20

UNCONVENTIONAL COMPUTING

As discussed in the introduction to this book, any (bio)physical mechanism that transforms some physical variable, such as the electrical potential across the membrane, in such a way that it can be mapped onto a meaningful formal mathematical operation, such as delay-and-correlate or convolution, can be treated as a computation. Traditionally only V_m, spike trains, and the firing rate $f(t)$ have been thought to play this role in the computations performed by the nervous system.

Due to the recent and widespread usage of high-resolution calcium-dependent fluorescent dyes, the concentration of free intracellular calcium $[Ca^{2+}]_i$ in presynaptic terminals, dendrites, and cell bodies has been promoted into the exalted rank of a variable that can act as a short-term memory and that can be manipulated using buffers, calcium-dependent enzymes, and diffusion in ways that can be said to instantiate specific computations.

But why stop here? Why not consider the vast number of signaling molecules that are localized to specific intra- or extracellular compartments to instantiate specific computations that can act over particular spatial and temporal time scales? And what about the peptides and hormones that are released into large areas of the brain or that circulate in the bloodstream? In this penultimate chapter, we will acquaint the reader with several examples of computations that use such unconventional means.

20.1 A Molecular Flip-Flop

The computation in question constitutes a *molecular switch* that stores a few bits of information at each of the thousands of synapses on a typical cortical cell. In order to describe its principle of operation, it will be necessary to introduce the reader to some basic concepts in biochemistry. The ability of individual synapses to potentially store analog variables is important enough that this modest intellectual investment will pay off. (For an introduction to biochemistry, consult Stryer, 1995).

20.1.1 Autophosphorylating Kinases

A group of proteins called *kinases* phosphorylate particular target proteins, that is they add a PO_4^{3-} group. The negative charge of the phosphate group changes the shape of the protein,

altering its function in very specific ways. *Phosphorylation* is a very common mechanism to modulate ionic currents and synapses (Kennedy and Marder, 1992; Hille, 1992; Kennedy, 1994). Indeed, for the most part metabotropic synaptic receptors act by causing the final target channel to be phosphorylated. Many of these kinases are activated by elevated levels of intracellular calcium.

In principle such a kinase could be used as a trigger to a 1-bit storage device. Calcium rushes into the cell, directly or indirectly activating the kinase, which in turn switches protein molecules into their phosphorylated "on" state. This "on" state is then assumed to be read out by another protein or process.

The problem with this is twofold. Firstly, another class of enzymes, the *phosphatases*, undoes the work of the kinase by snipping off the PO_4^{3-} group. This returns the protein to its "off" state. The time constant of this degrading action is on the order of minutes. Secondly, all the molecules making up biological systems (with the exception of DNA) degrade over a period of days, weeks, or, at the most, within a few months. This relentless *molecular turnover* means that each and every protein, phosphorylated or not, is eventually replaced by newly synthesized, and thereby unphosphorylated, proteins.

How can such unstable molecules be used to construct memories that can last for a lifetime? One solution is to store information in the very stable DNA molecules in the nucleus at the cell body. While it appears plausible that such storage occurs for properties that affect the entire neuron, such as the level of expression of its receptors or the overall state of its firing rate adaptation (Kandel and Schwartz, 1982), it is unclear how the DNA in the nucleus could control the synaptic strength of each and every one of the thousands of synapses in the dendritic tree of a typical neuron. This has lead to the proposal (Crick 1984b; Lisman, 1985; Saitoh and Schwartz, 1985; Miller and Kennedy, 1986) that a bistable kinase stores the information locally at each synapse. (For older ideas on the regulation of cellular growth and differentiation with the help of enzymatic networks see Monod and Jacob, 1961.)

The principle uses something called *autophosphorylation*, in which a particular kinase, here generically termed kinase-1, can phosphorylate itself in an autocatalytic reaction. The basic switch, illustrated in Fig. 20.1, is built from two proteins, kinase-1 and a phosphatase. Some neuronal stimulus, such as a brief but strong calcium transient at a dendritic spine, triggers a local kinase, kinase-2. As long as it is present, it phosphorylates kinase-1, transforming it from its inactive K_1 to its active K_1^* form. The presence of a phosphatase turns off this form, by returning it to its native K_1 state. This system can only store information as long as the original kinase-2 is present, for in its absence, all of the kinase-1 eventually ends up in its inactive K_1 form.

Things become interesting if *intermolecular autophosphorylation* occurs. Here the phosphorylated kinase-1 molecules can phosphorylate other, not yet phosphorylated kinase-1 molecules. The more K_1^* is present, the larger the rate with which K_1 is transformed into its active counterpart. Conversely, the larger the amount of K_1^*, the smaller the pool of remaining K_1 that can be phosphorylated. The kinetic equation describing this first-order reaction can be formulated as (Lisman, 1985)

$$\frac{d[K_1^*]}{dt} = c_1 \frac{[K_1][K_1^*]}{K_{d1} + [K_1]} - c_2 \frac{[P][K_1^*]}{K_{d1}^* + [K_1^*]} \tag{20.1}$$

where K_{d1} and K_{d1}^* are the Michaelis constants for the kinase-1 and the phosphatase reactions, respectively, and $[P]$ is the concentration of the phosphatase. The *Michaelis constant* of an enzymatic reaction is defined as the concentration of the substrate (here

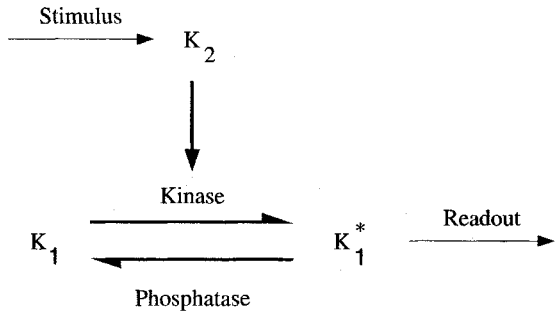

Fig. 20.1 MOLECULAR FLIP-FLOP SWITCH Basic building block of a bistable molecular switch that can retain its memory indefinitely in the face of constant protein turnover. The switch is constructed from an inactive unphosphorylated and an active phosphorylated form of a protein kinase, labeled K_1 and K_1^* respectively. The transition from the inactive to the active form of the protein can be initiated from the outside by another kinase K_2 (which, in turn, is triggered by some neuronal stimulus such as an elevation in the local calcium concentration). The critical component of the switch is the ability of the active form of kinase-1 to facilitate the phosphorylation of its own inactive form: the higher the concentration of K_1^*, the larger the reaction rate. The amount of K_1^*, in turn, controls some other process. Some phosphatase molecule is assumed to return the phosphorylated kinase-1 to its native unphosphorylated state. Reprinted in modified form by permission from Lisman (1985).

either K_1 or K_1^*) at which the reaction rate is half of its maximal value. c_1 and c_2 are the turnover numbers of the kinase-1 and phosphatase.

The first term on the right-hand side can be thought of as the product of $[K_1]$ and a forward rate constant. Due to autophosphorylation, the rate constant increases with $[K_1^*]$, but saturates at high concentrations of $[K_1]$. The backward rate constant saturates as well and is proportional to the fixed concentration $[P]$. Equation 20.1 is supplemented by the requirement that the total amount of kinase-1 in its active and inactive forms be conserved,

$$T_K = [K_1] + [K_1^*]. \tag{20.2}$$

Equation 20.1 can be expressed in normalized units ($K = [K_1^*]/T_K$ with $0 \leq K \leq 1$; $K'_{d1} = K_{d1}/T_K$ and $K^{*\prime}_{d1} = K^*_{d1}/T_K$) as

$$T_K \frac{dK}{dt} = c_1 \frac{T_K(1-K)K}{K_{d1}' + 1 - K} - c_2 \frac{[P]K}{K^{*\prime}_{d1} + K}. \tag{20.3}$$

With our choice of parameters (see legend to Fig. 20.2), the first reaction essentially does not saturate and has the shape of a parabola, while the second reaction is a strongly saturating function of K (Fig. 20.2A). The difference between the two terms is shown in Fig. 20.2B and displays, in general, three zero crossings.

The stability of Eq. 20.3 can be investigated using the methods introduced in Chap. 7, except that here everything is simpler. The system is stable if its first temporal derivative is zero and its second derivative negative. It is clear that the origin, $K = 0$, is a stable point, since any small perturbation $K = \epsilon > 0$ will lead to a negative value of dK/dt, bringing the system back to the origin. However, if the calcium stimulus that initiates the entire reaction by activating kinase-2 is present for a long enough time, it can phosphorylate enough kinase-1 into its active form to move K past the second zero crossing in Fig. 20.2B.

The system will converge to the third zero crossing and will remain there, even once the initial kinase-2 stimulus has subsided. The basis of attraction of the second stable point is large enough for it to remain stable even in the face of the ubiquitous turnover of all proteins

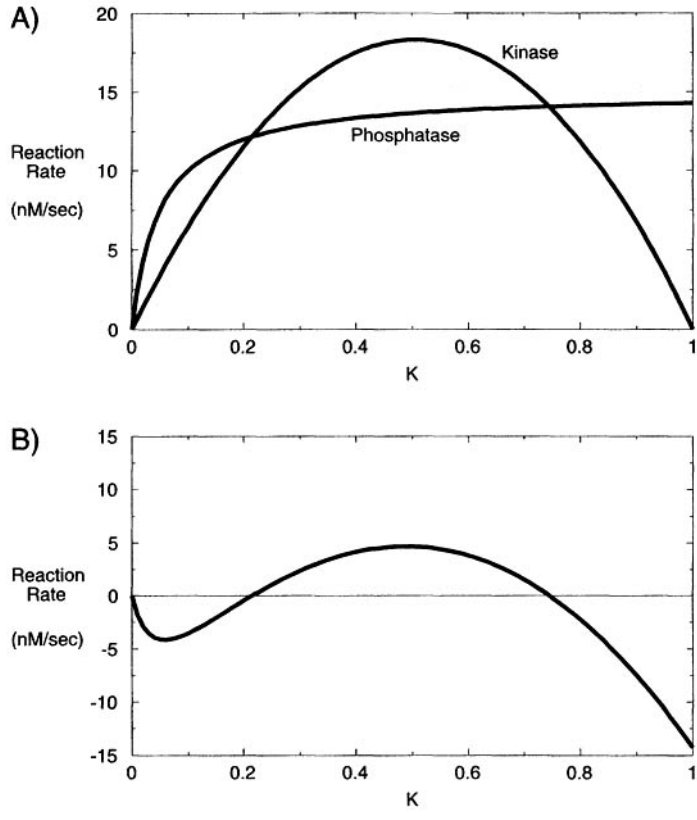

Fig. 20.2 Bistability of the Phosphorylated Form of Kinase-1 **(A)** Turnover, that is, the rate of the two reactions, as a function of the relative fraction of kinase-1 in its phosphorylated form, $K = [K_1^*]/T_K$ (Eq. 20.3 and Fig. 20.1). "Kinase" refers to the first term in Eq. 20.3, expressing the rate at which kinase-1 is moved from its inactive to its active phosphorylated form, while the inverse reaction, here assumed to saturate, is labeled "Phosphatase." **(B)** Net rate of change in the phosphorylated form of kinase-1, that is, the difference between the two curves in the upper panel. The stable points of the systems are at $K = 0$ and 0.74. That is, either no active form is present or about three-quarters of kinase-1 is in its phosphorylated form. Parameters are from Lisman (1985) with $c_1 = 30$/sec, $c_2 = 3$/sec, $K_{d1} = 1\ \mu M$, $K_{d1}^* = 2.5$ nM, $[P] = 5$ nM and $T_K = 50$ nM. Reprinted in modified form by permission from Lisman (1985).

(unless this turnover is faster than the rate of autophosphorylation). In other words, 1 bit of information can be stored in this system, making it a molecular *flip-flop*, even though all the molecules that make up the switch can themselves slowly turn over.

Nothing is free in nature. In this case the cost of maintaining the switch in its "on" position is represented by the ATP molecules supplying the energy needed to rephosphorylate those kinase molecules that are being dephosphorylated (similar to the power requirements for maintaining dynamic RAM).

This switch can be turned off by increasing the amount of phosphatase such that the loss of the phosphorylated form of kinase-1 cannot be compensated any longer by the autophosphorylation.

The important concept that has emerged from this proposal (Crick 1984b; Lisman, 1985; Miller and Kennedy, 1986) is the feasibility of long-term information storage by a molecular switch that attains its stability using a biochemical reaction with positive feedback.

20.1.2 CaM Kinase II and Synaptic Information Storage

What is the experimental status of the molecular switch idea? Work on the biochemistry and biophysics of long-term potentiation (LTP) and long-term depression (LTD; see Chap. 13) has shown that a brain protein, called Ca^{2+}/calmodulin-dependent protein kinase II (*CaM kinase II*), has many of the properties of the hypothetical kinase-1 discussed above. It has also a number of differences that make the proposal even more attractive from a computational point of view (Lisman and Goldring, 1988; Lisman, 1989; Patton, Molloy, and Kennedy, 1993; Lisman, 1994; Hanson et al., 1994).

Firstly, CaM kinase II exists in high concentrations at the *postsynaptic density* at synapses in the central nervous system (Fig. 4.1; Kennedy, 1993). Secondly, the kinase, immobilized within the postsynaptic density, is activated by the calcium-calmodulin complex (Hanson et al., 1994; Fig. 11.7). Finally, once a particular concentration of calcium has been exceeded, the molecule switches into an "on" state that retains its activity even after the removal of the initial Ca^{2+} stimulus (Miller and Kennedy, 1986). And this calcium-independent form of the phosphorylated CaM kinase II has been found at least up to an hour after the induction of LTP (Fukunaga et al., 1993).

One important difference between the hypothetical kinase-1 and CaM kinase II is that the latter works by *intramolecular autophosphorylation*. Figure 20.3A illustrates a schematic version of the structure of this enzyme, which consists of 12 subunits. Each subunit, in turn, contains three to four phosphorylation sites. Miller and Kennedy (1986) determined experimentally that calcium is only necessary for the addition of the first two to four phosphate groups onto these sites (out of a possible 30 or so sites) on a single CaM kinase II molecule. Subsequently, even if Ca^{2+} is removed, the other sites phosphorylate on their own in an autocatalytic reaction. If subunits become dephosphorylated by the action of phosphatase or if a new—unphosphorylated—subunit is inserted into the molecule as part of the general protein turnover, the remaining phosphorylated sites are more than sufficient to rephosphorylate all sites.

The fact that the autocatalytic reaction only occurs within a single molecule of CaM kinase II (that is, one molecule cannot phosphorylate a subunit in another molecule of CaM kinase II) opens the door to the possibility that analog information, rather than 1 bit of information, can be stored at an individual synapse (Lisman and Goldring, 1988; Fig. 20.3B). This assumes that the calcium transient, initiated by ongoing synaptic activity, does not saturate the postsynaptic density for so long that all CaM kinase II molecules will be in their phosphorylated states. Each calcium event will cause some molecules to switch. The more calcium, the more molecules will be switched. The amount of information that can be encoded in this manner depends on the uncertainty in the fraction of kinase molecules that is switched into their "on" state. Given the stochastic nature of molecular binding, Lisman and Goldring (1988) estimate that about 80 of these molecules could effectively store 3 to 4 bits of information at each synapse.

It is very tempting to estimate the storage capacity of the brain by simply multiplying this number by the total synaptic density of the cortex. But the implicit assumption that all synapses are independent of each other is most certainly incorrect. Furthermore, we know almost nothing about the spatial and temporal specificity of the read-out mechanism of the autophosphorylating molecular switch. Even the best RAM memory in the world serves little purpose if its states cannot be independently accessed.

The specific hypothesis we discussed, although exciting and with some experimental support, needs to be worked out in all of its messy biochemical details. What it does show, at least in principle, is that individual molecules can be used to instantiate computations using positive and negative feedback loops.

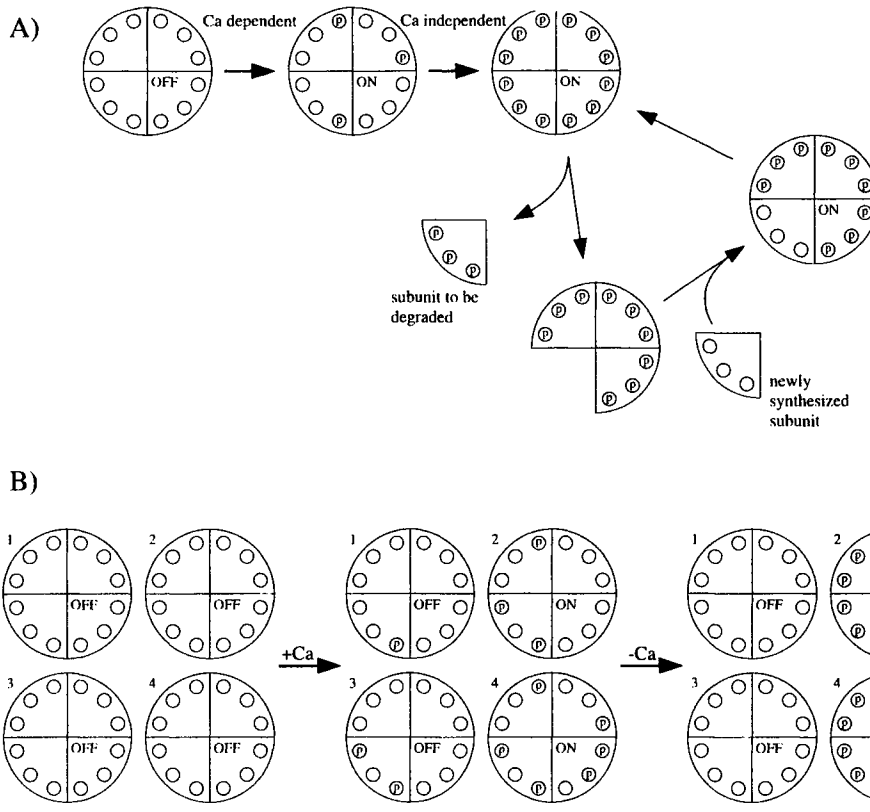

Fig. 20.3 Synaptic Information Storage via CaM Kinase II Experimental observations implicate the brain type II Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase II) as the crucial protein implementing the autocatalytic switching behavior proposed by Crick (1984b) and Lisman (1985). **(A)** One such molecule consists of a number of subunits with a total of about 30 phosphorylation sites, of which 12 are illustrated here. A rise in Ca^{2+} leads to formation of a calcium-calmodulin complex (Fig. 11.7) that induces phosphorylation of some of the sites. If a critical number of these sites (probably around three) has been phosphorylated, the molecule can itself phosphorylate the remaining sites in the absence of any further Ca^{2+}. This autocatalytic reaction also assures that the molecule remains completely phosphorylated in the face of the degrading action of phosphatase and the perpetually occurring protein turnover of individual subunits. **(B)** Because a phosphorylated molecule of this kinase cannot phosphorylate another molecule, an ensemble of molecules can encode graded information, as long as the initial calcium stimulus does not lead to phosphorylation occurring on all sites of all molecules. Due to the random nature of this process, the calcium transient causes a few sites on the four molecules to be phosphorylated (left and center). Here, it is assumed that if at least three sites per molecule are phosphorylated, the remaining sites on that molecule will be autophosphorylated, resulting in two completely activated and two inactivated molecules, even in the absence of elevated calcium (right). A longer calcium transient would have turned all four molecules on, allowing for the analog storage of information at each synapse (up to 4 bits). Reprinted in modified form by permission from Lisman and Goldring (1988).

In closing, let us recall the large number (on the order of 10^2–10^3) of regulatory proteins that can interact with calcium and other second messengers, giving rise to a complex web of dense interactions. We need to understand in what sense these molecules not only serve as metabolic intermediaries but also represent, store, and manipulate information. In principle, the computations involving V_m and $[Ca^{2+}]_i$ could be supplemented by molecular or protein computations. Their advantages are the minimal spatial requirements, usually operating at

the submicrometer scale, and the speed of the reaction, limited only by the associated chemical rate constants (Koshland, 1987; Bray, 1995; Barkai and Leibler, 1997).

20.2 Extracellular Resources and Presynaptic Inhibition

The designer of analog very large scale integrated (VLSI) electronic circuits needs to be careful when considering the spatial placement of various circuit components onto the silicon chip. For instance, the wire carrying the digital clock cannot be placed too closely to the wire carrying analog information since capacitive coupling between the two can induce sufficient noise so that the possibly very small analog variable can become corrupted. If done cleverly, the parasitic capacitance of a standard metal-oxide-semiconductor transistor can be exploited for a time-derivative computation rather than being an undesirable feature of this particular type of hardware.

We would argue that it is one of the defining characteristics of any efficient information processing system that the algorithms implemented are carefully matched to the physics of the machine. If we are willing to spend enough resources, of which there are fundamentally three—space, time, and power—we can violate such design principles, but at a price. In today's digital computers we take enormous amounts of time and power to implement functions that a house fly, with a brain volume of less than 1 mm^3, can carry out in real time.

It is reasonable to assume that evolutionary pressures will have acted on the nervous system in such a way as to optimize the placement of all circuit elements using constraints that we are only now beginning to be dimly aware of.

A case in point is the resource limitation imposed by the extracellular space (Montague, 1996). The amount of space accessible to ions outside neurons and glia cells is very small. Correcting for shrinkage during histological preparation of biological material leads to a *volume fraction* α, which is the fraction of the volume that is extracellular space. It is around 20% for most tissues (that is, $\alpha = 0.2$), with peak values of 30% for the parallel fiber system in the cerebellum (Nicholson, 1995; Syková, 1997; Barbour and Häusser, 1997). Of course, $\alpha = 1$ for an unencumbered volume (e.g., a beaker of water).

The smallness of this space could have important functional consequences, as proposed by Montague (1996). As discussed already in Sec. 4.2, the arrival of an action potential at the presynaptic terminal causes voltage-dependent calcium channels to open. The resulting influx of extracellular calcium is the crucial signal that triggers exocytosis, when the vesicle containing neurotransmitter molecules fuses with the membrane and dumps its content into the synaptic cleft (Bennett, 1997). When not enough calcium is present outside the presynaptic terminal, the probability of release, and therefore the average postsynaptic response, drops. Reducing the extracellular concentration $[Ca^{2+}]_o$ by a factor of 2, from 2 to 1 mM, reduces the postsynaptic response to 30% of its original value (Mintz, Sabatini, and Regehr, 1995). Reducing the extracellular Ca^{2+} concentration fourfold attenuates the postsynaptic response more than tenfold.

It has been estimated that on the order of 13,000 Ca^{2+} ions enter the presynaptic terminal to trigger release of a vesicle (Borst and Sakmann, 1996). Given the tight extracellular space, filled with membranes and other organelles that impede the rapid diffusion of ions, this influx of Ca^{2+} into the presynaptic terminal will deplete the calcium concentration just outside the terminal. This reduction in $[Ca^{2+}]_o$ is compounded if the postsynaptic terminal also demands calcium, for instance, if it contains significant numbers of NMDA receptors or voltage-dependent calcium channels.

When considering the diffusion of calcium, potassium, or other ions outside the cell one must account for the fact that the diffusion coefficient in the tight tortuous space between the glia cells and neurons is not the same as the diffusion coefficient in aqueous milieu. The free diffusion of ions is hindered by these membrane obstructions, by macromolecules, by charged molecules and so on. The reduction of D is accounted for by the so-called *tortuosity* factor λ (not to be confused with the electrotonic space constant). The effective diffusion coefficient D_{eff} as measured in the tissue is related to the coefficient in aqueous solution, D, via

$$D_{\text{eff}} = \frac{D}{\lambda^2}. \tag{20.4}$$

In an idealized aqueous solution $\lambda = 1$; experimentally measured values for brain tissue fall in the 1.5–1.9 range (Nicholson and Phillips, 1981; Nicholson, 1995). In other words, the effective diffusion coefficient is reduced by a factor 0.3 to 0.4 (see Eq. 11.26).

Taking both the volume fraction and tortuosity into account modifies the one-dimensional diffusion equation (Eq. 11.18) as follows:

$$\frac{\partial C(x,t)}{\partial t} = \frac{D}{\lambda^2}\frac{\partial^2 C(x,t)}{\partial x^2} + \frac{\text{sources and sinks}}{\alpha}, \tag{20.5}$$

where the sources and sinks include membrane currents, pumps, buffers, and so on.

Egelman and Montague (1997) have carried out exploratory simulations of the diffusion of Ca^{2+} ions in the extracellular space around the synaptic terminals on the basis of Eq. 20.5 and estimate that $[Ca^{2+}]_o$ can drop by as much as a quarter in response to a single action potential invading the presynaptic terminal. A high-frequency burst can further deplete extracellular calcium. Given packing densities on the order of one billion synapses per cubic millimeter of neuronal tissue (Sec. 4.1), a neighboring synaptic terminal might now have difficulties to release a synaptic vesicle successfully, since the needed Ca^{2+} ions have been "stolen" by the first synapse.

Depending on how long it will take to replenish extracellular $[Ca^{2+}]_o$ by pumps and calcium-release mechanisms, the first synapse can inhibit synaptic transmission at all closely adjacent synapses. And this irrespective of whether its postsynaptic action is excitatory or inhibitory, since this form of inhibition relies on calcium stealing by the presynaptic terminal. This is an instance of *presynaptic inhibition* in the absence of any postsynaptic conductance change.

It is well possible, of course, that the temporary reduction in $[Ca^{2+}]_o$ is too brief or too minute to significantly affect any but directly apposed synapses, or that the effect exists but that the brain does not exploit it for computational purposes. Or the reduction of $[Ca^{2+}]_o$ might only be significant during calcium spikes in the dendrites. Another interesting possibility is that synaptic microcircuits (Sec. 5.3), such as the spine-triad arrangement so prevalent in the thalamus (Sec. 12.3.4), implement a special form of presynaptic inhibition that relies on the exact spatial placement of the synaptic terminals relative to each other.

20.3 Computing with Puffs of Gas

Throughout this book, we have lived with the convenient assumption that the three-dimensional arrangements of synapses, dendrites, axons, and cell bodies do not matter and that all neurons can be reduced to sets of one-dimensional cylinders. This simplification is a powerful one since it allows us to study the spatio-temporal distribution of the membrane

potential and calcium with ease on the basis of one-dimensional cable and diffusion equations.

However, one of the most obvious features of almost any piece of nervous tissue is its high degree of structure: columns, layers, laminae, and other spatial organizational forms abound. It therefore behooves us to at least briefly consider the range of possible effects of three-dimensional geometry on neuronal computation.

As alluded to in Chap. 13, one or more retrograde messenger molecules have been postulated to mediate between the postsynaptic induction of LTP and its, at least partly, presynaptic expression. We also pointed out that the specificity of synapses during LTP and LTD might not be quite as high as frequently asserted. In the best explored model system for LTP, the synapses between the output fibers of CA3 pyramidal cells in the hippocampus and CA1 neurons, *spillover* exists. That is, synaptic plasticity is not only confined to the handful of synapses at the intersection of the single presynaptic axon and the postsynaptic cell recorded from, but is observed at "neighboring" synapses as well. LTP can be expressed at synapses from the same axon but made onto neighboring neurons that have not undergone the induction process (Bonhoeffer, Staiger, and Aertsen, 1989; Schuman and Madison 1994a). In a second form of spillover, excitatory synapses within 50–70 μm of the potentiated synapse onto the same neuron (but from nonstimulated axons) can be potentiated as well (Engert and Bonhoeffer, 1997; for a summary, see Murthy, 1997).

It has been hypothesized that these effects are mediated by a rather unusual class of neuronal messengers, of which the best known is the free radical gas *nitric oxide* (NO) (for reviews see Schuman and Madison, 1994b; Montague and Sejnowski, 1994; Schuman, 1995; Brenman and Bredt, 1997). Another possible candidate is the gas carbon monoxide (Zhuo et al., 1993).

While conventional neurotransmitters like glutamate, GABA, acetylcholine, or noradrenaline are packaged in synaptic vesicles and released from the presynaptic terminal in response to an invading action potential, nitric oxide is not released in vesicles but directly diffuses away from its site of production. Because it is gaseous and extremely membrane permeant, it readily moves *across cell membranes* irrespective of dendrites, axons, or other cellular processes. The second feature distinguishing it from a conventional neurotransmitter is that nitric oxide is "produced on demand" by a calcium-calmodulin-dependent enzyme.

Nitric oxide is limited in its spread by the fact that it is rapidly oxidized, with a half-life of 4 sec and possibly much less. Given its large diffusion coefficient (3.8 μm^2/msec; see Table 11.1) it can diffuse 160 μm in all directions in this time (Eq. 11.26), a sphere that encompasses around 20,000 synapses.

Nitric oxide has been implicated in the control of both LTP and LTD. In particular, inhibition of the enzyme responsible for producing NO, nitric oxide synthase, blocks the establishment of the NMDA-dependent form of LTP in the hippocampus. Furthermore, the correlation of presynaptic electrical activity and elevated levels of NO is sufficient to potentiate transmission at recently activated synaptic terminals (Zhuo et al., 1993).

A plausible scenario is based on conjoint pre- and postsynaptic electrical activity that causes the $[Ca^{2+}]_i$ in the spine to rise, triggering the production of NO by nitric oxide synthase (Montague et al., 1994). It immediately diffuses away from this site and into the local volume of tissue. Its sphere of influence includes its own presynaptic terminal as well as nearby synapses, those on the same postsynaptic neuron as well as those on other neurons (Fig. 20.4). At synapses that were active within some time window, for instance, where a vesicle was recently released, the NO leads to either LTP or LTD through a—as of yet—ill-characterized cascade of biochemical events (Kennedy, 1994).

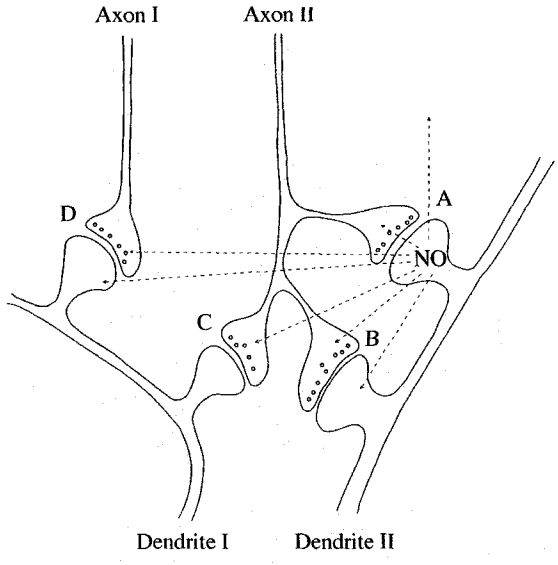

Fig. 20.4 Retrograde Messengers and Synaptic Specificity Schematic drawing illustrating the effect that the gas nitric oxide (NO) can have on synaptic weights (Gally et al., 1985). A sufficiently large calcium influx, reflecting an appropriate conjunction of pre- and postsynaptic activity, at spine A causes production of NO. Like a puff of gas, NO will freely diffuse from its site of production into the adjacent tissue volume. Presynaptic terminals that have recently been activated and that see an increase in the local concentration of NO (Eq. 20.6) upregulate their synaptic weight, leading to LTP. This *volume learning* (Gally et al., 1990) is less specific than classical associative Hebbian learning, since potentially thousands of synapses in the neighborhood of the primed one could be affected. Reprinted by permission from Gally et al., (1990).

As NO diffuses outward, its concentration drops, due to dilution, chemical degradation through oxidation to nitrates, and destruction by hemoglobin and other molecules. This imposes a limit on the volume throughout which the arrival of NO could trigger the biochemical cascade of events that eventually leads to the presynaptic expression of LTP.

Within this volume a large number of synapses could potentially change their synaptic weights, even those that lacked either pre- or postsynaptic activity to satisfy Hebb's rule (Eq. 13.7 or its variant Eq. 13.8). Experimental evidence suggests that synapses within 50 μm of a potentiated one do (Engert and Bonhoeffer, 1997).

The appropriate *volume learning* rule that needs to replace the covariance rule of Eq. 13.8 (Montague and Sejnowski, 1994) is

$$\Delta w_{kl} \propto (V_k - \langle V_k \rangle)([\mathrm{NO}]_k - \langle [\mathrm{NO}]_k \rangle) \tag{20.6}$$

where V_k is the presynaptic activity (and $\langle V_k \rangle$ its time average), $[\mathrm{NO}]_k$ the instantaneous concentration of NO (or any other retrograde messenger molecule) at the presynaptic terminal, and $\langle [\mathrm{NO}]_k \rangle$ its running average. The index l ranges over some neighborhood of the initially potentiated synapse (i, j) from which the NO diffuses. Of note is that Eq. 20.6 is independent of postsynaptic activity.

Conjoint increases in presynaptic activity and the concentration of NO (relative to their mean) cause LTP while a presynaptic increase in conjunction with a relative decrease in the concentration of the retrograde messenger (and vice versa) leads to LTD.

In other words, the unit of synaptic plasticity might not be individual synapses, as assumed by neural network learning algorithms, but groups of adjacent synapses, making for a more robust, albeit less specific learning rule. How specific depends, among other things, on the exact temporal relationship between the release of a presynaptic vesicle and the local change in $[NO]_i$. In any case, it is obvious that the detailed placement of axons and synapses in three dimensions will greatly affect their ability to locally store information.

It is important that plasticity rules, as in Eq. 20.6, be combined with realistic models of the three-dimensional configurations of axons and synapses in order to better understand developmental as well as ongoing learning processes in the brain (for an example, see Montague, Gally, and Edelman, 1991).

The picture that we are left with is one in which afferent patterns of activity are translated into local hot spots of calcium in spines and other postsynaptic terminals. These generate local puffs of gas that freely diffuse in three-dimensional space to up or down regulate synaptic weights at neighboring synapses.

20.4 Programming with Peptides

We mentioned in Chap. 4 the principle of synaptic *colocalization* of fast, classical neurotransmitters with much slower acting neuromodulators. In many synaptic terminals, neurotransmitters—small molecules such as ACh, GABA, or glutamate—are stored in small and clear vesicles while neuropeptides, short (typically 2–10) amino acid chains, are stored in *dense core vesicles* within the same terminal. The different vesicles can be released differentially, for instance, one preferentially at low stimulation frequencies and the other during high-frequency bursts. The number of neuroactive peptides in the brain, with very idiosyncratic names relating to the biological function they have historically first been identified with, totals 50 and keeps on rising. They are found throughout the animal kingdom and throughout all nervous structures (for reviews, see Kupfermann, 1991; Marder, Christie, and Kilman, 1995).

The study of their neuronal effects has progressed farthest in small nervous systems that are readily accessible to neurochemical methods. The model system of choice is the *stomatogastric ganglion* (STG) of the crab *Cancer borealis* (Selverston and Moulins, 1987). This ganglion consists of approximately 30 neurons and is responsible for controlling movement in the esophagus and stomach. The neurons are tightly coupled with both chemical and electrical synapses.

About 50 individually identifiable input fibers project from more forward located ganglia into the very dense central core of the STG. This neuroplex, consisting of nothing but axons, synapses, and dendrites, is about a quarter of a millimeter in diameter and is surrounded by the associated cell bodies (Fig. 20.5). Many of the input axons branch widely and throughout the ganglion while some have more restricted branching patterns. Some of the associated terminals resemble neurosecretory organs that are thought to release peptides into the hemolymph to act at distant sites while some resemble conventional point-to-point contacts. Colocalization of neuropeptides also fails to follow any simple rule, with any given peptide being associated with a different complement of cotransmitters in different neurons (for instance, all terminals staining immunocytochemically for peptide A may also stain for peptide B, but terminals positive for peptide B might be negative for A but positive for peptide C; Marder et al., 1997).

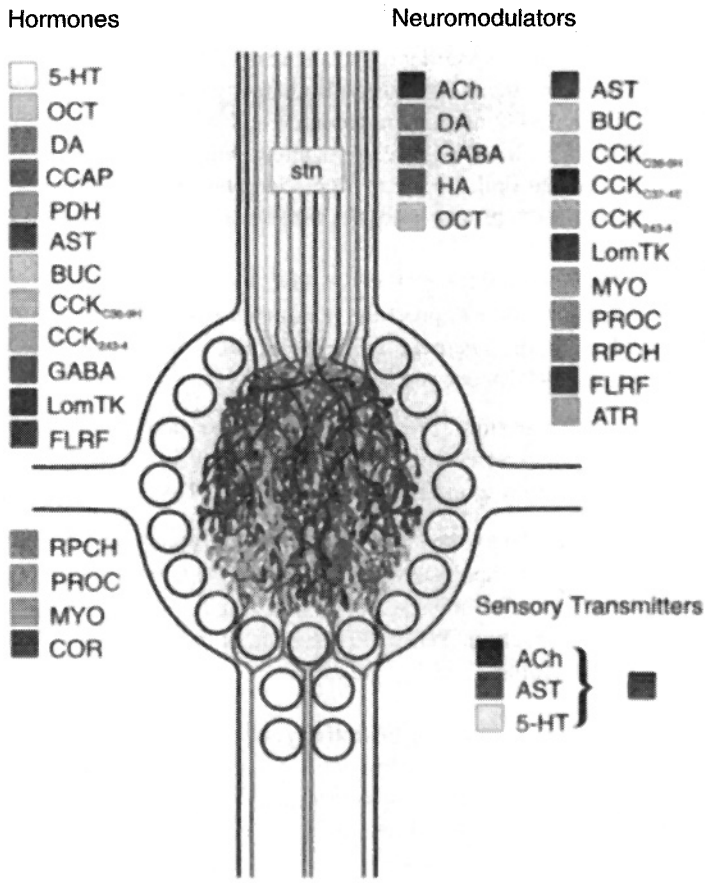

Fig. 20.5 Neuromodulators in a Small Nervous System Summary of neuroactive substances present in the inputs to the stomatogastric ganglion (STG) of the crab. *Hormones* are released into the circulating blood or hemolymph and can thus act globally, while *neuromodulators*—usually peptides—are released by conventional synaptic terminals and can modulate neuronal properties over long time scales. *Sensory transmitters* act locally and rapidly. Some substances, such as acetylcholine (ACh), bind to fast nicotinic as well as to slow muscarinic receptors. The input fibers project into the dense, central core of the ganglion, indicated schematically, with the 30 or so cell bodies (open circles) located in the periphery. The neuromodulators are present in various and complex subsets of the input fibers and are colocalized with conventional neurotransmitters. The area of influence of these peptides ranges from local synapses to the entire ganglion. As illustrated in Fig. 20.6, their effects vary widely in scope, time scale, and sign. Unpublished data from E. Marder, A. E. Christie, and M. P. Nusbaum, printed with permission.

While the distribution of neuropeptides and their postsynaptic receptors ranges from the very local to the global and defies any simple classification, their effects on their targets are equally varied.

When food moves from the mouth into the esophagus of the crab, the neurons in the STG generate a variety of different rhythmic motor patterns. Accordingly, most of the STG cells can be identified on the basis of a characteristic oscillatory pattern involving EPSPs, IPSPs, bursts, plateau potentials, and the like. Some neurons display these regular sequences in isolation, that is, when all synaptic input has been removed, while others rely on network

interactions. Such *central pattern generators* (CPG) are a common feature of vertebrate and invertebrate motor systems (Marder and Calabrese, 1996).

The action of peptides on these oscillatory discharges as well as on the 40 odd stomach muscles enervated by the STG neurons is complex and still ill-understood. We illustrate the possibilities in Fig. 20.6, involving the application of the peptide *proctolin*, released by fibers projecting into the neuropil. Applying proctolin to the entire ganglion (by adding it to the bath solution) changes the properties of the hardware at multiple organizational levels.

1. It affects specific motor patterns, here the so-called "pyloric rhythm" (Fig. 20.6A). Acting via a second-messenger cascade, proctolin modulates a voltage-dependent conductance in two identifiable neurons. The net effect is to increase the frequency and modulation depth of the oscillations (Hooper and Marder, 1987).
2. Even at very low concentrations, proctolin enhances the motor neuron evoked contractions of certain stomach muscles (Fig. 20.6B). The muscle may now be sensitive to firing rates it did not previously respond to (Marder et al., 1997).
3. Peptides can also affect the evoked amplitude of individual synaptic connections (Fig. 20.6C). Dual intracellular impalement in the IC and GM neurons in the absence of any spiking activity (by adding a sodium-channel blocking agent to the bath) fails to reveal any direct synaptic connection. Yet in the presence of proctolin a robust and profound inhibition can be observed.

A different substance, crustacean cardio-active peptide (CCAP), which is not present in any input fibers to the STG but is released into the hemolymph via neurosecretory structures, can initiate a switch from one pattern of neural activity into a qualitative different one (Weimann et al., 1997). The complex action of CCAP on various slow intrinsic membrane conductances changes the normal 1:1 alternation between two specific neurons to a 2:1, 3:1, or 4:1 alternation.

Peptides can be thought of as reprogramming the nervous system by changing its motor pattern, its synaptic gains, and its output. In an uncanny way this resembles loading a new program into an *application specific integrated circuit* (ASIC), with each peptide, or combination of different peptides, loading a slightly different motor program.

Because peptides rely on passive diffusion to influence an entire neural network, acting akin to a "global variable," the time scale of action is seconds, minutes, or longer (Jan and Jan, 1983; Kuffler and Sejnowski, 1983). An extreme form of this can be found in the mammalian *suprachiasmatic nucleus*, the central "clock" that transmits the circadian 24-hour rhythm to the rest of the brain and body. Grafting experiments have proven that circadian activity rhythms can be sustained via direct action of an as yet unidentified diffusible signal (Silver et al., 1996). The action of these modulatory signals must be viewed in constrast to the computational function of fast, synaptic input, which is quite local in time and space.

20.5 Routing Information Using Neuromodulators

We argued in Sec. 9.3 that the action of noradrenaline is contingent on excitatory synaptic input. By itself, its application causes only a small and long-lasting EPSP, which can be thought of as an epiphenomenon. Its real action is to close a potassium conductance and

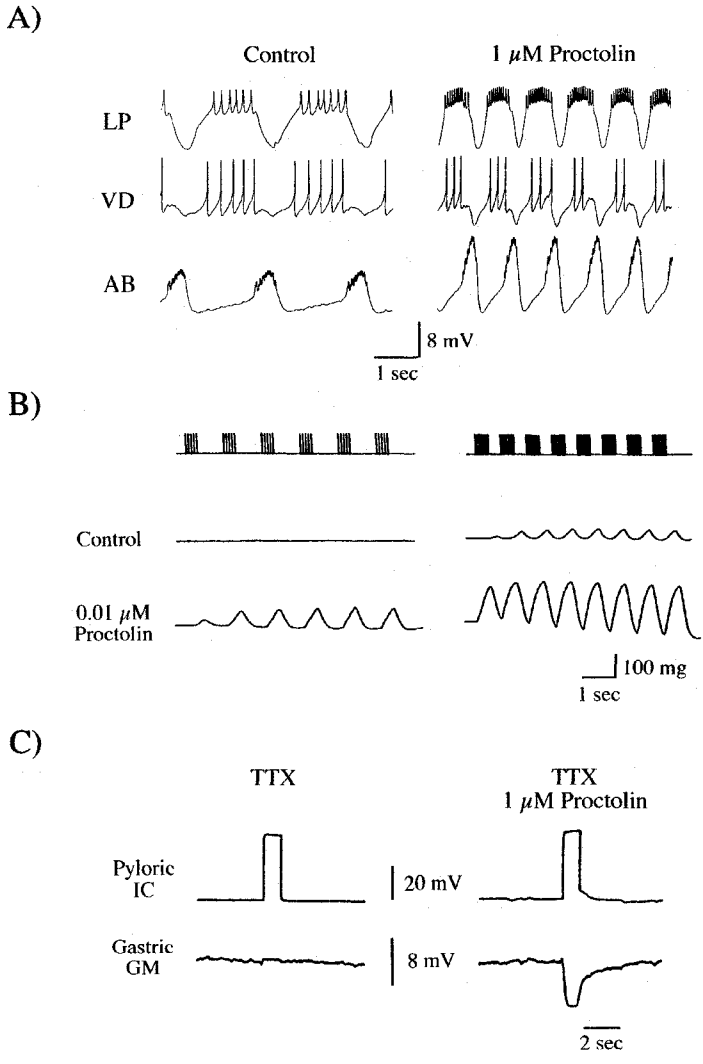

Fig. 20.6 Reprogramming a Neural Network via a Peptide Multifaceted action of proctolin, one of the many peptides that are released from afferent fibers into the stomatogastric ganglion of the lobster (PROC in Fig. 20.5). **(A)** Intracellular recordings from three identifiable STG neurons reveal a complex oscillatory behavior in their membrane potential, characteristic of central pattern generators. The effect of 1 μM of proctolin on this "pyloric" rhythm is complex; among others, it increases the frequency of oscillation in the AB neuron. Reprinted by permission from Hooper and Marder (1987). **(B)** A hundredfold lower concentration of proctolin enhances contractions of two stomach muscles controlled by STG neurons. The motor nerve was stimulated repetitively (upper row); at low stimulation frequencies (left column) no muscle contraction is apparent while a weak one can be observed at high frequencies (right column). In the presence of proctolin, these contractions are much enhanced. Unpublished data from J. C. Jorge-Rivera, printed with permission. **(C)** Proctolin also affects synaptic gain. In this experiment, all spikes are blocked by the application of TTX. A depolarizing pulse in one identifiable neuron fails to cause any change in the membrane potential of another. Adding proctolin to the bath reveals a profound and long-lasting inhibitory synaptic connection from one to the other. Unpublished data from J. M. Weimann, printed with permission.

thereby increase the gain of the cell's discharge curve (as in Figs. 9.13 and 21.3) without affecting the cell's excitability. This mechanism might enable the nervous system to send information selectively this or that way in a dynamic manner.

Efforts to build massively parallel computers have lead to the realization that a major challenge facing the computer architect is the problem of routing information efficiently among the individual processors, that is, using the least amount of time and/or space (here, transistors). The cortex and similar structures, with upward of 10^{10} to 10^{11} processors operating in parallel, most likely face a similar conundrum. How is information routed among different neurons without quickly exceeding space by connecting every neuron with every other neuron? One possible mechanism to deal with this could be based on neuromodulators (Koch and Poggio, 1987).

Let us assume that a particular input neuron projects to a large number of neurons, that is, establishes conventional synapses with them. In the absence of any modulatory input, spikes in an afferent fiber evoke synaptic activity in all of its targets. Assume that in the presence of the neuromodulator A for which only a subset of neurons M_A, has receptors, the excitability of neurons in M_A is enhanced (and possibly suppressed in other neurons). Substance A could be released either from a local interneuron—as in Fig. 20.7—or from the axon terminal of a neuron far away (e.g., in the locus coeruleus). By itself A does not induce a signal. Yet in the presence of conventional synaptic input, a neuron that is part of M_A signals more vigorously than before while neurons that are not part of M_A respond in a much weaker manner. Conceptually, this can be thought of as routing synaptic input to the subset M_A of neurons. If another population M_B has receptors for neuromodulator B, the information can be routed to a different target.

In order for such an addressing scheme to work efficiently, a large number of neuromodulators is required to target specific subsets of neurons. Addressing works by selectively and temporarily (on the order of seconds) increasing the output gain of the class of neurons that are meant to be targeted without directly exciting them. This solution to the addressing problem is similar to the traditional telephone exchange system, in which connections are made and broken as required for exchanging information (in contrast to a dedicated-line solution).

20.6 Recapitulation

We here dealt with a number of mechanisms that are not conventionally thought of as subserving specific neuronal computations. None of them involve the membrane potential, the firing rate, or the intracellular calcium concentration.

The entire realm of biochemical computations has been neglected. Yet at present there are no solid arguments ruling out why specific molecular reactions might not subserve specific computations. As one instance we introduced a molecular flip-flop switch that relies on positive feedback—via autophosphorylation—to implement a long-term memory device with the storage capacity of a few bits that could reside at individual synapses. This is but one example of a realm of computation about which we know almost nothing. Given the extremely large and complex regulatory cascades and networks of proteins and enzymes, the possibilities for nested multilevel computations are staggering. The crucial question is whether the brain avails itself of these possibilities or whether such computations cannot be implemented for reasons having to do with lack of bandwidth, signal-to-noise ratio or specificity.

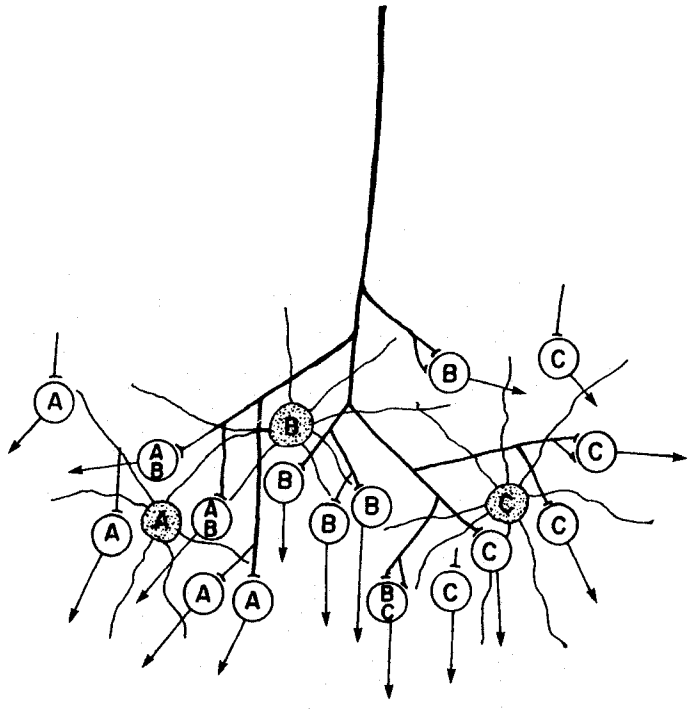

Fig. 20.7 Routing Information among Neurons Using Neuromodulators Speculative addressing scheme based on neuromodulators (Koch and Poggio, 1987). The input axon (heavy line) is presynaptic to a variety of cells that project both within and outside the system. Neurons have receptors for many neuromodulators (here only three types are indicated schematically, A, B, and C). Neuromodulators can be released by an interneuron (as shown here) or by some external neuron, diffuse throughout the ganglion, and bind to their receptor sites on a specific subset of neurons (M_A and so on). The postsynaptic effect of a neuromodulatory substance is to change the gain of the firing response of the neuron, that is, the same neuron responds more or less vigorously to the same presynaptic input as before (e.g. Fig. 20.6C). Depending on which neuromodulatory substance has been released, action potentials coming in on the axon will only activate a subset of neurons. Functionally, this enables information to be selectively routed to neurons within the ganglion. Reprinted by permission from Koch and Poggio (1987).

Two other candidate mechanisms rely on the precise three-dimensional arrangements of neuronal components, either at the subcellular or at the cellular level. Whether or not the short-term depletion of extracellular Ca^{2+} ions implements a universal presynaptic inhibition that works without any conductance changes is pure speculation at the moment, but is too important to neglect from an experimental point of view.

That puffs of nitric oxide (and possibly carbon monoxide) are released in nervous tissue following local hot spots of synaptic-induced calcium activity opens up new avenues of spreading information in a retrograde manner, back across the synapse. Given the inexorable square-root law of diffusion and the aggressive chemical nature of nitric oxide, NO is unlikely to be effective beyond a small fraction of a millimeter from the site of its synthesis. This sphere of influence does include a potentially very large number of synapses. One of the "unfortunate" consequences of such a diffusing substance is that the specificity of Hebb's synaptic plasticity rule would be significantly reduced. The unit of learning would not be individual synapses, but groups of adjacent synapses.

The last two mechanisms exploit the very large laundry list of neuroactive substances (biogenic amines, neuropeptides, hormones) known to be present in any nervous system to implement global variables that act over a fraction of a millimeter and longer distances and on a seconds to minutes and longer time scale. We speculated on the role of neuromodulators in routing information, for reprogramming a particular neural network to change its mode of operation, for adapting the retina or other sensory surfaces to different operating conditions, and the like.