A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory

(calcium/calmodulin-dependent protein kinase II/protein phosphatase 1/adenylyl cyclase/calciurein)

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ABSTRACT In a previous paper, a model was presented showing how the group of synaptic knobs within a postsynaptic density could stably store a graded synaptic weight. This paper completes the model by showing how bidirectional control of synaptic weight could be achieved. It is proposed that the quantitative level of the activity-dependent rise in postsynaptic Ca$^{2+}$ determines whether the synaptic weight will increase or decrease. It is further proposed that reduction of synaptic weight is governed by protein phosphatase 1, an enzyme indirectly controlled by Ca$^{2+}$ through reactions involving phosphatase inhibitor 1, cAMP-dependent protein kinase, calciurein, and adenylyl cyclase. Modeling of this biochemical system shows that it can function as an analog computer that can store a synaptic weight and modify it in accord with the Hebb and anti-Hebb learning rules.

In many types of neural network models, learning occurs by the bidirectional modification of synaptic weights according to simple activity-dependent rules that resemble the Hebb and anti-Hebb rules described below. Such networks can store multiple memories (1), develop selectivity for input patterns (2), find optimum solutions (3), and organize topographic maps (4). These theoretical results suggest that understanding how synaptic weights are stored and modified is important for understanding brain function in general, and learning and memory in particular.

The best experimental evidence for activity-dependent changes in synaptic weight comes from work in the hippocampus (5) demonstrating that high-frequency stimulation of a group of excitatory synaptic inputs leads to a long-term potentiation (LTP) of the synaptic efficacy of the stimulated synapses. Analysis (6) of LTP in the CA1 region of the hippocampus has shown that the process is governed by the Hebb rule (7); the synaptic weight of a synapse increases when there is both presynaptic activity at that synapse and a critical level of postsynaptic depolarization. Induction of LTP involves the N-methyl-D-aspartate (NMDA) class of glutamate-activated channels in the postsynaptic membrane (8). These channels open only if there is both presynaptic release of glutamate and substantial postsynaptic depolarization (9, 10). Opening of the NMDA channel leads to an influx of Ca$^{2+}$ (11, 12) that triggers the increase in synaptic weight (13, 14). The final expression of the increase in synaptic weight is enhancement of the synaptic current carried by the non-NMDA class of glutamate-activated channels (15).

Fundamental questions about how synaptic weights are stored and modified remain unanswered. Theoretical considerations suggest that there must be processes that decrease synaptic weight (16) when presynaptic and postsynaptic activity do not occur together. Such processes have in fact been observed experimentally (see below) and have been labeled "anti-Hebb" processes (17), but almost nothing is known about their mechanism. Furthermore, the central problem of memory storage—how synaptic weights are stored—remains mysterious, though work on related questions in invertebrates has made progress (18).

In recent theoretical papers (19, 20), Marc Goldring and I explored the possibility that the synaptic weight of each synapse could be stored locally by the group of Ca$^{2+}$/calmodulin kinase II (CaM-kinase II) molecules contained in the postsynaptic density (21, 22), a cytoplasmic structure that directly abuts the postsynaptic receptors within each dendritic spine (23). In vitro work on this kinase (24, 25) has demonstrated that Ca$^{2+}$ stimulates this enzyme to phosphorylate other substrates and itself (autophosphorylation). After Ca$^{2+}$ stimulates autophosphorylation of 2 or 3 of the Ca$^{2+}$ phosphorylation sites on this enzyme, the enzyme switches to an "on" state in which it phosphorylates itself and other substrates, even after Ca$^{2+}$ is removed. Our calculations showed that, in this on state, the Ca$^{2+}$-independent autophosphorylation could enable kinase molecules to resist the resetting effects of phosphatase and protein turnover and that individual molecules could therefore retain information with the stability required for long-term memory. We further showed that the group of CaM-kinase II molecules in the postsynaptic density could encode gradations in synaptic weight through gradations in the fraction of enzymes switched. According to this model, the large Ca$^{2+}$ influx that occurs under Hebb conditions increases the synaptic weight by increasing the fraction of CaM-kinase II molecules in the on state. The model did not, however, address the question of how synaptic weights decrease under anti-Hebb conditions. The purpose of this paper is to extend the previous model to show how bidirectional control of synaptic weight might be achieved.

Physiological Evidence for Anti-Hebb Processes

The Hebb process increases synaptic weight when presynaptic and postsynaptic activity occur together. Anti-Hebb processes decrease synaptic weight when presynaptic and postsynaptic activity do not occur together. It is useful to distinguish between two types of such processes. One type weakens a synapse when the presynaptic input is inactive but the postsynaptic cell is active because of other inputs. I will refer to this as a post-not-pre anti-Hebb process. The second type weakens a synapse when the presynaptic input is active but the postsynaptic cell is not active because of inadequate excitation by other inputs or too much inhibition by other inputs. I will refer to this as a pre-not-post anti-Hebb process.

Evidence for a post-not-pre anti-Hebb process has been obtained in the dentate gyrus of the hippocampus (26). Cells there receive inputs from both the ipsilateral and contra-
eral entorhinal cortex. Strong activation of one set of inputs depolarizes the postsynaptic cell and produces a long-lasting decrease in the synaptic weight of the inactive inputs.

Indirect evidence for a pre-not-post anti-Hebb process has been obtained in experiments that perturb the normal binocular innervation of cells in the visual cortex (27). If one eye is deprived of visual experience during the critical period of development, cortical cells subsequently respond preferentially to the nondeprived eye. If, however, the postsynaptic cells are prevented from firing by long-term application of inhibitory transmitter agonist, the cells subsequently respond preferentially to the deprived eye. This is the expected outcome of a pre-not-post anti-Hebb process; active inputs from the non-deprived eye are weakened because there is never simultaneous postsynaptic activity. In contrast, inactive inputs from the deprived eye are not weakened. Recent work demonstrates that the pre-not-post process is also present in the hippocampus and can be studied at the cellular level (28).

Ca$^{2+}$ Levels During Hebb and Anti-Hebb Processes

As mentioned above, entry of Ca$^{2+}$ is thought to trigger the increase in synaptic weight that occurs under Hebb conditions. To develop a model for how synaptic weights are decreased, we need to consider which second messenger might initiate this process. Under post-not-pre anti-Hebb conditions, what is required is a mechanism by which the critical level of net excitation produced by active synapses triggers a biochemical process that reduces the synaptic weight of inactive excitatory synapses. Clearly, depolarization can spread from active to inactive synapses, but how does this depolarization produce a second-messenger change? Voltage-dependent Ca$^{2+}$ channels are the only known mechanism that can convert depolarization into a large second-messenger change. I have thus explored models in which Ca$^{2+}$ initiates the anti-Hebb process in addition to initiating the Hebb process. As argued in the next paragraph, the rise in Ca$^{2+}$ will be smaller under anti-Hebb conditions than under Hebb conditions. The magnitude of the rise in Ca$^{2+}$ could therefore be used to determine whether synaptic weights increase or decrease.

The pathways affecting the local concentration of postsynaptic Ca$^{2+}$ at an active or inactive synapses are outlined in the left part of Fig. 1. Under pre-not-post anti-Hebb conditions, a moderate rise in Ca$^{2+}$ will be produced due to Ca$^{2+}$ entry through non-NMDA channels (12), due to a partial activation of NMDA channels at resting potential (not shown in Fig. 1) and to glutamate-stimulated Ca$^{2+}$ release from internal stores (29). Similarly, under post-not-pre anti-Hebb conditions, a moderate rise in Ca$^{2+}$ will occur due to Ca$^{2+}$ entry through the voltage-dependent Ca$^{2+}$ channels known to exist in dendritic membranes (30, 31). Under Hebb conditions, there is both presynaptic and postsynaptic activity, and all the Ca$^{2+}$ entry mechanisms will contribute. Thus, because of simple summation, the rise in Ca$^{2+}$ should be higher than under anti-Hebb conditions. In addition, the NMDA channel specifically opens under Hebb conditions and allows a further large influx of Ca$^{2+}$.

Regulation of Protein Phosphatase 1

We now need to consider how different levels of Ca$^{2+}$ might be detected and how the moderate elevation of Ca$^{2+}$ that occurs under anti-Hebb conditions could lead to dephosphorylation of CaM-kinase II, whereas the high elevation of Ca$^{2+}$ that occurs under Hebb conditions could lead to phosphorylation of CaM-kinase II. The dephosphorylation of CaM-kinase II required to reduce synaptic weight must result from the activation of a phosphatase. Cells contain a variety of phosphatases (32), but only protein phosphatase 1 and protein phosphatase 2a are able to dephosphorylate CaM-kinase II (25, 33). Since only phosphatase 1 is associated with the postsynaptic density (33) and only phosphatase 1 is a regulated enzyme, this phosphatase is likely to be more important in the process of decreasing synaptic weights than protein phosphatase 2a.

Fig. 1 summarizes what is known about the complex regulation of phosphatase 1. The enzyme is inhibited when it binds to the phosphorylated form of a small protein, protein

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**Fig. 1.** Biochemical mechanism for bidirectional control of synaptic weight by Hebb and anti-Hebb processes. CaM-K, CaM-kinase II; PDE, phosphodiesterase.
phosphatase inhibitor 1 (34). Two other proteins that inhibit phosphatase 1, DARPP-32 and protein phosphatase inhibitor 2, are not considered here because of their low concentration in the hippocampus (35, 36). Because inhibitor 1 is phosphorylated by cAMP-dependent protein kinase and dephosphorylated by the Ca\textsuperscript{2+}/CaM-dependent protein phosphatase 2b (calcineurin) (37), phosphatase 1 is indirectly activated by Ca\textsuperscript{2+} and inhibited by cAMP (32). The biochemistry is further complicated by the fact that the cAMP concentration itself depends on Ca\textsuperscript{2+}. Adenylate cyclase, the enzyme that synthesizes cAMP, is activated by Ca\textsuperscript{2+}/CaM (38–40) (and inhibited again at very high Ca\textsuperscript{2+}). Phosphodiesterase, the enzyme that hydrolyzes cAMP, is also activated by Ca\textsuperscript{2+}/CaM, but activation occurs at a higher Ca\textsuperscript{2+} concentration than is required for the activation of adenylate cyclase (36). These enzymes are known to occur in the forebrain and most have been directly demonstrated to exist in the hippocampus. This complex biochemical circuit has been discussed elsewhere (41), but its role is unclear.

I have attempted to model these reactions with the goal of asking whether they could provide a means of detecting different Ca\textsuperscript{2+} levels and producing the required phosphorylation and dephosphorylation of CaM-kinase II. It might at first be thought that this system would not be suitable for detecting different levels of Ca\textsuperscript{2+} since all the Ca\textsuperscript{2+} detection is done by the same protein, CaM. It has been shown, however, that the activation of different CaM-dependent enzymes can occur at different Ca\textsuperscript{2+} levels because of differences in the interaction of Ca\textsuperscript{2+}/CaM with the enzymes (42). An exact comparison of the Ca\textsuperscript{2+} activation of the CaM-dependent enzymes cannot be made on the basis of the available data because of differences in experimental conditions as well as in the methods used to calibrate Ca\textsuperscript{2+} concentrations. A further difficulty is the existence of large spatial gradients of Ca\textsuperscript{2+} within dendritic spines (43). The activation of different enzymes by Ca\textsuperscript{2+} will therefore also depend on their position within the gradient. Because detailed information about these biochemical parameters is lacking, the question I have asked is whether the system of reactions shown in Fig. 1 can produce bidirectional control of synaptic weight if plausible parameters are chosen. As discussed in the next paragraph, the answer to this question is yes. The most critical assumption made is that, as Ca\textsuperscript{2+} is elevated, calcineurin is activated before adenylate cyclase; either because calcineurin has a higher apparent affinity for Ca\textsuperscript{2+} or because it is closer to the source of Ca\textsuperscript{2+}.

Fig. 2 shows how the level of Ca\textsuperscript{2+} affects the net phosphorylation rate of CaM-kinase II according to the steady-state model described in the Appendix. For the purpose of understanding how this system works, let us assume that, under anti-Hebb conditions, the Ca\textsuperscript{2+} concentration rises from a resting value of 0.03 \( \mu \)M to a level of about 0.1 \( \mu \)M, whereas under Hebb conditions, the Ca\textsuperscript{2+} concentration rises to close to 1 \( \mu \)M. The moderate rise in Ca\textsuperscript{2+} that occurs under anti-Hebb conditions activates calcineurin, leading to dephosphorylation of inhibitor 1. The consequent activation of phosphatase 1 is so large that the protein phosphatase 1 activity is greater than the Ca\textsuperscript{2+}-independent autophosphorylation rate of CaM-kinase II molecules that previously have been switched on. These molecules will undergo net dephosphorylation (stippled region in Fig. 2 Upper) and some or all of them will switch off, thereby producing a decrease in synaptic weight.

The higher elevations of Ca\textsuperscript{2+} that occur under Hebb conditions will increase the number of on CaM-kinase II molecules by producing a net phosphorylation of off CaM-kinase II molecules, as shown by the stippled region in Fig. 2 Lower. High Ca\textsuperscript{2+} activates the Ca\textsuperscript{2+}-dependent autophosphorylation of off CaM-kinase II molecules and, importantly, prevents activation of protein phosphatase 1 activity (i.e., phosphatase 1 activity is a bell-shaped function of Ca\textsuperscript{2+}). Inhibition of phosphatase 1 occurs at high levels of Ca\textsuperscript{2+} because adenylate cyclase becomes activated; the resulting rise in cAMP and stimulation of cAMP-dependent protein kinase vetoes the action of calcineurin on inhibitor 1. Because there is net phosphorylation of off CaM-kinase II molecules, some or all of these will turn on, and the synaptic weight will increase. Fig. 2 thus demonstrates that the biochemical scheme shown in Fig. 1 is capable of bidirectional control of synaptic weights.

**Discussion**

In a previous paper (19), a model was developed for how CaM-kinase II molecules could store a synaptic weight with the stability required for long-term memory. This model is under active investigation, but its validity remains unclear; there is some indirect evidence both for and against it (44, 45, 56). This paper extends the previous model by showing how synaptic weights stored by CaM-kinase II could be bidirectionally modified by physiological activity (Fig. 2), as required to implement the Hebb and anti-Hebb learning rules.

A key aspect of the model shown in Fig. 1 is that elevation of Ca\textsuperscript{2+} triggers both upward and downward modifications of synaptic weight; the magnitude of the elevation determines
whether weights will increase or decrease. Work on growth cones has led to an hypothesis (46) similar to that proposed here in which very different processes are set in motion by quantitatively different levels of Ca\(^{2+}\). One prediction of the model outlined in Fig. 1 is that it should be possible to change synaptic weights either upwards or downwards by appropriate manipulation of postsynaptic Ca\(^{2+}\). Recent work using a photoactivatable Ca\(^{2+}\) source suggests that weights can be increased by elevation of Ca\(^{2+}\) (14). Whether smaller elevations of Ca\(^{2+}\) cause a decrease in synaptic weight was not tested, nor has it been determined whether decreases in synaptic weight can be blocked by addition of intracellular Ca\(^{2+}\) buffer, as would be predicted by the model.

Another key aspect of the model is the postulate that phosphatase 1 and its associated control mechanisms (Fig. 2) are involved in the modification of synaptic weights. There are no data bearing directly on this question. However, according to the model, increasing cAMP should promote the process of increasing synaptic weight, and there are some data consistent with this prediction. When cAMP is injected directly into postsynaptic cells of the CA3 region of the hippocampus (48), induction of LTP is facilitated. Whether cAMP acts similarly in the CA1 region has not been determined. Other work (48) in CA3 indicates that norepinephrine, acting through cAMP, can slowly increase synaptic weight (presumably without a rise in Ca\(^{2+}\)). The model presented here could account for this if the elevation of cAMP was sufficient to reduce the phosphatase 1 activity to a level lower than CaM-kinase activity at resting Ca\(^{2+}\). The resulting net phosphorylation would lead to a slow increase in synaptic weight. Similar arguments could be used to explain why the Drosophila mutations that affect long-term cyclic nucleotide metabolism disrupt learning (49, 50).

Several additional features of the model are worth pointing out. Fig. 1 shows how both types of anti-Hebb rules could be implemented at the same synapse, but both rules need not always be operative. Which rules are operative will strongly affect the properties of a neuron in a network (51). Consider a cell that has several groups of inputs and assume that a group of inputs must be active together to make the cell fire. If the post-not-pre anti-Hebb rule is operative, the firing of any one group weakens all others; i.e., there is competition between groups and the cell will eventually come to represent the most active group. If only the pre-not-post anti-Hebb rule is operative, there is no competition between groups and the cell fires whenever any group is active. In this connection, it is interesting that a single change, the removal or attenuation of dendritic voltage-dependent Ca\(^{2+}\) channels, would selectively eliminate the post-not-pre anti-Hebb process.

Another interesting aspect of Fig. 1 is that CaM is required for both upward and downward modifications of synaptic weight. However, CaM is not required for CaM-kinase II molecules to express their Ca\(^{2+}\)-independent catalytic activity, and it is this activity that presumably controls synaptic efficacy. Thus, in the absence of CaM, the synapses would be in a "read only" mode. Recent work (52) indicates that presynaptic terminals contain a CaM-sequestering molecule (GAP43), which releases CaM when phosphorylated by protein kinase C (53). It would be of great interest if a related mechanism for binding CaM were found in postsynaptic sites.

It is now well established that phosphatases and kinases can be highly regulated by second messengers, that electrical activity can influence second messenger concentrations, and that different second messenger systems can interact with each other. It has generally been unclear, however, what function this bewildering array of biochemical processes perform. In this paper, I have started with a well-defined function and explored how this function might be accomplished through the interaction of known biochemical processes. The resulting model (Fig. 1) shows how a large number of reactions can interact to perform an integrated function. The ensemble of reactions can be viewed as a biochemical analog computer in which different reactions detect second messengers, perform logical operations, store information, and modify the stored information.

Appendix

The curves in Fig. 2 show how the net phosphorylation rate of CaM-kinase II depends on Ca\(^{2+}\). These were calculated as follows: on CaM-kinase II molecules have a Ca\(^{2+}\)-independent kinase activity, \(k_{\text{max}}\). The net phosphorylation rate for on enzymes is given by

\[ k_{\text{max}} - P, \]

where protein phosphatase 1 activity, \(P\), is as given by the last equation in the Appendix. A value of \(k_{\text{max}} = 0.5\) was used, indicating that the maximal kinase rate was 50% that of the maximal phosphatase 1 rate. Net phosphorylation of off enzymes is given by

\[ k_{\text{max}} \cdot Ca^4/(Ca^4 + 0.74) - P, \]

where the half-maximal activation (\(k_{1/2}\)) of the kinase by Ca\(^{2+}\) is 0.7 \(\mu\)M. All CaM-dependent enzymes were taken to depend on the fourth power of Ca\(^{2+}\) (54, 55). For mathematical simplicity, sites have been assumed to be phosphorylated serially.

To calculate the rate of the phosphatase 1 reaction, it is necessary to calculate the effect of Ca\(^{2+}\) on cAMP levels. The rate of the adenylyl cyclase reaction, \(C\), is enhanced by Ca\(^{2+}\)/CaM (\(k_{1/2} = 0.4\) \(\mu\)M) and then decreased at somewhat higher Ca\(^{2+}\) concentration (\(k_{1/2} = 0.8\) \(\mu\)M):

\[ C = 0.8^d/(Ca^4 + 0.8^d) \cdot Ca^4/(Ca^4 + 0.4^d) + C_0. \]

The basal cyclase activity, \(C_0\), was taken to be 0.01 \(\mu\)mol/sec. The rate constant for phosphodiesterase, \(D\), is activated by Ca\(^{2+}\)/CaM (\(k_{1/2} = 1.0\) \(\mu\)M):

\[ D = Ca^4/(Ca^4 + 1^d) + D_0. \]

The basal phosphodiesterase rate constant, \(D_0\), was taken to be 0.1.

In the steady state, the rate of cAMP synthesis equals the rate of degradation. Thus, the dependence of cAMP on Ca\(^{2+}\) can be calculated as follows:

\[ C = cAMP \cdot D, \]

which can be rearranged to give

\[ cAMP = C/D. \]

The value 1.5 is an adjustable constant that signifies the maximum phosphorylation rate of inhibitor 1 by cAMP kinase relative to the maximum dephosphorylation by calcineurin.

Calcineurin is activated by Ca\(^{2+}\)/CaM (\(k_{1/2} = 0.1\) \(\mu\)M). The rate constant of this enzyme, \(B\), is given by

\[ B = Ca^4/(Ca^4 + 0.1^d). \]

In the steady state, the rate at which inhibitor 1 is phosphorylated equals the rate at which it is dephosphorylated. Thus,

\[ B \cdot I_P = A \cdot (I_t - I_P) \text{ and } I_P = I_t \cdot A/(A + B), \]
where \( I_r = 0.01 \mu M \) is the total concentration of inhibitor 1, and \( I_p \) is the concentration of phosphorylated inhibitor 1. The fraction, \( f \), of phosphatase 1 that is inactivated because of binding to phosphorylated inhibitor 1 is given by

\[ f = I_p / (I_p + 0.001) \]

where 0.001 \( \mu M \) is the concentration of inhibitor 1 that gives half-maximal inhibition. The rate constant of phosphatase 1 (\( P \)) is proportional to the free phosphatase 1 concentration

\[ P = 1 - f \]

Phosphatase 1 activity for any given value of Ca\(^{2+} \) can be calculated by starting with the third equation and working down.

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