'selection-at-a-distance', or for efficient triage of presynaptic candidates, the worst-case scenario could require that the postsynaptic unit sequentially, physically, 'interviews' all possible groups of ten candidate axons by first forming actual synaptic connections with them, and then testing their correlations through a postsynaptic signalling pathway. As should be evident from this example, an exhaustive physical search through the space of all accessible wiring diagrams is intractable.

Computer simulations of learning rules involving wiring plasticity confirm the need for a large number of generate-and-test operations[21,22] — as are known to occur during development[55,56] — but have also pointed to heuristics that can accelerate the learning process and boost storage capacity. In experiments with a structural rewiring learning rule[22], it was found that when a new candidate synapse was brought in to replace a poorly performing synapse within a postsynaptic unit, the learning rate was accelerated and the final capacity was substantially increased, if at each iteration the new synapse was drawn from the top of a pre-screened candidate pool, rather than at random[22]. The pre-screened pool in the simulation experiments could be analogous to the pool of 'silent' synapses (lacking AMPA receptors) that exists in pyramidal neurons[57–59]. The physical convergence of a group of like-activated axons onto a compatible postsynaptic unit could also be accelerated through activity-dependent release of diffusible factors from axons and/or dendrites[60], or through electric fields[61]. Clearly, many open questions remain as to what biological mechanisms are needed, and which actually exist, to manage the search for new partnerships between unconnected presynaptic and postsynaptic units.

An additional question involves the rate and extent of synapse turnover that we should expect to see as learning progresses in a structure-based rewiring mode[10,18]. Without a theoretical handle on this issue, we will not know whether, say, 1% synapse turnover per day is too little plasticity to be interesting, in that it signals that the system is virtually hardwired; whether it is too much plasticity to be interesting, in that virtually every plastic synapse will have turned over within a few weeks; or whether it is the optimal rate of turnover given the learning task at hand within the cortical area in question. Theoretical and modelling studies could help to shed more light on these questions.

Interdependence of weight and wiring changes

Although we have adopted the view that weight changes and wiring changes should be distinguished, it is nonetheless likely that if both modes of learning operate in the adult cortex, they will be mechanistically linked. In particular, the process of generating and testing new partnerships between presynaptic and postsynaptic units, a core operation in wiring plasticity mode, necessitates a hebbian LTP-like mechanism (see below) to stabilize newly formed connections when they correlate strongly with the overall postsynaptic response. Similarly, an LTD-like mechanism is required for the elimination of poorly correlated connections. This reflects the fact that at a very local level, the formation or deletion of a synaptic contact can simultaneously reflect a weight and a wiring change, with LTP and LTD as the bridging mechanisms. As a definitional matter, although the term LTP has been used to describe a wide variety of plasticity phenomena at diverse synapses with unknown mechanisms[62], we use the terms LTP and LTD here to refer strictly to changes in synaptic efficacy at existing synapses. Candidate biological mechanisms for synaptic strength changes include modulation of the amount of neurotransmitter released per action potential, and the number and properties of synaptic glutamate receptors[7].

Weight changes in the adult brain

What is the evidence for pure weight changes in adult learning? Detecting synaptic strength changes induced by experience-dependent plasticity[63] or learning[64] remains a great challenge. In the adult motor cortex, behavioural training can produce LTP-like potentiation of horizontal connections[65]. However, in these experiments the synaptic mechanisms are not known and could involve structural, including wiring, changes. In the developing neocortex, deprivation-induced plasticity seems to be associated with changes in release probability[66] and changes in glutamate receptor number[67,68]. However, plasticity in the developing neocortex also produces large-scale rearrangements of axonal[69] and dendritic arbors[70], and synapse formation and elimination[71]. It is therefore unlikely that changes in synaptic strength alone will comprise most of the circuit changes underlying experience-dependent plasticity in the developing brain.

Can the strengths of individual synapses be maintained for sufficiently long periods to explain long-term memory? A priori the answer to this question is uncertain because synapses often function with only a small number (about ten) of channels and receptors[72,73]. Strength changes might therefore involve the modulation of only a few copies of proteins with short lifetimes[74]. Long-term stability of synaptic strengths would then demand essentially single-molecule precision from the cell biological mechanisms that maintain

synapses. Information about synapse stability could come from long-term imaging of individual synapses *in vivo*. For example, imaging of synaptic receptors tagged with fluorescent proteins[75] over time would give an indication of the stability of synaptic structure and synaptic strength.

Causal relationship

Experiences that induce changes in synaptic function can also cause structural changes and wiring changes. This has led to the view that changes in synaptic efficacy, and synapse formation and elimination might not be exclusive, but might operate on distinct timescales. Modification of synaptic function could operate in seconds to hours, whereas structural changes become important over longer periods[2]. This view is supported by studies of the gill-withdrawal reflex of *Aplysia*[2,15]. Somewhat analogous results come from studies in cultured hippocampal brain slices, where stimuli that induce LTP also lead to the growth of dendritic spines[76,77] that make new synapses[78]. These new synapses appear delayed compared with synaptic potentiation, indicating that they could be part of a late phase of synaptic plasticity.

Shared cell biological and molecular mechanisms

Can molecular techniques help to distinguish between the roles for weight versus wiring changes in experience-dependent plasticity and in learning and memory? Several interdependencies could complicate the interpretation of molecular interventions. In the process of synapse formation, contact formation between dendrite and axon triggers the delivery of presynaptic release machinery and postsynaptic receptors to synapses[79]. Maturation of synapses involves hebbian forms of synaptic plasticity[80,81]. Consistent with this, LTP is especially pronounced during developmental periods of massive synapse formation[82]. The cell biology of synapse formation and elimination, and synaptic strength changes, therefore share cell biological mechanisms.

Shared molecular pathways also exist at the level of induction of plasticity. For example, one of the better-studied pathways involves the calcium/calmodulin-dependent protein kinase (CaMKII). CaMKII clearly has a prominent role in LTP: it is necessary for the induction of LTP and is activated persistently by stimuli that produce LTP. Moreover, activated CaMKII is sufficient to potentiate synaptic transmission. CaMKII also has a role in plasticity *in vivo*: genetic disruption of CaMKII function prevents experience-dependent plasticity of receptive fields and hippocampal-dependent learning. Does this mean that CaMKII and LTP are the molecular and cellular substrates of memory? The problem with this interpretation is that the CaMKII pathway is not specific to LTP. Rather, a large class of activity-dependent responses involve CaMKII signalling, including dendritic and axonal branching in the developing brain[83], the formation of spine synapses[84], and changes in the wiring diagram in cultured neurons[85]. Genetic perturbations of CaMKII therefore probably interfere with both LTP and wiring plasticity. Experiments involving perturbations of other molecular pathways are similarly difficult to interpret in terms of circuit mechanisms.

An important question for future research is whether a core of molecular pathways exists that is specific to modulation of synaptic transmission as opposed to structural change. Given knowledge of such pathways, spatially and temporally precise molecular perturbations could yield important information on the role of structural plasticity and wiring change in the adult brain. However, even if such core pathways are identified, molecular perturbations could be difficult to interpret. Genetic perturbations of structural plasticity would presumably change the patterns of activity in neural circuits, which could change synaptic strength.

Future directions

We have argued that learning-related plasticity in the cortical wiring diagram, mediated by structural changes in spines, dendrites and axons, could underlie a second mode of long-term information storage in the adult cortex that operates in addition to the more commonly accepted learning mode based on changes in synaptic weights. Proof that wiring changes have a major role in adult learning will depend on further developments in imaging technologies to allow subcellular visualization of neural activity and morphological changes in the brains of behaving adult animals. An alternative approach could involve the development of new technologies to allow rapid analysis of synaptic circuits on a large scale. This might include high-throughput serial-section electron microscopy to allow the reconstruction of the synaptic circuits defining entire cortical columns in individual animals. Data of this kind would allow comparison of cortical circuits in animals that have, and have not, undergone particular forms of training.

We have emphasized that a fuller understanding of the role of wiring plasticity in adult learning depends not just on gathering more and better data showing the dynamics, spatial extent and longevity of learning-related structural changes in the adult brain. It also depends on: (1) a fuller description of the integrative properties of individual cortical neurons; (2) better models of the representational redundancies that exist among the neurons within the cortical column; (3) better geometric models of pyramidal cell morphologies and of the spatial intercalation of axons and dendrites in the cortical neuropil; and (4) a more complete description of the guidance and triage mechanisms that, just as in early development, promote the gathering together of correlated axon terminals onto postsynaptic targets.

More global 'systems' issues ought to be considered as well. For example, given that the encoding of information through learning-induced wiring changes is an inherently slow process, we must consider what strategies the brain might have adopted to buffer the flow of incoming information while it is being (slowly) structurally encoded. The proposal that information is rapidly encoded in the hippocampus during episodic learning (weight plasticity?), and later consolidated in cortical tissue over many months (wiring plasticity?), is highly relevant to the present discussion[86–88]. It may also be possible to search for congenital and/or disease-related long-term memory deficits that can be causally connected to the absence or dysfunction of factors contributing to structural plasticity and neurite guidance.

The identification of the engram — the physical change(s) encoding a particular long-term memory — remains a key aim of the field. In approaching this and other difficult questions relating to the physical substrate for long-term storage in the adult brain, an interdisciplinary approach that combines anatomical, physiological, molecular and theoretical methods seems the most likely to succeed. □

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