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Cytoskeletal involvement in neuronal learning: a review

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Abstract. This paper introduces the ideas of neural networks in the context of currently recognized cellular structures within neurons. Neural network models and paradigms require adaptation of synapses for learning to occur in the network. Some models of learning paradigms require information to move from axon to dendrite. This motivated us to examine the possibility of intracellular signaling to mediate such signals. The cytoskeleton forms a substrate for intracellular signaling via material transport and other putative mechanisms. Furthermore, many experimental results suggest a link between the cytoskeleton and cognitive processing. In this paper we review research on intracellular signaling in the context of neural network learning.

Key words: Neural network – Cytoskeleton – Synaptic adaptation

1. Introduction

A fundamental challenge of current research is to explain the possible mechanisms for learning and cognitive processing in the brain. Such an explanation will entail concepts at multiple levels: the systems level (network of neurons), the neuron level (processing and adaptation of the neuron and its synapses), and the molecular level (synaptic and intracellular proteins and trafficking). Results from systems modeling of neural networks show us effective and important aspects for the individual neurons or processing elements that enable the neural networks to learn to recognize patterns. Biophysical mechanisms for these functional capabilities can then be examined at the level of protein molecules.

Neural network learning paradigms, a focus of recent attention, show how networks of neuron-like components can learn through adapting synaptic strengths. Learning is done in response to patterns that stimulate the network, which learns to classify those patterns. Powerful learning capabilities have been discovered for neural network paradigms such as back propagation, counter propagation, sigma-pi networks, time-delay neural networks, adaptive resonance theory (ART), and networks with competitive layers. Many of these paradigms require reciprocal communications between pairs of neuron-like components and require information flow to go backwards through the network as well as forwards. But real neurons have membrane mediated signaling that travels in the "forwards" direction – dendrite to axon terminus – as post-synaptic potentials signal from dendrites to the spike initiation zone and action potentials travel down axons, away from the soma. Two-way communication could occur by nerve impulses but world require pairs of neurons to synapse onto each others dendrites, at sites already receiving synapses from other neurons. This regular and intricate arrangement has not been observed. Reciprocal communication could more plausibly be mediated by intracellular signals going in the reverse direction, with the neuronal cytoskeleton mediating such intracellular signals. This arrangement requires anatomical structures that are readily observed.

In this paper we introduce the ideas of neural networks in the context of cytoskeletal structures within neurons and the role such structures could play with respect to intracellular signaling. We review the neural network approach, in which synaptic strengths are adapted to produce learning in the network. We identify specific neural network learning paradigms that would require "backwards" propagation of signals – axon to dendrite – and other intracellular signals. The structure of the neuronal-cytoskeleton is then described with emphasis on candidate mechanisms for supporting intracellular signals. Although one mechanism, material transport, has been verified by extensive experimentation, such transport is relatively slow. Faster mechanisms would enable learning to take place more quickly. Thus we summarize additional mechanisms for faster signaling along the cytoskeleton

Abbreviations: MT, microtubule; MTs, microtubules; ART, adaptive resonance theory; RCE, restricted coulomb energy; MAP, microtubule associated protein; NO, nitric oxide

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that have been proposed and that appear plausible. Experimental and theoretical studies concerning these faster signaling mechanisms are reviewed. We then include a review of experimental evidence for a relationship between cognition and cytoskeleton, and conclude with a discussion on the impact of the neural network approach.

2. Neural network approach

The term "neural network" covers a broad spectrum of learning models composed of networks of interconnected processing units or neuron-like components that adjust their synaptic strengths according to training examples. A variety of such learning models have been proposed, and many have become key techniques for engineering applications in which pattern recognition must be learned from examples found in data (Maren et al. 1990; Hecht-Nielsen 1990; Dayhoff 1990). Neural networks that employ processing units inspired by biological neurons but greatly simplified or altered are considered "artificial neural networks" but even these paradigms may be biologically plausible (Dayhoff et al. 1992). Recently, much attention has focused on back propagation, an artificial network paradigm that has led to a large number of applications. Back propagation trains feed-forward networks; such trained networks have been shown to have powerful mathematical properties, including the ability to approximate an arbitrary functional mapping and thus the ability to discern arbitrary differences among input patterns (Hornik et al. 1990). Other paradigms include adaptive resonance theory (ART) (Carpenter and Grossberg 1987, 1988), the RCE network (Reilly et al. 1982), time-delay networks (Waibel et al. 1989), sigma-pi networks and networks with competitive layers including the self-organizing feature map (Rumelhart et al. 1986; Kohonen 1988).

Back propagation networks usually consist of layers of processing units that forwards propagate activation layer by layer as stimulated by an input pattern. The last layer of activation is read out as the output of the network. Each processing unit performs a weighted sum of incoming activations, computes a sigmoid function (a "soft" threshold), and sends its resulting activation level as output to the next layer (see Fig. 1). Such networks can adjust their interconnection weights (synaptic strengths) in response to training patterns that consist of input vectors with an associated target vector (the desired output vector). With appropriate adjustment of interconnection strengths, the network can learn to produce the target vectors as output. In back propagation, the network's output is compared to the target vector and an error value is computed for each unit in the output layer. Calculation of inner, "hidden" unit error values propagates backwards through the network. Each processing unit then adjusts the weights on incoming connections according to its calculated error value.

Back propagation has long been considered not to be biologically plausible (Stork 1989; Hecht-Nielsen 1989; Churchland and Sejnowski 1989). Most studies regarding biological back-error propagation have assumed that error feedback must be provided by action potentials and axodendritic synapses. This assumption requires a heavy density of bidirectionally connected cells, in which many pairs of cells must be connected in both directions via axo-dendritic synapses. For each forwards connection to a target neuron, there is assumed to be a backwards connection from the target neuron to the dendrites of the original neuron, to send error difference signals backwards to the previous layer of synapses. There is a lack of evidence for the occurrence of such arrangements, and, in addition, a lack of proposed mechanisms for causing such interconnection topologies to be built. Hence the biological plausibility of back-error propagation has been considered extremely unlikely.

Recently, Dayhoff et al. (1992, 1993) have taken a radically different approach to the biological plausibility of back-error propagation, and have utilized the internal complexity of the neuron to construct a model in which back-error signals are propagated within a nerve cell through the cell cytoskeleton, the cell's structural support system, which includes microtubules (MTs), protein polymer strands (actin filaments and neurofilaments), and microtubule associated proteins (MAPs), some of which crosslink MTs.

The possibility of intracellular cytoskeletal signaling fits strikingly with a variety of experimental evidence from studies of the cytoskeleton and its associated biochemistry. Previous authors have suggested that MTs are indeed involved in back-errors propagation (Werbos 1990, 1993; Hameroff et al. 1989; Rasmussen et al. 1990) or in neuronal learning (Grossberg 1969), but did not develop a description of the underlying biophysics or suggest anatomical sites at which each of the necessary computations take place. Specific sites and biophysical mechanisms have been suggested by Dayhoff et al. (1992a, 1993) and are summarized below.

The equations of the back-propagation learning algorithm are as follows. An input pattern (vector) is presented to the network, and the values of the vector are activation levels of input units (firing rates of input neurons). Each successive layer calculates its activation levels as

$$a_{j,h} = f\left(\sum_{i=1}^{n_1} a_{j,h-1}\, w_{ji,h-1}\right) \tag{1}$$

where n_h is the number of processing units in layer h, $a_{i,h}$ is the activation level of unit i in layer h, $w_{ji,h}$ is the weight to unit j (layer $h+1$) from unit i (layer h), and f is a squashing function, usually the sigmoid $f(x) = 1/(1+e^{-x})$.

Error delta values are computed for each processing unit, first at the output layer, where the delta value is

$$\delta_{j,3} = (t_j - a_{j,3})\, f'(S_{j,3}) \tag{2}$$

where $\delta_{j,h}$ is the delta value for unit j, layer h, f' is the derivative of the squashing function, and $S_{j,h}$ denotes the incoming sum to unit j, layer h:

$$S_{j,h} = \sum_{i=1}^{n_{h-1}} a_{j,h-1}\, w_{ji,h-1}$$

For the hidden layer, the delta values are

$$\delta_{j,2} = \left(\sum_{k=1}^{n_3} w_{kj,2}\, \delta_{k,3}\right) f'(S_{j,2}) \tag{3}$$

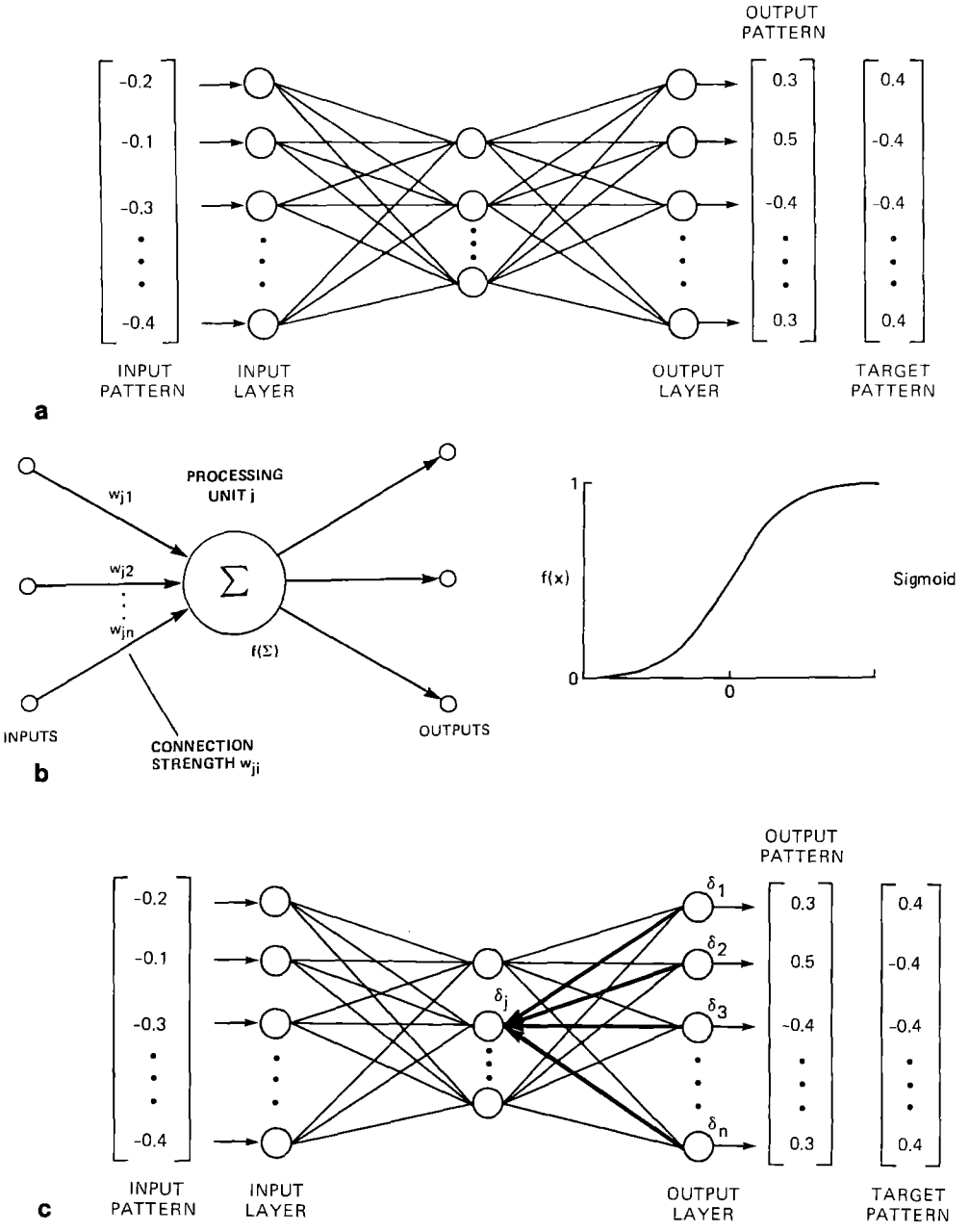

Fig. 1. **a** Feed-forward layered network with three layers of processing units: input, hidden, and output layers. Each layer is fully interconnected to the next layer in a forwards direction. A weight is associated with each interconnection and an activation level is associated with each processing unit. **b** Individual processing units perform a weighted sum of arriving inputs and then compute a squashing function, usually a sigmoid. **c** Network weights are trained by back-error propagation in which error delta values for each unit are computed and used to correct incoming weights. Target vectors are the source for the error delta value computations. Reprinted from Dayhoff (1990) with permission

Weights associated with interconnections that go to the output layer are changed as follows:

$$\Delta w_{ji,h-1} = \eta \, \delta_{j,h} \, a_{i,h-1} \tag{4}$$

where η is the learning rate parameter. Although the weight adjustment in (4) is specified precisely in a mathematical equation, approximations will suffice. For example, quantized increments or decrements in weights are sufficient for learning; later iterations can compensate for non-optimal weights, as training is completed. Also, the value of η can be raised or lowered during training; usually lowering η slowly provides better learning.

The above description assumes a layered and fully interconnected network, but the back-propagation learning paradigm applies to more irregular configurations, with interconnections skipping layers, going to lower layers, and sparse interconnections. Figure 2 depicts a layered fully connected network in a biological context.

Error-differencing can take place between the output signal of the neural network and an external signal indicating the target value. If each signal is coded by its firing

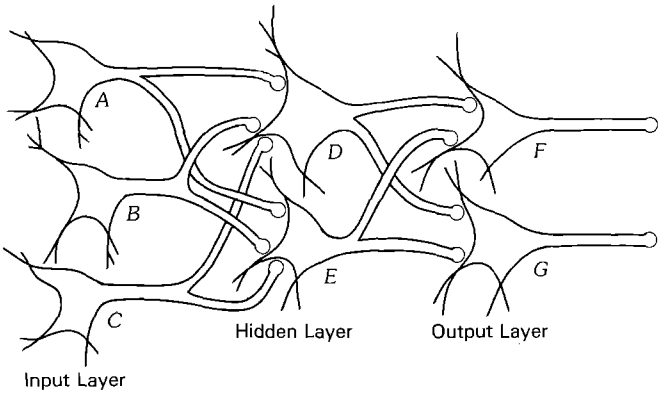

Fig. 2. Depiction of a biological feed-forward network with three layers of neurons, each layer fully interconnected to the next layer. Reprinted from Dayhoff et al. (1993)

frequency, then error differencing could take place from any of the three circuits shown in Fig. 3. Figure 3a shows an intermediary neuron taking the error difference by having one positive (excitatory) input, the target signal, and one negative (inhibitory) input, the network output. Figure 3b shows error differencing at a reciprocal synapse, where the forwards synapse is excitatory and the reverse synapse is inhibitory. Figure 3c illustrates an error differencing all in the same synapse, with the use of a reverse transmitter, perhaps NO. In each case a signal could be initiated along the presynaptic cytoskeleton proportional to the error difference.

The error signal is presumed to arrive at the post-synaptic sites in the dendrite, and there to influence the amount of change in synaptic strength. In a realistic model of synaptic strength, the strength of the synapse depends on the number of active receptor molecules at the postsynaptic site. Thus positive cytoskeletal signals could trigger stabilization and anchoring of more of these receptors, and negative signals could trigger the deactivation of receptor molecules. Receptor molecules may be anchored to the cytoskeleton through proteins such as anchorin and fodrin (Burgoyne 1991) which could be influenced by the cytoskeleton and, if deactivated, would lower the number of active receptor molecules.

In the training of a three-layered network there is an additional step not required in a two-layer network. In this step a neuron in the intermediate layer uses error signals from neurons in the outer layer. To model in a biological context, the intermediate neuron would have to pick up error signals that arrive at presynaptic sites in the axon and transmit them back to post-synaptic sites in the dendrite. A reverse transmitter could be responsible for the transmission of error signals backwards across synapses at the axon (Barinaga 1991; Gardner 1993). The error signal would be multiplied by the strength of the synapses, as in (3). If the strength of the synapse were modeled as the synaptic area, then this multiplication would take place as the reverse transmitter binds to receptors at the pre-synaptic site. Figure 4a depicts transmission of the error signal backwards to the hidden unit and Fig. 4b shows the merging (or summation) of intracellular signals within the neuron, corresponding to the sum in (3).

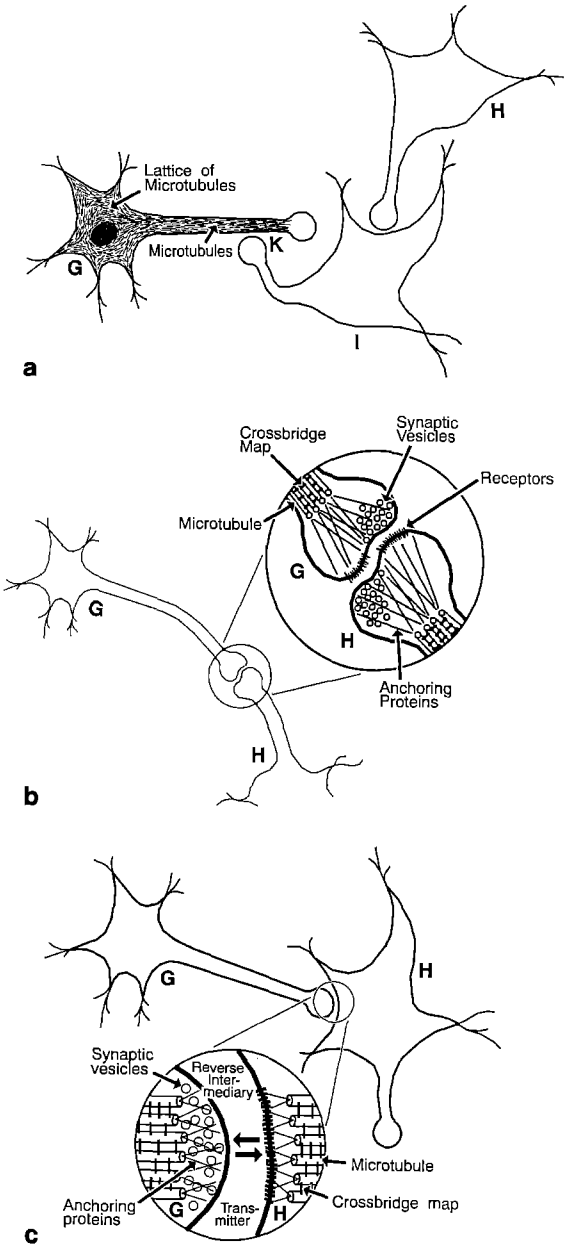

Fig. 3a–c. Three mechanisms by which error differencing could be computed biologically. **a** Intermediary neuron. **b** Reciprocal synapses. **c** Single synapse with reverse transmitter. Reprinted from Dayhoff et al. (1993)

The above summarizes briefly the sites at which each of the computations could plausibly take place, and identifies appropriate biophysical substrates at each site suitable to mediate the needed computations for a learning paradigm that utilizes backwards propagation (axon-to-dendrite) of error signals, as proposed by Dayhoff et al. (1993). Detailed experimentation is yet required to explore whether the activity of the molecules is consistent with such a learning paradigm, or whether a different (but perhaps similar) learning paradigm is used.

The classical back-propagation algorithm shown above is only one of many neural network paradigms that employ feedback signals that go backwards through a network. A variety of neural paradigms back propagate

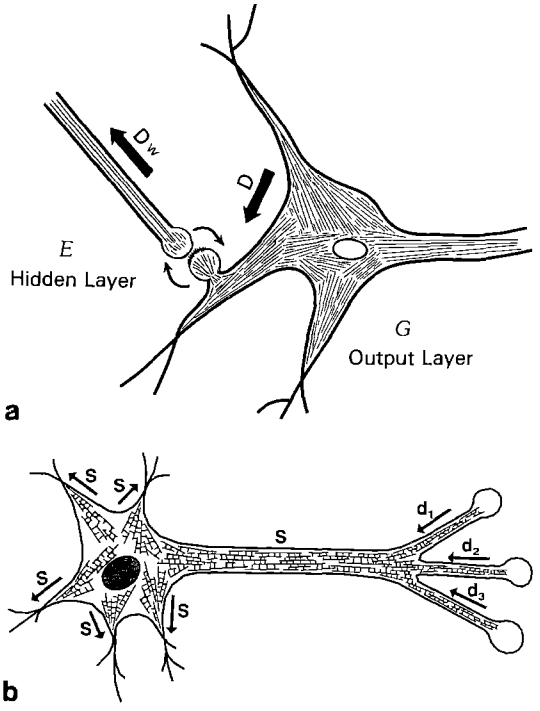

Fig. 4. **a** If the network has more than two layers of neurons, then error feedback signals would need to be carried backwards across synapses via a reverse transmitter (e.g., NO) **b** Internal signals traveling up axons would be merged at the axonal trunk, resulting in a summation of signal intensity. Reprinted from Dayhoff et al. (1993)

error signals through the network and could be considered variations of the original back propagation paradigm. For example, the sigma-pi network multiplies groups of incoming signals before a summation is done at the target processing unit (Rumelhart et al. 1986). Errors are then back propagated through the network to correct the weights.

The time-delay neural network (TDNN) propagates error backwards to adjust weights in spite of time delays along interconnections, and the ATNN back-propagates error correction signals that are used to adapt time delays along interconnections (Waibel et al. 1989; Day and Davenport 1993; Lin et al. 1992). These paradigms allow for learning of spatiotemporal patterns.

Recurrent networks allow for lateral and backwards interconnections in the network and adapt weights along all interconnections; the connections may be arbitrarily placed (Werbos 1988). Back propagation itself does not have to adhere to a fully-connected layered topology but can also have arbitrarily placed forwards connections. These networks all depend on the backwards flow of information (about errors) through the network, the construct that requires axon-to-dendrite signaling biologically.

Other learning paradigms have powerful functional approximation and pattern mapping capabilities. The work of Copper and coworkers on the RCE networks includes schemes for recruiting hidden units as needed by an externally connected layer of output units (Reilly et al. 1982). Thresholds of hidden units are adjusted according to information sent backwards from the output layer; this information depends on error differences. These networks map input patterns to desired output patterns. This also requires reverse intracellular signaling, possibly mediated by the cytoskeleton in biological systems. In this case, however, the signal may only have to propagate backwards along the axon to the cell body where the threshold is set.

An important class of neural network architectures consists of two layers of bidirectionally connected cells. The most notable of these architectures is ART (adaptive resonance theory) (Carpenter and Grossberg 1987, 1988). The top-down and bottom-up weights have separate rules for adjustment during learning. Recognition of incoming patterns occurs with "resonance", a state in which activation flowing downwards reinforces that flowing upwards. Since each unit in the bottom layer is bidirectionally connected to each unit in the top layer, the obvious biological analog is forwards signaling along axonal membranes and backwards signaling intracellularly along the cytoskeleton (Dayhoff et al. 1992c).

Other researchers have proposed learning mechanisms that employ communication between dendritic sites. Heterosynaptic mechanisms proposed (Finkel et al. 1989) utilize a selectionist population approach in which other dendrites on the same neuron communicate to modify a given dendritic synapse. Pribram (1991) has proposed dendritic influences acting as a field effect and having mechanisms of communication other than that channeled along membranes.

3. The neuronal cytoskeleton

The cytoskeleton, a lattice-like network of protein polymers and associated proteins, is found in the interior of neurons and collectively supports the cell structure and internal cell processes. This network includes microtubules (MT), centrioles, actin filaments and neurofilaments, membrane anchoring proteins, and microtubule associated proteins (MAPs) that crosslink MTs and other structures. Microtubules and other cytoskeletal components are found in virtually all eukaryotic cells, and provide physical and structural support to the cell (see Fig. 5). In addition, microtubules provide a communication and transport channel between remote cell parts. In most cells, the MTs connect the cell center (centriole) to the cell periphery in a radial pattern. In nerve cells, MTs have evolved a striking adaptation in that the microtubular lattice extends into the extremities of the axons and the dendrites, and some MTs may bypass the cell center. These internal highly ordered structures allow not only for the mechanical transport of material particles, but recent models have suggested that the cytoskeleton might be capable of transmitting fast signals and could do so internally within a nerve cell (Rasmussen et al. 1990). A variety of candidate mechanisms have been proposed for microtubule signal propagation, including propagating conformational changes and ionic motions (Hameroff 1987; Hameroff et al. 1989; Rasmussen et al. 1990).

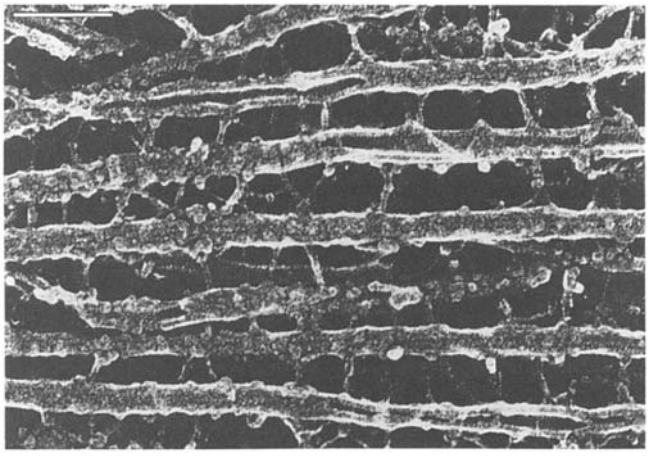

Fig. 5. Electron micrograph of microtubule/MAP complex assembled in vitro. From Sato-Yoshitake et al. (1989) with permission

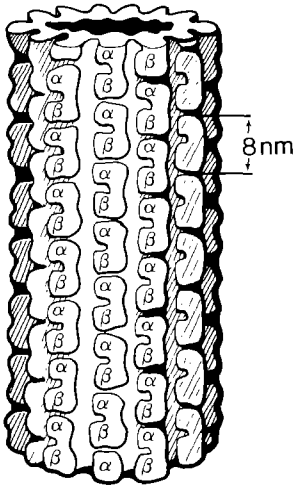

Fig. 6. MTs are comprised of approximately 13 protofilaments arranged in parallel to form a tubular structure. Protofilaments are composed of tubulin dimers consisting of α and β tubulin monomers. Dimers are 8 nm long. Reprinted with permission from Hameroff (1987). Different profilament arrangements are possible (Amos and Amos 1991)

Primary organizing elements of the cytoskeleton are called microtubules (MTs), which are arrayed in parallel and are inter-connected by microtubule associated proteins (MAPs) (Dustin 1984). Other interconnecting networks of smaller filamentous proteins (actin, neurofilaments, etc.) intersperse with MTs to form a dynamic net whose activities are essential to the living state. The cytoskeleton participates in activities such as mitosis, growth and differentiation, locomotion, food ingestion or phagocytosis, and cytoplasmic formation. In nerve cells, the cytoskeleton participates in synapse modification, dendritic spine formation, and neurotransmitter release (Burgoyne 1991).

MTs are cylindrical in shape, with a 25 nanometer (10^{-9} m) diameter, and MT bundles may span meters in some mammalian neurons. MT walls consists of longitudinal protofilaments that are each a series of subunit proteins known as tubulin (see Fig. 6). The protofilament number varies, but is usually 13 or 14. Each tubulin subunit is a polar dimer, measuring 8 nm (nanometers) in length, that consists of two slightly different classes of 4 nm monomers known as α and β tubulin (Amos and Klug 1974). The tubulin dimer subunits within MTs are arranged in a helix of tubulin subunits that resembles a hexagonal lattice that is slightly twisted, resulting in differing neighbor relationship among each subunit and its six nearest neighbors. MTs can be assembled from a wide variety of tubulin isozymes, as extensive heterogeneity exists owing to genetic diversity of tubulin and post-translational modifications.

Interconnected by crosslinking MAPs, MTs from a lattice network configuration within cells that provides structural support. In cells such as neurons they interconnect the cell membrane with cell organelles, and play a role in material transport and possibly in signaling that promotes protein synthesis. MTs generally radiate from a MT organizing center (the centriole) located near, but outside, the cell nucleus. A centriole consists of a pair of cylindrical super-assemblies comprised of nine MT triplets. In neurons, some MTs bypass the centriole and span areas of the cell soma, axons, and dendrites. Cytoskeletal elements apparently play a role in gene expression and cell shape changes, and may allow the environment of the cell to trigger protein synthesis (Ben Ze'ev 1991). Distal ends of MTs typically connect via anchoring proteins (such as fodrin) with membrane proteins, including receptors and ion channels.

MTs are polar; the tubulin dimer is oriented with a negative charge on the alpha monomer and a positive charge on the beta monomer (DeBrabander 1982). The entire MT, as well as individual subunits, have the same polarity, and MTs are proposed to behave as "electret polymers" (Mascarenhas 1974; Athenstaedt 1974). The polar nature of MTs introduces an asymmetric into their orientation within the cells. For MTs in axons, almost all have been reported to be oriented with their beta (+) end towards the axon terminus and their alpha (−) end towards the cell body (Burton and Paige 1981; Heidemann et al. 1981). MTs in the dendritic part of the cell are mixed with approximately half oriented with their plus ends away from the cell body (Burton 1988) and half oriented in the opposite direction.

MTs undergo dynamic assembly and disassembly processes, in which MTs either grow or shrink, and may shift back and forth between these two processes (Kirschner and Michison 1986). MTs can quickly change from depolymerization to reassembly in another direction. MT assembly and disassembly are complex processes that depend on various factors including temperature, calcium ion concentration and the availability of GTP. GTP-tubulin is required for assembly, and the hydrolysis of GTP-tubulin to GDP-tubulin occurs in the assembled MT.

Microtubule associated proteins (MAPs) form crosslinks between parallel MTs, producing a ladder-like structure. Crosslinking MAPs attach to two MTs at specific dimer sites, and patterns of MAP attachments can be irregular, or can occur in periodic patterns that may be super-helical on the MT surface (Kim et al. 1986;

Burns 1978). Similar patterns can be obtained by coherent phonon mode maxima calculated for MTs (Samsonovich et al. 1992), and coherent phonons have been proposed as a candidate mechanism for signaling along MTs. Some MAPs form bridges that laterally connect specific subunits on parallel-arrayed MTs to neurofilaments, membrane proteins, and organelles.

Dynein and kinesin are motor proteins that use biochemical energy from ATP hydrolysis for mechanical movement along MTs. These molecules bind to cell organelles and expend energy to cause the organelles to move along MTs like trains on a track (Allan et al. 1991). Similar movements can be caused in vitro with beads. Movement requires ATP as an energy source, hence dynein and kinesin can be considered motor proteins. The result is a form of material movement called axoplasmic transport or dendritoplasmic transport. Extensive experimental evidence exists for these motorized movements throughout the nerve cell. Material transport can be considered a mode of intracellular signaling in which information is represented by the presence, absence, or movement of particular substances.

Neurons are distinguished from other cells by their highly elongated processes (axons and dendrites) and must maintain their nutrient competence by a flow of material from regions near the soma to their elongated extremities. Since most cellular biosynthesis occurs in the region of the cell body, enzymes, receptors, neurotransmitters, and other substances must be transported. The transport of material away from the cell body towards axonal and dendritic extremities is called anterograde transport, whereas transport towards the soma is termed retrograde transport. Retrograde transport may recycle some material or provide feedback information. Axonal anterograde transport is generally classified by the rates at which transport of material occurs. There are different, distinct forms of fast anterograde transport with rates of 100–400 mm/day, 20–70 mm/day, and 3–20 mm/day (Vallee and Bloom 1991), but rates of 400–2000 mm/day have also been reported (Ochs 1982). They are, in all likelihood, mediated by different mechanisms and involve the transport of different material particles, possibly different sizes of polypeptides (Grafstein and Forman 1980). The two forms of slow anterograde transport have transport rates on the order of 0.1–4 mm/day and presumably involve movement of the cytoskeleton and its membrane connections (Brady and Lasek 1981). Our knowledge of retrograde material transport is not as extensive as anterograde transport although reputed rates are generally of the same of magnitude, about 300 mm/day (Grafstein and Forman 1980).

The polar nature of MTs noted previously offers directionality to the transport motor which somehow allows for the coupling and uncoupling of selective carrier proteins. Kinesin and cytoplasmic dynein proteins appear to have a role in fast anterograde and retrograde axonal transport respectively, whereas dynein has been implicated in slow transport. MTs also play a role in transporting material from the cell body to the dendritic terminals (Allan et al. 1991). Thus axonal retrograde transport and dendritic anterograde material transport allow for an efffective coupling between axon terminals and the dendrites.

Axonal and dendritic transport, important for synaptic maintenance and modulation, depend on motor proteins which hydrolyze ATP for energy. Transported materials bind to motor proteins kinesin, cytosplasmic dynein and/or dynamin which then, as a complex, interact transiently with the MT structure like trains on a track (Pfister et al. 1989; Brady et al. 1990). The movement consumes ATP hydrolysis energy in the process; however, the mechanism for orchestration and sequential signaling within MTs is unknown. Material can travel in opposite directions along a single microtubule, and can travel simultaneously, but particles carried by kinesin always move towards the + end and those carried by dynein move towards the − end.

Other cytoskeletal activities involve the molecular machinery of cell division, growth, differentiation, formation of synapses and dendritic spines (Burgoyne 1991; Hirokawa 1991). Formation of synapses, including dendritic spines, involves neurite and growth cone extension by polymerization of MT, actin, and other cytoskeletal proteins. Once established, synapses are maintained and modulated by axoplasmic transport and other mechanisms. In single cell organisms such as amoeba and paramecium, which do not have synapses or brains to explain their adaptive behaviors, relatively complex behaviors are controlled by or involve the cytoskeleton (Wichterman 1985; Hameroff et al. 1993).

Actin filaments are another major part of the cytoskeleton in addition to MTs. Actin filaments are polymerized actin strands that form their own lattice-like network throughout the cell. These filaments cross each other, and are interspersed with MT/MAP lattices and neurofilaments. They are more flexible than MTs, capable of more bending because tubules have some rigidity. Usually a dense lattice of actin is present. Actin strands can be seen in pre- and post-synaptic areas, but cover the cell as well.

Actin strands play an important role in the pre-synaptic nerve terminal, where they are the major cytoskeletal element (Hirokawa et al. 1989). Although arranged in a meshwork, actin filaments are mainly perpendicular to the membrane at the site of neurotransmitter release. Secretion of transmitter vesicles is accompanied by changes in the assembly and disassembly of actin filaments (Sontag et al. 1988). Blocking the disassembly or assembly of actin filaments changes the amounts of neutrotransmitter released in response to depolarization (Bamburg and Bernstein 1991). Actin disassembly is required for higher release and reassembly appears to cause more limited release.

Synapsin 1, present in pre-synaptic terminals, can bind to tubulin, actin, and synaptic vesicles, according to biochemical studies (Goldenring et al. 1986). Synapsin 1 crosslinks actin filaments to each other and to synaptic vesicles (Hirokawa et al. 1989). Cytoskeletal binding to synapsin 1 is reported modulated by phosphorylation of synapsin 1 via protein kinase II, dependent on Ca^{2+} calmodulin (Schiebler et al. 1986). Phosphorylation of synapsin 1 could regulate the release of neurotransmitter,

as the phosphorylation is thought to detach vesicles from the actin filament, allowing them to fuse to the membrane and release transmitter (Llinas et al. 1985). It has been proposed that release of the vesicles from the actin network and the disassembly of actin filaments both enhance the release of neurotransmitter following arrival of an action potential (Bamburg and Bernstein 1991).

Growth cone extension occurs as actin polymerizes towards the end of the axon, followed by MT polymerization. Assembly and disassembly of actin strands thus mediate axonal growth and, consequently, synaptic formations.

Neurofilaments are formed from polymerized keratin-like proteins. These polymer strands are without the tubular structure of MTs, hence they are lacking in rigidity. They are interspersed with actin strands, MTs, and other associated proteins of the cytoskeleton.

4. Signal transmission

In this section we propose and review candidate mechanisms for intracellular signaling in neurons. These mechanisms would be mediated by the neuronal cytoskeleton. Although material transport is the most widely considered mechanisms, as much experimental evidence exists, other mechanisms have been proposed, including propagated conformational changes, ionic movements and translation of the polymer strands. Material transport is relatively slow (one or more hours to traverse a neuron); in contrast, the alternative mechanisms proposed would be faster, some within a nanosecond time frame.

Direct evidence for fast signal propagation in MTs has been generated by Vassiliev et al. (1985) who suspended parallel, excitable membranes in ionic solution containing unpolymerized tubulin. Excitation in one membrane affected excitation in the other only when tubulin was assembled and the two membranes were connected by MTs. These authors suggested that similar communication signals may occur routinely within the cytoskeleton. Experimental evidence for fast signal propagation in actin filaments was reported by Cantiello and Kim (1993). In a dual patch-clamp recording experiment, a voltage imposed at one patch clamp cause a signal that was detected by another patch clamp electrode attached to the other end of the same actin filament.

4.1. Material transport

Material transport along MTs could be considered an intracellular signal. Much experimental evidence exists for such transport. Because axoplasmic (and dendritoplasmic) transport is bidirectional and includes a component from axon to dendrite, the transported material itself is one possible mediator of neuronal learning signals. Such transport is relatively slow, but a variety of specific rates have been observed. Rates of 400 mm/day and even 2000 mm/day have been reported (Ochs 1982). The fastest estimate could propel particles or organelles along one centimeter in about 1.2 h.

Material transport could be considered as a "signal" in the following ways. Arrival of a vesicle to a distal position such as a dendritic post-synaptic site could initiate synaptic strengthening if the vesicle contained receptor molecules or other proteins that mediate post-synaptic responsiveness. Transport of substances from pre-synaptic sites in the axon to the nucleus or dendrites could signal specific phenomena taking place at the synapse, such as the presence of compounds created or modified during synaptic acivity, or detection of reverse transmitters such as NO (Barinaga 1991) that may bind to or perfuse into the pre-synaptic site.

4.2. Propagated conformational changes

Propagated conformational changes along MTs have been proposed to mediate signal propagation. Propagation of tubulin conformational states are implicated in ciliary MT (Atema 1973) and by Cianci et al. (1986), who claim that MTs are capable of ATP-induced "gelation-contraction" which presumably results from all tubulin subunits assuming shorter conformational states. Melki et al. (1989) have observed tubulin polymerizing in a curve; this could result from "bending" of each tubulin molecule, a phenomenon explained by the existence of two conformational states of tubulin. Sequential shortening or bending of subunits along the MT would propagate a wave carried by conformational changes of the tubulin subunits (Hameroff and Watt 1982).

In a propagated conformational change, individual tubulin molecules would alter their conformational state in response to neighboring tubulin molecules, resulting in a signal that propagates down the MT. Energy pumping would be required to counteract energy dissipation, perhaps by GTP hydrolysis and MAP dephosphorylation. Energy pumping would not require GTP hydrolysis for every conformational change that occurs, but rather could provide a background of activity (e.g. molecular states, vibrations or other movements) that enhance conformational transitions needed for signaling.

Hameroff et al. (1989) and Rasmussen et al. (1990) considered conformational states (α, β) in each tubulin dimer characterized by a negative charge redistribution towards either the alpha or beta monomer. They developed models in which dipole coupling among neighboring tubulins occurred, and resulted in traveling patterns of tubulin conformational state changes. Specific types of patterns (e.g. "bus gliders") propagated due to tubulin conformational changes brought about by nearest-neighbor interactions. Figure 7 illustrates this work. The propagating patterns (gliders) varied in size, and could represent signals of different polarities and magnitudes. Since the patterns were discrete entities, each different pattern could stand for a different discrete magnitude being signaled. Such signals would be sufficient for neural learning paradigms, because quantized magnitudes of error signals are sufficient for learning. The velocity of pattern propagation from such MT/MAP models is predicted to be 8 nm per excitation time (10^{-9} to 10^{-11} s), yielding a velocity of 8 or more meters per second – similar to the

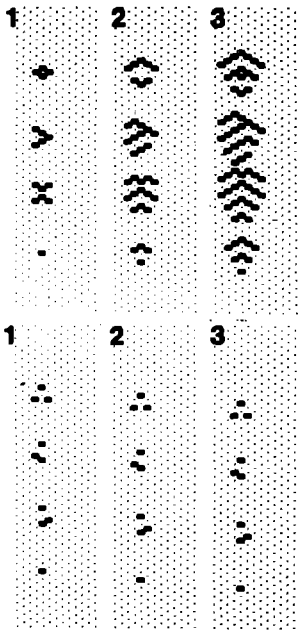

Fig. 7. MT pattern signaling, predicted by computations of MT automata models (Rasmussen et al. 1990). Black elements are tubulin dimers in the β conformation state; white elements are background α conformational states. *Top*: Three successive time frames for four object (gliders) moving downward, leaving "wakes" which result in traveling wave patterns. *Bottom*: Three successive time frames for a "dot glider" and three other gliders. Simulated with different automata threshold parameters. These gliders travel leftward without a wake. From Rasmussen et al. (1990) with permission

velocity of traveling membrane potentials. Thus cytoskeletal signals, if utilizing this mechanism, would propagate in concert with action potentials, in either direction.

Cellular automata are computational systems described by Von Neumann (1966). The idea of cellular automata models within neurons as a basis for intracellular signaling or information processing related to cognition was introduced by Conrad and coworkers (1973), who originated the term "molecular automata" for such models. Automata models require a lattice with subunits that can exist in two or more states at discrete time steps ("clocking frequency") governed by transition rules between states. Transition rules depend on neighboring subunits in the lattice. Depending on the pattern of initial states and transition rules, patterns can propagate ("gliders"), interact, compute and store information, often in exceedingly complex ways (Wolfram 1984 a, b). Von Neumann (1966) proved mathematically that cellular automata are capable of universal computation.

A model can be constructed in which tubulin has two conformational states, α and β. A signal can then be encoded as a "glider", a traveling cluster of one state on a background of the other state. For example, a positive polarity could be a signal in state β on a background of state α, and a negative polarity could be a signal in state α on a background of state β. In this case the size of the traveling cluster would represent the signal's magnitude. In an alternative mode, the signal could be a traveling cluster of (mostly) α states, on the background configuration of β, or vice versa. The percent density of α states could then represent the signal magnitude. There could be a "baseline" signal of size b so that a signal of size D is interpreted as D−b. This would allow the encoding of both negative and positive numbers by a signal that has only a magnitude. From models of learning in neural networks, it appears preferable for the internal signal to cary both a magnitude and polarity, although for many learning paradigms simply a binary signal of a magnitude alone would suffice.

Propagated conformational changes could occur on an extremely fast time scale, as proteins exhibit conformational alterations over a wide range of time scales, and very rapid (10^{-15} s) changes occur in protein side chains or local regions. Conformational transitions involving the protein globally generally occur in the nanosecond to 10 picosecond (10^{-11} s) range (Frauenfelder et al. 1988; Karplus and McCammon 1983). If tubulin were to have more than one stable conformational state, then these conformational states could store information, and if changes in state occurred then these changes could possibly process information. Many models that relate tubulin conformational states within MTs to possibilities for signaling and information processing have appeared in the literature. These models include propagating tubulin conformational changes mediating sensory transduction in ciliary MT (Atema 1973), conformational gradients among tubulin subunits (Roth and Pihlaja 1977), changes in MT-tubulin lattice symmetry (Koruga 1984), memory storage in neurofilaments (Barnett 1987) automata-behavior in MT (Hameroff et al. 1989; Rasmussen et al. 1990), and molecular computing (Lahoz-Beltra et al. 1993). Although most of these studies are theoretical or speculative, they open up a new approach towards understanding the potential impact of protein conformational changes.

Frohlich (1970, 1975, 1986) proposed that protein conformational states are coupled to charge redistributions such as dipole oscillations within specific regions of proteins. His model also predicted that proteins which have an interconnected lattice structure, are joined within a common voltage field, and are "pumped" with biochemical energy (e.g. protein phosphorylation, GTP or ATP hydrolysis) will display long-range coherent excitations in the range from 10^{-9} to 10^{-11} s. Such excitations may also be described as acousto-conformational transitions ("phonons") in the GHz to microwave frequency range. Experimental evidence for such coherent excitations includes observation of GHz-range phonon excitations in proteins (Genberg et al. 1991), sharp-resonant, nonthermal effects of microwave irradiation on living cells (Grundler and Keilman 1983), GHz induced activation of MT pinocytosis in rat brain (Neubauer et al. 1990) and long-range regularities in cytoskeletal structures, such as the super-lattice attachment pattern of MAPs on MT (Kim et al. 1986). A model that predicts MAP attachment sites is based on phonon excitations (Samsonovich et al. 1992).

Traveling waves that propagate without changing form and maintain localized shape are called "solitary waves". In special cases where they can pass through each

other without change of form and with only a slight change in phase, the solitary waves are called "solitons". Initially observed by John Scott Russell in 1934 in the movement of water waves on the surface of a canal, solitons were introduced into the biological literature by Davydov (1973) as a means of temporarily storing the dephosphated energy of ATP within a protein and conveying it to active sites such in actin-myosin muscle contraction. According to Davydov, the hydrolysis of ATP (about 0.49 eV/mol) injects energy into the protein alpha-helical chains. Due to nonlinear coupling of the energies of dispersion and stretching, stable, pulse-like waves would move within the protein (Scott 1982). Although presently unproven experimentally in proteins, DNA has ben conjectured to support solitons and solitons have been used to explain the hydrogen-deuterium exchange reaction of double-helical-polynucleotides (Nakanishi and Tsuboi 1978; Teitelbaum and Englander 1975). In this system the soliton is viewed as a configuration formed by the localized partial unwinding of the DNA helix over a few base pairs. Using model Hamiltonian parameters, Yamosa (1984) demonstrated the non-local character of this excited mode: it extends over 10 base pairs, and moves with a velocity of about 8×10^3 cm/s, a value significantly less than the velocity of sound in DNA (Hakim et al. 1984; Swenberg and Miller 1989). On a microscopic scale, however, this is a fast mode of signaling compared to material transport discussed above. In addition to alpha-helical solitons, DNA solitons, and membrane solitons, lattice solitons appropriate for cytoskeletal structures have been proposed. Sataric et al. (1992) and Manka and Ogrodnik (1992) have proposed lattice soliton models in MTs as mechanisms of signaling and information transport.

4.3. Ionic movements

Movements of ions that are clustered or trapped along MTs and filaments is within the realm of possibility. According to experimental evidence of Urry et al. (1988), ions may be held or trapped within folded proteins and along protein polymer strands. Both tubulin and actin have highly electronegative portions, a property that could enable this phenomenon. Ion transfer across a membrane may initiate ionic movements along actin strands or MTs.

Although ions could in principle flow along filaments or along or within MTs, there is a lack of experimental evidence for such movement occurring. However, a signal could be carried even if ions did not flow along the strand, because a traveling wave could be supported by motions of trapped ions. Trapping charges and forces would have to be strong enough to prevent dissipation of the ions into the surrounding fluid. Experimental evidence that can be explained by trapped ions supporting fast signals has been provided by Lin and Cantiello (1993).

4.4. Movement and tension

The actual movement of a MT is a possibility, in a direction towards one of its terminals. This movement alone could be initiated by appropriate anchoring proteins that connect the MTs to the synapse. MTs may be under tension because of the anchoring proteins; thus the cytoskeleton may be a "tensegrity net" (Joshi et al. 1985) capable of end-to-end movements or responsive to end-to-end pressure. Because of the rigidity of the tubular structure, MTs could be pulled or pushed under pressure. Lacking rigidity, filaments bend under similar pressure. Recent evidence from the laboratory of Ingber has shown that some membrane proteins (integrins) are anchored to the cytoskeleton, and show resistance to lateral movement along the membrane (Wang et al. 1993; Heideman 1993).

Bone cellular networks, which resemble networks of neurons, respond to tension by secreting calcium to build bone. The cytoskeleton could mediate the detection of tension because of the tension and rigidity in the cytoskeletal network. A model for cellular networks in bone in which cytoskeletal detection of tension results in secretion of calcium from vesicles is plausible and resembles the model for neuronal learning in which cytoskeletal movement or tension mediates the strength of release of neurotransmitter.

In an alternative form of movement, MTs "grow" by polymerizing at one end and depolymerizing at the other end. This translational mechanism has been observed at (1–3 mm/day) (Kirschner and Michison 1986). Actin filaments may also undergo assembly and disassembly that translate their positions, or cause growth or shrinkage. However, assembly at one end does not force disassembly at the other end, or vice versa, so this mechanism is not an appropriate signal carrier. However, assembly is known to play a role in the formation and growth of axonal and dendritic processes, which in turn effects the network of synapses that is eventually formed. Neurite growth occurs when actin polymerizes followed by MT assembly along the growing process. Such growth could potentially be triggered by cytoskeletal signals, and, furthermore, if cytoskeletal signals were to represent error magnitudes (as in the back-propagation model), growth of new processes would be appropriately triggered by larger error signals.

4.5. Signal initiation

In this section we propose mechanisms that may initiate signals along the cytoskeleton.

1. *Presence of a compound.* The presence of material to be transported along with motor proteins and energy source (ATP) may initiate material transport, which could then be considered a signal.

2. *Phosphorylation/dephosphorylation of* MAPs. Since MAPs are bound to MTs, and MAPs are ATPases, a phosphorylation-induced conformational change could

initiate MT signals. For example, protein kinase C (activated by membrane events, such as binding of NMDA and glutamate to receptors) phosphorylates MAP-2 in dendrites, and similar events could occur with other MAPs in axons.

3. *Calcium-initiated events.* Calcium is well-known to be released during synaptic activity, and is capable of binding to calmodulin. Since calmodulin is bound to MTs and MAPs, this coupling could in turn cause conformational or other changes in the MTs which initiate a signal up the MT. Calcium might also directly bind to MTs, initiating tubulin conformational changes.

4. *Binding.* If a specialized protein or molecule binds at the end of the MT, to a MAP, an anchoring protein, or to the MT itself, then binding could cause a conformational change that initiates a MT signal. Particular proteins might even be responsible for initiating different signal patterns. Furthermore, such a molecule might be a compound such as NO, that diffuses in a retrograde fashion across synapses, or another compound that is activated by such a retrograde messenger.

5. *Anchoring proteins.* Fodrin, anchorin, and other membrane-coupled proteins might initiate signals at the axonal end of a MT, because they are known to form bridges from the MTs to the synaptic membrane. In this case the signal might be initiated by the membrane potential, membrane proteins, second messengers, or other activity at the membrane, or by modulations in the distance between the membrane and the MT.

6. *Chemical messengers.* Other chemical messengers might interact with MTs, MAPs, or anchoring proteins to effect a signal initiation at the microtubular end. Ben Ze-ev (1991) reviews possibilities that involve a receptor bonding to an intermediary which in turn interacts with the cytoskeleton. An important chemical messenger to consider is a retrograde synaptic transmitter such as NO, that might initiate signals along cytoskeleton in the presynaptic area based on activity in the synaptic cleft or activity at the post-synaptic site.

5. Cognitive processes

Experimental evidence suggests that the cytoskeleton may be directly involved in neuronal information processing, cognition, and learning. For example, Mileusnic et al. (1980) correlated tubulin production and microtubule activities with peak learning in baby chickens. A study by Cronly-Dillon et al. (1974) on baby rats showed that at the beginning of their critical learning phase for the visual system (when the rats first open their eyes), neurons in the visual cortex begin producing vast quantities of tubulin. When the critical learning phase is over (when the rats are 35 days old), tubulin production is drastically reduced. Kudo et al. (1990) correlated the amount of reduction in MAP2 levels with the degree of cognitive impairment in gerbils exposed to cerebral ischemia (lack of brain blood flow). Bensimon and Chermat (1991) found that selective disruption of brain MTs by the drug colchicine caused cognitive defects in learning and memory which mimic the clinical symptoms of Alzheimer's disease. One of the neuronal MAPs is axon-specific "tau protein", the major constituent of the neuropathological correlate of Alzheimer's disease (Matsuyama and Jarvik 1989).

Conventional wisdom generally links cognitive functions including learning with synaptic plasticity, and evidence linking plasticity to the cytoskeleton includes the following. Lynch and Baudry (1987) have studied synaptic changes in hippocampal neurons. They have examined long-term potentiation (LTP) of synaptic efficacy in glutamate-NMDA hippocampal neurons, a correlative model of learning. They find, as does Friedrich (1990), that LTP depends on rearrangement of the subsynaptic cytoskeleton. Other studies have suggested that cytoskeletal proteins directly link to nerve membrane ion channels and receptors and that the intra-neuronal cytoskeleton is linked to nerve membrane excitability and synaptic transmission (Matsumoto and Sakai 1979; Hirokawa 1991).

Desmond and Levy (1988) have studied dendritic spine structural changes during a synaptic learning paradigm. They find mechanical shape changes in spines mediated by cytoskeletal actin connected to microtubules in dendrites. Kwak and Matus (1988) and Aoki and Siekevitz (1985) have shown that in neurons deprived of input, the cytoskeleton depolymerizes. The latter authors have also found that signaling and regulation for dendrite spine synapse function depends on phosphorylation of a cytoskeletal protein: the dendrite-specific MAP, called MAP-2.

Halpain and Greengard (1990) have shown that activation of glutamate-NMDA receptors induces rapid dephosphorylation of MAP-2. MAP-2 is responsible for the consumption of a significant portion of brain biochemical energy. MAP-2 phosphorylation and de-phosphorylation are regulated by cyclic AMP-dependent protein kinase and calcium-calmodulin protein kinase, second messenger systems activated by neurotransmitter-receptor binding. Theurkauf and Vallee (1983) have found MAP-2 to be the major substrate for endogenous cyclic AMP-dependent phosphorylation in cytosolic brain tissue, and they concluded that MAP-2 phosphorylation may be an important reaction in response to neurotransmitter stimulation. Schulman and Lou (1989) and Aszodi et al. (1991) have shown that membrane receptor initiated second messengers (e.g. Ca^{++}, cyclic AMP) converge on protein kinase A and/or Ca^{++}/calmodulin dependent protein kinases which respond by prolonged phosphorylation of intracellular proteins including MAPs, MTs and neurofilaments (Vallano et al. 1986). These authors suggest that such prolonged phosphorylation, which supplies biochemical energy, also participates in learning.

Bigot and Hunt (1990) showed that glutamate and NMDA stimulation of cultured neurons cause a redistribution of tau and MAP2. Other couplings between the cytoskeleton and membrane/synaptic function include second messenger systems such as G-proteins (Rasenick et al. 1990), calcium ion fluxes, and direct links via fodrin,

synapsin, or other proteins to ion channel mediated membrane excitability and synaptic transmission (Matsumoto and Sakai 1979; Hirokawa 1991).

In neural learning paradigms, synaptic strengths must be adjusted for learning to take place. Although there are many alternative candidate mechanisms for synaptic strength, a key model is the number of post-synaptic receptor molecules. There is experimental evidence that anchoring proteins connect to MTs and fasten the receptor molecules in place (Hirokawa 1991). Different states of these complexes might occur in response to different signals arriving via cytoskeleton. Friedrich (1990) has proposed that, in learning, sub-synaptic cytoskeletal receptor complexes become fortified and increase in complexity, a mechanism that would strengthen the synapse. In learning, sometimes the number of post-synaptic receptors would need to decrease to weaken the synapse. This might happen by degradation of anchoring proteins that connect the MTs to the synapse, as degradation might serve to release receptor molecules thereby decreasing the number of active receptors available at the post-synaptic site. Instead of degradation it is possible that a deactivation or conformational change in the anchoring proteins could bring about a decrease in the number of active receptor molecules. Receptor molecules are known to constantly "turn over"; thus non-replacement would lead to a dwindling of numbers. Degradation of fodrin has been observed experimentally (Siman et al. 1984).

6. Discussion

During the course of evolution, the cytoskeleton has developed more than one role that is critical to the functioning of nerve cells: transport of nutrients, support and formation of spatial branching structures of axons and dendrites, organization of cell division and support of membrane processes. It is common during evolution for a structure to evolve towards one purpose and then to take on other new purposes later. The cytoskeleton is positioned strategically to mediate intracellular signals during learning. Since signaling mechanisms are proposed that are experimentally observed and/or theoretically plausible, the only remaining issue is whether or how these signaling mechanisms are actually used by the cell. The result of evolution is a system that is complex enough to implement learning paradigms based on intracellular signals and evolutionary processes could result in using the cytoskeleton as a medium for carrying error signals during learning. Perhaps we have caught evolution in the act.

In this paper we have reviewed findings that impact on potential neural network learning paradigms that could utilize intracellular signaling. These findings relate to the role of the cytoskeleton and its putative capability for carrying error feedback signals during learning. The importance of this work is demonstrated by models of neuron-like components that perform simplified cognitive and pattern-recognition tasks. In these models, synaptic changes are the key to learning, and intracellular signaling could enable a broad spectrum of powerful learning rules to take place. Furthermore, experimental evidence shows that the cytoskeleton plays a role in cognition and learning. Ample evidence exists to motivate further research on intracellular signaling in the context of neural network learning.

Evaluating the biological plausibility of artificial neural network paradigms is directly relevant to developing a better understanding of how biological neural networks may behave during learning. The evaluation of back-error propagation models opens the door for a class of studies in which learning rules known to be effective in neural network models can be evaluated for biological plausibility. It is exciting that in this review there are identified effective biological mechanisms that could plausibly implement a well-established neural network paradigm.

Although we do not believe that back-error propagation is utilized biologically the same way that engineers utilize this technique, signals and processing required by back-error propagation are possible in biological systems. Since a variety of learning rules are demonstrated to be effective in artificial neural network learning paradigms, more studies of plausibility and related biological schemes are needed.

Knowledge from the combined sources of biological experimentation and experimentation with models of neural learning paradigms have underscored some basic tenets for neural systems. First, synaptic weights are of central importance, and paradigms for adapting those weights are crucial to attaining learning and performance of a neural network. Many neural learning paradigms of the artificial variety have been proposed through simulation studies, and could plausibly utilize intracellular signals if the learning rules were to occur in biological systems.

Based on the evidence in this review, it is not possible to make the case that back-error propagation specifically is used in biological systems. It is certainly true, however, that back-error propagation is biologically plausible. It would not be surprising, however, if real neural systems utilized a learning law in which an intracellular signal were responsible for modulating the amounts of weight adaptations at biological synapses.

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