

November 1998

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Louis D. Matzel

Rutgers University - New Brunswick/Piscataway

Andrew C. Talk

Rutgers University - New Brunswick/Piscataway

Isabel A. Muzzio

University of Pennsylvania, imimuzzio@sas.upenn.edu

Ronald F. Rogers

San Jose State University, ronald.rogers@sjsu.edu

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Recommended Citation

Louis D. Matzel, Andrew C. Talk, Isabel A. Muzzio, and Ronald F. Rogers. "Ubiquitous molecular substrates for associative learning and activity-dependent neuronal facilitation." *Reviews in Neuroscience* (1998): 129-167.

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Ubiquitous Molecular Substrates for Associative Learning and Activity-Dependent Neuronal Facilitation

Louis D. Matzel, Andrew C. Talk, Isabel A. Muzzio, and Ronald F. Rogers¹

*Department of Psychology, Program in Biopsychology and Behavioral Neuroscience
Rutgers University, New Brunswick NJ 08854-8020*

¹*Department of Psychology, Program in Neural Science
Indiana University, Bloomington Indiana 47405, USA*

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Reprint address:

Louis D. Matzel

Department of Psychology

Program in Biopsychology and Behavioral Neuroscience

Rutgers University, Busch Campus

Piscataway, NJ 08854-8020, USA

SYNOPSIS

Recent evidence suggests that many of the molecular cascades and substrates that contribute to learning-related forms of neuronal plasticity may be conserved across ostensibly disparate model systems. Notably, the facilitation of neuronal excitability and synaptic transmission that contribute to associative learning in *Aplysia* and *Hermissenda*, as well as associative LTP in hippocampal CA1 cells, all require (or are enhanced by) the convergence of a transient elevation in intracellular Ca^{2+} with transmitter binding to metabotropic cell-surface receptors. This temporal convergence of Ca^{2+} and G-protein-stimulated second-messenger cascades synergistically stimulates several classes of serine/threonine protein kinases, which in turn modulate receptor function or cell excitability through the phosphorylation of ion channels. We present a summary of the biophysical and molecular constituents of neuronal and synaptic facilitation in each of these three model systems. Although specific components of the underlying molecular cascades differ across these three systems, fundamental aspects of these cascades are widely conserved, leading to the conclusion that the conceptual semblance of these superficially disparate systems is far greater than is generally acknowledged. We suggest that the elucidation of mechanistic similarities between different systems will ultimately fulfill the goal of

the model systems approach, that is, the description of critical and ubiquitous features of neuronal and synaptic events that contribute to memory induction.

KEY WORDS

activity-dependent facilitation, calcium, protein kinases, metabotropic receptors, phospholipases, ionotropic receptors, *Aplysia*, *Hermissenda*, long-term potentiation, memory

1. INTRODUCTION

Prior to 1965, empirical insights pertaining to the mechanisms by which brain cells might encode new memories were infrequent. Progress was slowed in part by the complexity and technical difficulties associated with studies of the mammalian brain. In 1965, however, a pair of seminal papers by Eric Kandel and Ladisav Tauc /137,138/ appeared that addressed the neurophysiological basis for a learned response in the marine mollusc *Aplysia*, the relatively "simple" nervous system of which is comprised of identifiable cells. In this early work, Kandel and Tauc reported that synaptic facilitation of a weak pathway developed as a consequence of repeated stimulation of that pathway concomitant with the stimulation of a stronger afferent input. Furthermore, Kandel and Tauc reported that this "heterosynaptic facilitation" was mediated by an increase in presynaptic transmitter release. Not only did these early papers provide a conceptual basis for understanding brain mechanisms of memory, but perhaps more importantly, they served as the impetus for a major redirection of effort in the field of neuroscience. Since the original description of their results, literally thousands of papers have helped to delineate the mechanisms by which this and other forms of synaptic facilitation occur in *Aplysia* as well as in other "simple" systems. Through this body of work, it has become more fully appreciated that mechanisms of memory storage might be discernible if simple forms of learning were studied in species with tractable nervous systems. Moreover, this paradigm foreshadowed similar approaches which utilize reduced preparations of mammalian brains.

Abbreviations: AA (arachidonic acid); ACPD (aminocyclopentane-1S,3R-dicarboxylic acid); AlF_4^- (aluminum/fluoride complex); AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate); ATP (adenosine 5'-triphosphate); CaMK (calcium/calmodulin-dependent protein kinase); CS (conditioned stimulus); DAG (diacylglycerol); EPSP (excitatory postsynaptic potential); ER (endoplasmic reticulum); GABA (γ -amino-*n*-butyric acid); G-protein (guanosine 5'-triphosphate-binding protein); GDP (trilithium salt of guanosine); GTP (tetralithium salt of guanosine); I (ion current); IP_3 (inositol trisphosphate); LTP (long-term potentiation); mGluR (metabotropic glutamate receptor); NMDA (N-methyl-D-aspartate); PLC (phospholipase C); PLA_2 (phospholipase A_2); PIP_2 (phosphatidylinositol-4,5-bisphosphate); PKA (cyclic AMP-dependent kinase); PKC (protein kinase C); TEA (tetraethylammonium chloride); UFA (cis unsaturated fatty acid); US (unconditioned stimulus); 5-HT (5-hydroxytryptamine; serotonin).

The introduction of the "model systems" approach by Kandel and Tauc motivated a proliferation of invertebrate models (for reviews, see /51,56,99,146/), and has been applied in a similar fashion to vertebrate systems /153,177/, although in the latter case without the benefits afforded by the ability to identify single cells. This proliferation is in part attributable to the recognition that basic principles of cell biology, e.g., biophysical, biochemical, and molecular determinants of neuronal activity and transmission, are widely conserved across species and cell types. By implication, key elements of neuronal plasticity elucidated in simpler nervous systems or other reduced preparations might be conserved into the mammalian brain, and likewise, from simpler mammalian brains to humans. Support for this assumption would of course be strengthened to the extent that a similar conservation was found across various invertebrate systems. To some degree, the promise suggested by this approach has been slow to materialize. Many reviews of the model systems literature have suggested that common mechanisms underlying memory induction had not emerged, suggesting the unappealing possibility that the study of memory in these model systems might provide little insight into ubiquitous cellular properties of learning (but see /51,66,128,146/). In the present article, we review a body of evidence which suggests that any such conclusion may have been premature. At the time of many earlier treatments of this subject, specific details of the mechanisms underlying learning in species other than *Aplysia* were somewhat sparse. In the case of *Hermissenda*, the mechanisms of learning appeared in some respects different from those described in *Aplysia*, and in other respects even antithetical /17,56/. In contrast to these earlier disparities, recent evidence suggests that learning in *Hermissenda* may share many of the molecular constituents employed by *Aplysia*. Moreover, the recent elucidation of the contribution of the metabotropic glutamate receptor to some forms of long-term potentiation (LTP) in the mammalian brain suggests potential points of convergence between LTP and the forms of plasticity previously found to contribute to learning in invertebrates.

In the present review, we describe the behavioral processes and synaptic interactions within neural networks that underlie the induction of associative

learning in *Aplysia* and *Hermissenda*, followed by a summary of the neurophysiological and molecular constituents of that learning. These learning-related forms of neuronal plasticity in the invertebrate nervous systems will be briefly compared to a prominent form of hippocampal LTP that has been suggested to contribute to some aspects of memory formation. We believe that these ostensibly disparate systems share many important and fundamental similarities, even though particular molecular constituents differ. Since learning-induced neuronal modifications are sure to be widespread throughout any nervous system, we suggest that many of the specific differences observed across preparations are not necessarily indicative of a lack of generality between species, but rather, reflect the diversity within the nervous system itself. It is our view that it is the ubiquitous features of learning-related neuronal modifications that are derived from these interspecies comparisons which are most likely to advance an understanding of the necessary and sufficient cellular constituents of memory.

The present review is limited primarily to mechanisms underlying memory *induction*, with only an occasional passing reference to long-term storage. This approach is necessary both for reasons of economy and because less is known (particularly in the cases of *Hermissenda* and LTP) regarding the maintenance of memory beyond the parameters imposed by normal protein degradation and turnover. Moreover, our principal concern will be with associative forms of learning, in which the response to a stimulus (referred to as a conditioned stimulus, or CS) is modified as a result of its predictive relationship with some other stimulus of (usually) strong affective valence (referred to as an unconditioned stimulus, or US). This focus on associative learning is predicated on its pervasiveness among both simple and complex organisms, because the behavioral processes that govern the acquisition and expression of associatively conditioned responses are widely conserved among organisms with seemingly little else in common, and because it is thought to be a primary element of more complex forms of learning /265/.

2. LEARNING IN *APLYSIA*

Since *Aplysia* is the prototypic (and the most fully developed) model system employed in the search for cellular memory mechanisms, we begin with a review of this preparation. We intentionally describe only the most critical features of this system as more extensive descriptions have been presented elsewhere (e.g., /51,54,128,135/).

2.1 Behavioral processes underlying associative learning in *Aplysia californica*

Stimulated by the early examples of heterosynaptic facilitation in neurons of the abdominal ganglion /137/, the marine mollusc *Aplysia californica* has enjoyed an extensive history of analysis directed at both associative and non-associative forms of learning. Although our focus here is on the cellular and molecular substrates of associative conditioning, associative and nonassociative forms of learning may share common mechanisms in *Aplysia* /124/, and this continuity between the processes and mechanisms will become apparent in the following pages.

While behavioral studies have demonstrated the capacity of *Aplysia* to learn a large repertoire of associations (e.g., /71,158,258,267,268/), most investigations of associative learning in this animal have been directed at the acquisition of defensive withdrawal reflexes. Studies of associative learning in *Aplysia* have primarily focused on two simple defensive reflexes: gill/siphon withdrawal and tail withdrawal. In the experimentally naive *Aplysia*, application of a weak tactile stimulus to the siphon (CS) produces only a small withdrawal of the siphon and gill organs, while a brief electric shock (US) to the animal's tail results in a vigorous and more sustained withdrawal of both the siphon and gill. Carew, Walters, and Kandel /57/ found that as few as 15 temporally paired presentations of the CS and US were sufficient to induce a long-lasting (several days) conditioned siphon and gill withdrawal reflex that was expressed as both an increase in amplitude and persistence of the response to the CS alone. Consistent with an underlying associative process, random or unpaired presentations of the CS and US produced comparatively small changes in the animal's responses to the CS, although US

presentations alone did result in a small enhancement of the withdrawal reflex which was attributable to nonassociative sensitization. This work was extended with the demonstration that the gill withdrawal reflex could be differentially conditioned using a light touch to the siphon (CS+) or mantle (CS-) as discriminative stimuli /55/. In this procedure the CS+ is always presented contiguously with the US (tail shock), while the CS- is presented in isolation such that the animal's experience with the two CSs differs only in their temporal relationship with the US. Using this well balanced procedure and optimal training parameters, evidence of learning and 24 hour retention could be observed with as little as one conditioning trial. Furthermore, this form of conditioning seems analogous to vertebrate learning in that differential conditioning in *Aplysia* is degraded by backward or simultaneous CS and US presentations, and is sensitive to interstimulus interval and reduced CS-US contingency /65,125/.

2.2 Network interactions underlying sensitization and associative learning in *Aplysia*

It is an appealing feature of an invertebrate model system when some concordance of the processes underlying learning are observed between that system and its vertebrate counterparts. Although this phylogenetic conservation is interesting in itself (and in this case underscores the fundamental import of associative learning), the ultimate goal of this approach is to account for these processes at a neuronal level of analysis. To that end, cellular networks of sensory, interneuronal and motor cells that transduce CS and US information in *Aplysia* have been identified, and an overview of this network is presented in Figure 1. Tactile stimulation of the siphon or mantle excites the afferent terminals of mechanoreceptors whose cell bodies are found within the abdominal ganglion /53/. These sensory cells then synapse with excitatory projections onto motor neurons and interneurons also located in the abdominal ganglion /149/. Gill and siphon contractions arise from excitatory monosynaptic and polysynaptic input, respectively, from abdominal ganglion motor neurons. Tail shock (US) results in the stimulation of interneurons that synapse onto sensory neuron

(siphon or mantle) terminals that are integral to the CS (siphon or mantle touch) pathways /136/. It is the innervation of the sensory neurons by the "facilitatory" interneurons that is critical to both nonassociative (US-induced sensitization) and associative modifications of the CS pathway.

2.3 Induction of presynaptic facilitation during sensitization training

As described, associative learning in *Aplysia* is at least in part attributable to an amplification of the sensitizing response to the US alone. In *Aplysia*, the small withdrawal of the gill induced by touch to the siphon is greatly enhanced if the animal had recently been exposed to a noxious stimulus such as tail shock /217/, and *in vitro* preparations that permit the direct conditioning of identified neural pathways in the isolated abdominal ganglion have been instrumental in elucidating the cellular basis of this learned response. Sensitization in this reduced preparation is accompanied by an enhancement of the EPSP in a monosynaptic pathway between siphon sensory neurons and postsynaptic motor neurons mediating gill withdrawal, and this enhancement has been attributed to a presynaptic alteration in transmitter release /58/.

As depicted in Figure 1, sensory information from the tail interacts with the siphon sensory neurons via a polysynaptic serotonergic (5-HT) pathway /126,127,166/. The mechanism of enhanced transmitter release in the siphon sensory neuron has its origin in two parallel 5-HT-induced second messenger cascades that culminate in the stimulation of a cAMP-dependent protein kinase (PKA) and Ca^{2+} /phospholipid-dependent protein kinase (PKC) /46/. The stimulation of PKA and PKC influences transmitter release in three general ways. These include: 1) the broadening of the action potential; 2) modulation of L-type Ca^{2+} currents, and 3) transmitter vesicle mobilization.

Surface receptors for 5-HT interact with at least two G-proteins: G_s , which is coupled to the cAMP pathway, and G_o , which is coupled to a parallel PKC pathway. G_s stimulates the membrane bound enzyme, adenylate cyclase, which catalyzes the formation of cAMP from ATP. The newly formed cAMP binds the regulatory subunits of the cAMP-dependent protein kinase (PKA), causing the

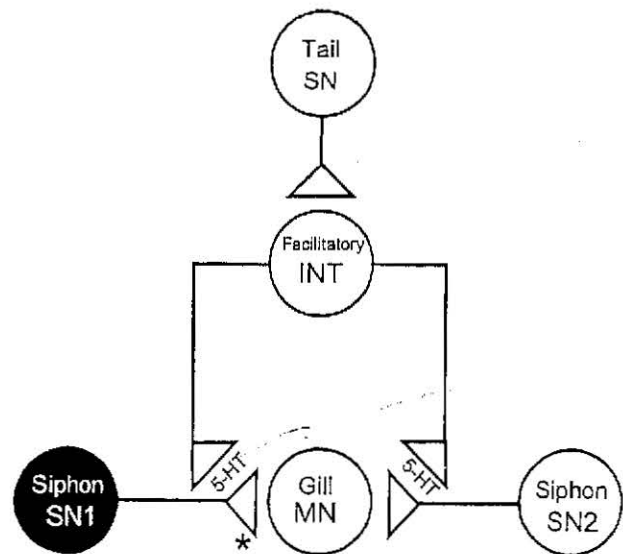


Fig. 1: A simplified depiction of the neural network that underlies associative learning in *Aplysia*. During associative learning, a light touch to the siphon (CS) in the area innervated by siphon sensory neuron 1 (SN1) depolarizes that cell and normally induces a weak gill-withdrawal reflex via monosynaptic projections to gill motor neurons (MN). Approximately 0.5 sec after the siphon touch, a tail shock (US) is presented that stimulates tail sensory neurons which project via interneurons onto siphon sensory cell axons. The binding of 5-HT to the SN1 during a period of depolarization-induced activity modifies the cell in such a way that its synaptic interaction with gill motor neurons (MN) is facilitated (*), inducing an enhanced gill withdrawal in response to the CS alone. Note that in the absence of conjoint transmitter binding and activity, the synaptic interaction between SN2 and the gill MN does not undergo facilitation beyond what may be caused by 5-HT alone (i.e., sensitization).

regulatory subunits of the enzyme to dissociate from the catalytic subunits. G_o stimulates the activity of phospholipase C (PLC), which catalyzes the breakdown of membrane phospholipids into inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 -induced release of Ca^{2+} from cytosolic storage pools in combination with DAG induces the translocation of PKC from the cytosol to the membrane of the sensory cell /233,251/. Upon translocation, PKC phosphorylates membrane proteins associated with voltage-dependent K^+ channels, reducing conduct-

ance through these channels /73/ and further prolonging action potential duration /257/. Thus, 5-HT-induced activation of PKC is also associated with the movement of transmitter vesicles to the releasable pool, increasing the transmitter available for release /112/.

5-HT has been reported to phosphorylate two specific classes of K^+ channels, the S-type K^+ channels (I_{KS}) /144,246/ and the transient voltage-dependent channel (I_{KV}) /33,129/. Reduction of these repolarizing currents (I_{KS} and I_{KV}) has been implicated in the broadening of action potentials, although their relative contributions are different. While I_{KV} plays a prominent role in spike broadening, I_{KS} is more important for regulating other measures of cell excitability /34,52/. Moreover, spike broadening is mediated by both PKC and PKA /257/ whereas increases in evoked spike rate or input resistance appear to be mediated primarily by PKA /112,145/.

One consequence of the prolonged depolarizing phase of the sensory cell's action potential following sensitization training is an enhancement of voltage-dependent influx of Ca^{2+} through N-type Ca^{2+} channels /90/. This potentiated elevation of intracellular Ca^{2+} appears to account for a large portion of the training-induced facilitation of the EPSP owing to the direct relationship between intracellular Ca^{2+} levels and presynaptic transmitter release. Additionally, Edmonds *et al.* /90/ report that 5-HT (or cAMP) application leads to a direct increase in conductance through the L-type Ca^{2+} channel (as opposed to a secondary effect as a result of decreased outward K^+ flux), presumably further increasing the availability of transmitter vesicles for release. The transduction pathway underlying this direct enhancement of Ca^{2+} conductance has not been elucidated, and we will not address it further here. In summary, the synaptic enhancement observed following sensitization training is the direct result of a protein kinase mediated increase in the availability of transmitter vesicles for release as well as a reduction of several classes of K^+ channels which facilitate that release.

2.4 Stimulus convergence underlying associative learning in *Aplysia*

Given the similarities between sensitization training and classical conditioning in *Aplysia*, initial studies of associative forms of learning in *Aplysia* were guided by the hypothesis that the mechanism underlying the induction of associative learning may be similar to, or an amplification of, nonassociative presynaptic facilitation. As such, the convergence of signals originating in the CS and US sensory pathways might serve to amplify and prolong the synaptic facilitation that accompanies sensitization /64,137/. Support for this hypothesis was reported using differential classical conditioning of siphon sensory neurons /124/, and independently with a similar procedure in which tail sensory neuron stimulation served as the CS /266/. In the partially intact preparation described above, Hawkins *et al.* /124/ performed a differential conditioning experiment in which CS+ and CS- stimuli consisted of current-evoked spike activity in two individual siphon sensory neurons. This *in vitro* conditioning arrangement can be discerned from Figure 1. Stimulation of the CS+ pathway (depicted as the darkened sensory neuron in Figure 1) immediately preceded electrical shock to the tail (US), while stimulation of the CS- neuron was explicitly unpaired with US presentation. Conditioning was measured as a change in the magnitude of excitatory postsynaptic potentials (EPSPs) recorded from a common motor neuron (either siphon or gill motor neuron) efferent to both the CS+ and CS- sensory neurons. Under these conditions a moderate facilitation of the EPSP was recorded for both sensory neurons (as would arise from sensitization training). However, if the US (shock) was preceded by spike activity in the sensory neuron, as in the CS+ condition, the magnitude of this facilitation was significantly enhanced (and its maintenance prolonged) relative to the sensory cells that had received unpaired (CS-) training. Additional work with this *in vitro* preparation investigated the temporal constraints on effective CS-US inter-stimulus intervals, and found them to be in close agreement with the behavioral data previously described /64/. Two conclusions are immediately apparent based on these results as well as the similar results of Walters and Byrne. First, these bio-

physical data are consistent with behavioral studies that indicate a requirement for temporal contiguity between a CS and US for efficient learning. This is evident in the finding that associative modifications are restricted to sensory pathways whose neuronal activities were correlated in time. In addition, the ability to differentially condition two pathways converging onto a common postsynaptic motor neuron indicates that the pairing-specific amplification of the EPSP must originate presynaptically, i.e., in the sensory neuron.

Consistent with a presynaptic locus of modulation, Hawkins *et al.* /124/ observed a pairing-specific increase in the duration of action potentials in the sensory neuron following differential conditioning in the reduced preparation. This broadening of the action potential was attributable in part to a reduction of conductance through K^+ channels that resemble the S type- K^+ channel known to play a causal role in nonassociative presynaptic facilitation (cf. /143/). Moreover, it was reported that the spike-broadening normally induced by 5-HT (i.e., as in sensitization) was significantly enhanced if its application was paired with a burst of activity in the sensory neuron /106/. These data suggest that 5-HT is playing a modulatory role during associative learning similar to its role in sensitization, and that this modulatory action of 5-HT may be enhanced by contiguous activity in the sensory neuron.

The results described above indicate that the contribution of the US during associative learning in *Aplysia* is accounted for by the release of 5-HT onto the sensory neuron, raising the question as to how the CS (spike activity) induces an enhancement of the 5-HT-dependent mechanism. Several converging lines of evidence have implicated an interaction between a CS-induced elevation in intracellular Ca^{2+} and 5-HT's stimulation of second messenger systems. First, the enhanced spike-broadening induced by the combined effects of 5-HT and activity was found to be dependent on the presence of external Ca^{2+} /2/. Furthermore, 5-HT, which is known to elevate cAMP levels in the sensory cell /35/, induces a greater elevation if its application is closely preceded by spike activity in the sensory neuron /45/ (see also /208/). These findings suggest that the transmitter-induced elevation of intracellular cAMP levels might be amplified by a contiguous rise in intracellular Ca^{2+}

during a train of spike activity. Previous work had demonstrated that adenylate cyclase, the enzyme responsible for cAMP production, possessed certain characteristics that made it attractive as a point of convergence between these two sensory signals /271/ and may be modulated in a Ca^{2+} -dependent manner. This was confirmed with the observation that adenylate cyclase was stimulated by Ca^{2+} /calmodulin /92/, and that the cyclase was activated by physiologically relevant rates of firing /279/. Furthermore, the same cyclase molecules that are modulated by Ca^{2+} /calmodulin also exhibit an increase in activity as a result of the 5-HT-induced stimulation of the G-protein-coupled receptor that mediates the US pathway /3/. Consequently, it can be surmised that both Ca^{2+} and 5-HT converge at the point of the sensory cell to synergistically activate adenylate cyclase during the *in vitro* conditioning procedure. Relevant to a role for this mechanism in associative conditioning, the synergistic activation of the cyclase by Ca^{2+} and 5-HT is most efficient when the Ca^{2+} signal generated by stimulation of the sensory neuron (i.e., the CS) precedes 5-HT binding by roughly 500 ms. This interval should be recognized as the optimal interval for forward conditioning in this preparation /3,278/.

Figure 2 presents a molecular model that summarizes the data described above and illustrates the presumed mechanism of activity-dependent presynaptic facilitation in *Aplysia*. This illustration is based on previously published models /66,114,128/. Figure 2A illustrates a US-alone presentation as would occur during sensitization training. Upon tail stimulation (US), 5-HT is released from a facilitatory interneuron and interacts with its postsynaptic receptor on the siphon sensory neuron. This receptor site is associated with a G_s protein that stimulates the activity of adenylate cyclase. The calmodulin binding site on the cyclase remains open due to the lack of Ca^{2+} elevation necessary for calmodulin activation. Following stimulation, adenylate cyclase catalyzes the production of cAMP from ATP. The moderate activation of PKA by cAMP results in the phosphorylation of K^+ channels leading to a modest degree of presynaptic facilitation. A parallel transduction pathway, arising from the binding of 5-HT to G_o receptor proteins, stimulates the transient translocation of PKC to the membrane where it phosphorylates a class of

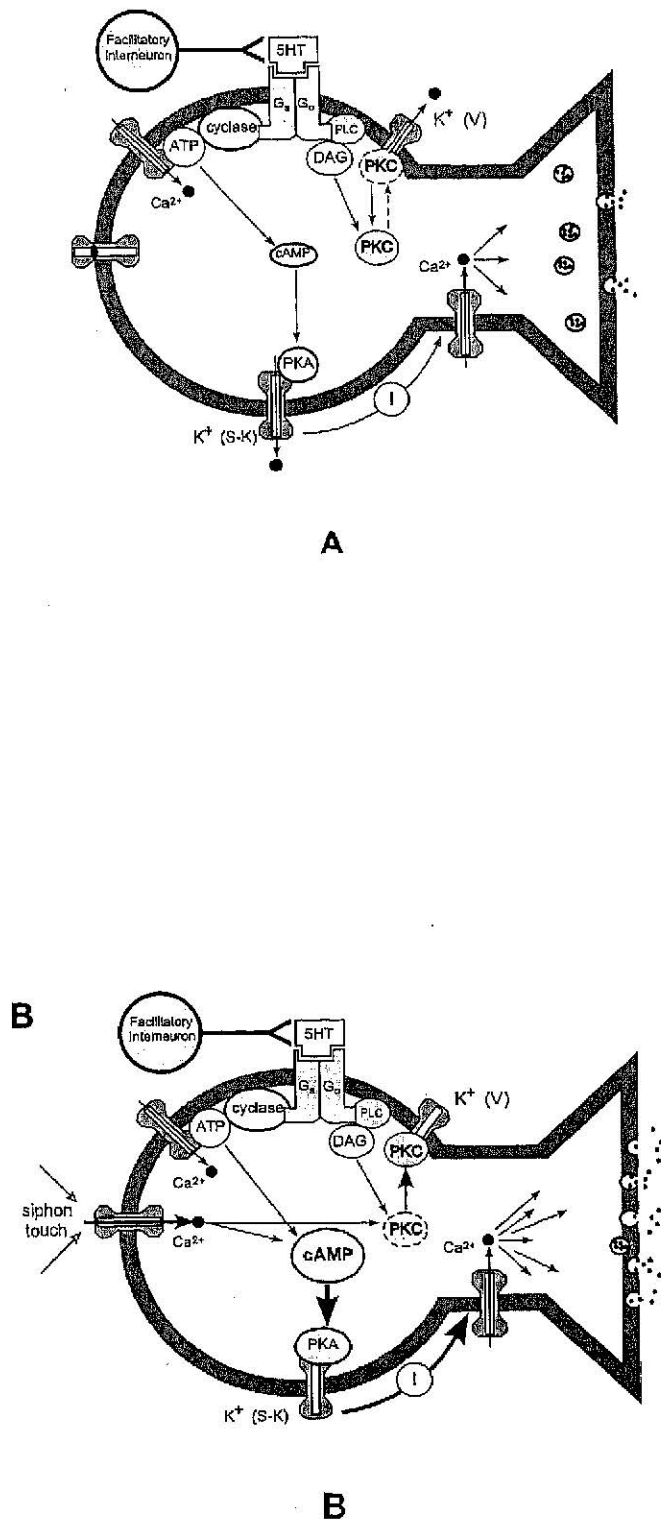


Fig. 2: Molecular constituents of activity-dependent facilitation in *Aplysia* sensory cells.

A. During the presentation of tail shock (as in sensitization training), stimulation of facilitatory interneurons induces a release of 5-HT that binds to two classes of metabotropic receptors (G_s and G_o dependent) on the axon of the siphon sensory neuron (the location of receptors in the figure is only illustrative). The G_s pathway stimulated by 5-HT induces a small Ca^{2+} influx through the generation of ATP, which also stimulates the production of cAMP. cAMP weakly activates PKA which phosphorylates the S-K class of K^+ channel, causing a small and transient reduction of current flow through that channel. The G_o pathway stimulated by 5-HT induces a cleavage of PLC which in turn generates DAG, an activator of PKC. (This pathway is also likely to induce Ca^{2+} release from cytosolic pools, although this has not been demonstrated in these cells.) PKC transiently translocates to the cell membrane and phosphorylates a voltage dependent K^+ channel, further reducing K^+ efflux. The weak and transient reduction of these outward K^+ currents results in a reduced threshold for elicitation, as well as a broadening, of evoked action potentials, ultimately increasing Ca^{2+} influx in the axon. Through this increase in Ca^{2+} influx, any subsequent stimulation of the sensory cell will cause an enhancement of transmitter release and the interaction with postsynaptic motor neurons, thus accounting for sensitization.

B. If the siphon is touched just prior to the application of tail shock (as in classical conditioning), stretch receptors in the sensory cell induce a large Ca^{2+} influx through mechanically-coupled ion channels. This Ca^{2+} influx (several times greater than is induced by 5-HT binding) greatly potentiates the generation of cAMP from ATP, causing a more effective activation of PKA. Likewise, the higher Ca^{2+} concentration is presumed to facilitate the activation of PKC by DAG. As a result, PKA and PKC phosphorylate their target proteins more efficiently and the phosphorylation is more persistent. Thus in *Aplysia*, the facilitation of the synaptic interaction between sensory and motor neurons that underlies classical conditioning is a synergistic consequence of a contiguous rise in intracellular Ca^{2+} concentration (CS induced) and stimulation of G-protein coupled receptors by 5-HT (US induced) in the sensory cells.

voltage-dependent K^+ channels. If the facilitatory transmitter release is preceded by an elevation in intracellular Ca^{2+} , an amplification of this biochemical response system is observed. Figure 2B illustrates the cellular events underlying paired CS-US presentations. Consequent to the voltage-dependent elevation of postsynaptic Ca^{2+} levels, Ca^{2+} /calmodulin binds to adenylate cyclase causing an enhanced production of cAMP and a potentiation in activated levels of PKA, while PKC binding in the neuronal membrane is presumably enhanced and prolonged by the same elevation of Ca^{2+} (although this latter assumption is untested). The result of this sensory convergence is a Ca^{2+} -dependent enhancement and prolongation of the effects of presynaptic facilitation. As a consequence, the CS-induced withdrawal reflex is amplified relative to both naive and sensitized animals, indicative of an associative "memory".

Interestingly, the relative contribution of PKA and PKC to facilitation appears to depend on the history of the sensory-motor synapse (for a review see /54/). In previously untreated synapses, 5HT-induced facilitation of evoked transmitter release is blocked by inhibitors of PKA, although in depressed synapses, PKC inhibitors are more effective /112/. Moreover, the involvement of these second messengers was also dependent on the time at which the inhibitors were applied after 5-HT application. PKA inhibitors attenuate facilitation immediately or soon after 5-HT application /129/ whereas PKC inhibitors are effective at later times /257/. The time dependence of the PKA and PKC effects was corroborated with data obtained using two dimensional gel electrophoresis evaluating degree of phosphorylation. These studies found that the effects of brief 5-HT exposure on protein phosphorylation could be mimicked by cAMP exposure but not by phorbol esters /259/, whereas the effect of long 5-HT exposures was only replicated with phorbol esters /130/. Analysis of the biophysical effects induced by these two kinases also showed that their effects could be dissociated. While PKA appears to exert its effects on I_{KS} and I_{KV} , PKC only affects I_{KV} /257/.

The time dependence of facilitation produced by PKA and PKC is of particular importance due to the fact that PKC appears to underlie an intermediate type of memory that links short and long-term

processes. In the *Aplysia* nervous system two phorbol ester sensitive isoenzymes have been identified, Apl I, a Ca^{2+} dependent isoform, and Apl II, a Ca^{2+} independent isoform /147/. While short term facilitation leads to the activation of Apl I, intermediate facilitation leads to the activation of Apl I and an autonomous kinase, which arises from Apl II /276/. Activation of PKC during this intermediate period of time is paralleled by an increase in cell excitability (evoked spike rate) that is independent of protein synthesis /175/.

The molecular events that underlie the presynaptic modifications of *Aplysia* sensory neurons during associative learning were originally referred to as "activity-dependent amplification" of presynaptic facilitation /124/, and this description, or close variants of it, is typically used to refer to this mechanism. Although many of the individual components of this mechanism differ in detail from those to be described in the following sections, there is also a clear emergence of many critical similarities. Given these similarities, these seemingly disparate mechanisms might best be viewed as variants of the theme exemplified by activity-dependent facilitation.

It should be noted that recent evidence suggests that modifications of the postsynaptic motor neuron (see Figure 1) might contribute to some forms of facilitation at the sensorimotor synapse in *Aplysia*. This conclusion is warranted by the observations in cell culture that conjoint activity in the sensory neuron and the motor neuron induces at least a short-term form of synaptic facilitation between them, and that this facilitation is attenuated by chelation of Ca^{2+} in the postsynaptic motor neuron /31,114,195/. Moreover, this form of potentiation can be blocked by antagonists of glutamate, an isoform of which appears to mediate the interaction between the sensory cell and the motor neuron /161/. Although these earlier experiments were conducted in integrated sensory-motor cultures, a recent experiment makes an important connection between these results and the induction of memory during associative training in *Aplysia*. Using an *in vitro* associative conditioning procedure like those previously used to elucidate activity-dependent amplification of presynaptic facilitation /124/, Murphy and Glanzman /196/ have reported that NMDA receptor antagonists disrupt synaptic

enhancement at the sensory-motor synapse, but had no effect on the enhancement (presumably pre-synaptic) that followed nonassociative (sensitization) training. These results suggest that the synaptic enhancement which underlies the associative memory may involve post- as well as pre-synaptic modification. Although the presynaptic mechanism of facilitation described above is apparently sufficient to account for the behavioral modifications indicative of memory, these data suggest the possibility that the story is not yet complete and that memory storage may involve the complex interaction of several mechanisms. These results remind us of the caveat by Glanzman /114/ that classical conditioning, even in such "simple" systems, is clearly not as simple as we may have believed.

The warning by Glanzman /114/ that the cellular basis of classical conditioning in *Aplysia* may not be "simple" can certainly be applied to either of the remaining models contained in this review. However, since the molecular constituents of learning in *Aplysia* are more clearly delineated than in *Hermissenda* or hippocampal LTP, the evidence that learning in *Aplysia* cannot be subsumed by a single mechanism is a clear reminder that learning mechanisms, like its processes /222,223,269/, are not yet well delineated. For instance, the "simple" network purported to subserve defensive conditioning in *Aplysia* (see Figure 1) suggests that a well delineated circuit underlies the generation of the gill-withdrawal reflex. However, it has been reported that *Aplysia*'s gill withdrawal reflex is not likely to arise from activity in a small, dedicated circuit. Rather, the gill withdrawal response involves at least 1000 cells distributed in at least three distinct ganglia /264,273/. Likewise, Colebrook and Lukowiak /68/ trained the semi-intact *Aplysia* to express a conditioned gill withdrawal reflex using parameters developed in earlier studies /45,124/. Unlike earlier demonstrations, Colebrook and Lukowiak simultaneously recorded the amplitude of the conditioned gill-withdrawal reflex and the enhanced EPSP in the gill motor neuron (presumably indicative of presynaptic activity-dependent facilitation). Across animals, the amplitudes of the EPSPs and the gill-withdrawal reflex were enhanced; however, no evidence of a correlation between the two responses was detected. This

suggested to Colebrook and Lukowiak that multiple mechanisms contribute to the generation of the conditioned response in *Aplysia*, and that no single mechanism or locus of change was necessary or sufficient to support learning (also see /164/). Given the apparent disparity within a model system, results such as these might suggest a certain futility in attempting to discern common themes arising from the comparison of different model systems. On the other hand, an opposite consideration is raised by this intrinsic diversity. We will take the position that in light of such diversity, it is the *commonalities* that are expressed across systems that are likely to be most critical to our understanding of the molecular properties of experience-dependent plasticity.

3. LEARNING IN *HERMISSENDA*

Prior descriptions of learning-related neuronal modifications in *Hermissenda* have been somewhat preliminary. However, recent work has more fully elucidated the underlying mechanisms of learning in this system, and we will provide a complete summary of those data here.

3.1 Behavioral processes of associative learning in *Hermissenda*

Several of *Hermissenda*'s responses to light are subject to modification using a simple associative learning paradigm in which a light (CS) is paired with rotation or water turbulence (US). These modifications of phototactic behavior have been observed following paired, but not unpaired or random, presentations of light and rotation, and can persist for days following 150 pairings spread over 3 days /79/, or for several weeks following more extensive training /122,180/. As with *Aplysia*, associative learning in *Hermissenda* exhibits many of the characteristics of traditional vertebrate conditioning preparations, including sensitivity to the contingency and temporal order of the CS and US /96,117/, interstimulus interval /188/, extinction /226/, facilitated reacquisition following degradation of memory /79,180/, and a deficit in learning as a consequence of massed training trials /197,231/.

Numerous measures have been used to index phototactic behavior in *Hermissenda*, including light-initiated locomotion /78,98/, orientation

towards a light source /79/, and locomotion within a light gradient /96,98/. Light-induced phototactic behavior is accompanied by an extension of the animal's muscular "foot", its organ of locomotion. In contrast to its responses to light, exposure to rotation elicits unconditioned responses that are opposite in nature to those elicited by light, i.e., reduced rate of locomotion and contraction of the foot /79,154,187/. Lederhendler *et al.* /154/ demonstrated that a light CS comes to elicit a foot contraction (CR) following paired presentations of light and high-speed rotation, suggesting that the conditioned response reflected the transference of some of the properties of the US (rotation) to the CS (light). A similar effect was reported by Matzel *et al.* /187/, who demonstrated a strong correlation between conditioned reductions in phototaxis and conditioned foot contraction, although the former was more subject to nonassociative modification suggesting some degree of dimorphism between the responses. Lederhendler *et al.* and Matzel *et al.* speculated that conditioned foot contraction served a "preparatory" role /154,187,215/, reducing the likelihood that rotation would dislodge the animal from its substrate. This speculation received support with the demonstration that animals for which rotation is predicted by light cling more effectively to their substrate during a period of rotation /231/.

The behavioral analysis of association formation in *Hermissenda* has aided the development of a model system that, like *Aplysia*, shares a host of similarities with analogous vertebrate learning and yet provides the simplicity and specificity to investigate the network, cellular, and molecular components of that learning. Prior to any description of the molecular constituents of this learning, we will describe the interaction of visual and vestibular systems in *Hermissenda* as well as the mechanisms of sensory transduction in these systems.

3.2 Network interactions underlying *Hermissenda* associative learning

Sensory information about the light CS and rotation US is transduced in the *Hermissenda* nervous system through a symmetrical pair of eyes and statocysts. Previous work has elucidated numerous intersensory interactions between these sensory systems as well as within the sensory-motor

networks /8,82,84/. A simplified version of the neural network underlying these interactions and classically conditioned responses in *Hermissenda* is depicted in Figure 3.

Light (CS) Pathway. Visual information about the CS is encoded through the eyes of the *Hermissenda* which each contain five photoreceptors that are situated within a lattice of nonneuronal pigment cells /89/. These photoreceptors have been distinguished electrophysiologically into two type A and three type B photoreceptors /13/. Type B receptors exhibit synaptically mediated, reciprocal inhibition with each other and more weakly inhibit type A photoreceptors /82/. Light-evoked responses in type A and B photoreceptors consist of complex potential changes carried by selective alterations in ion permeability /83,97/. An early, peak depolarization of the B photoreceptor in response to light is carried by an inward, light-activated Na^+ current ($I_{\text{Na-light}}$) that is opposed by a fast, rapidly inactivating outward potassium current (I_A). Following the peak response, the light-induced depolarization begins to reverse and then plateaus at an intermediate level of depolarization. A sustained depolarization during light is maintained by a light-activated, nonspecific cation currents (I_{cation}) as well as a voltage-dependent Ca^{2+} current (I_{Ca}). These depolarizing currents are opposed in part by voltage and Ca^{2+} -dependent potassium currents (I_{KV} and I_{KCa}).

Axons from all five photoreceptors exit the eye and send projections to motor neurons via an ipsilateral optic ganglion and efferent interneurons. Although these interneurons are known to project onto motor neurons which innervate the foot, the interactions between interneurons as well as their efferent pathways have been only partially characterized /5,116,225/.

Rotation (US) Pathway. Early morphological analysis revealed that the statocyst was a spherical structure formed by 13 ciliated hair cells which when acted upon by centrifugal forces, are mechanically displaced by statoconia suspended within the cyst /150/. In response to this stimulation, hair cells exhibit a depolarizing generator potential. Axons from the hair cells travel to the neuropil of the ipsilateral cerebropleural ganglion where they give rise to terminal branches

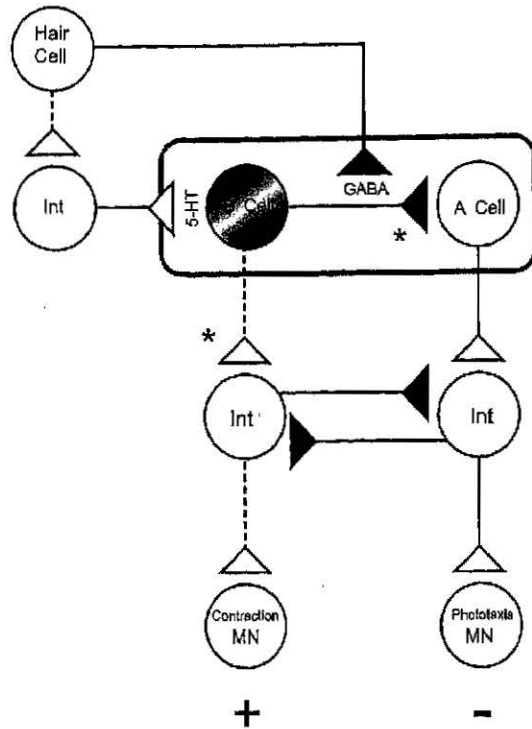


Fig. 3: A simplified depiction of the neural network that underlies associative learning in *Hermissenda*. During associative conditioning, light (CS) depolarizes the type A and type B photoreceptors and the mechanical perturbation of cilia induced by rotation (US) depolarizes and induces action potentials in the hair cells. Hair cells monosynaptically project onto the photoreceptor axons where they release GABA. It is also presumed, though not confirmed (dashed line) that the hair cells stimulate interneurons (INT) which project onto the B cell soma and release 5-HT. The binding of GABA and/or 5-HT to the B cell during a period of light-induced depolarization induces a persistent increase in the excitability of the B cell which in turn facilitates (*) its inhibitory synaptic interaction with the A photoreceptors as well as a presumed, though not well characterized (dashed line) direct projection onto interneurons. As a result, subsequent light presentations bias the output of the eye toward the B photoreceptor, shunting A cell-mediated phototactic responses to light and inducing an otherwise sublimated foot contraction. It should be explicitly noted that the light-responsive interneuronal network is quite complex and not well characterized. Given that unidirectional, bidirectional, excitatory, inhibitory, or polysynaptic interactions exist between each of at least 24 such interneurons, the depiction of this network is only illustrative, although it is at least consistent with known pathways and can account for the behavioral modifications that reflect associative learning.

and project monosynaptically onto ipsilateral photoreceptors /115/, as well as central visual interneurons /5/. Depolarization of the caudal hair cells results in a hyperpolarization in the B photoreceptor. This hyperpolarization can be as large as -12 mV at resting potentials and is accompanied by a cessation of spontaneous spikes in the photoreceptor /11,228/. This inhibitory response of the photoreceptors to statocyst stimulation is mediated by GABAergic receptors localized in the terminal region of the B photoreceptor's axon /11,179,228/. Although there was some initial confusion concerning the basis of the postsynaptic response to GABA, it is now clear that this response is mediated in large part by an increase in K^+ conductivity in that the response is dependent on extracellular K^+ but not Cl^- , reverses between -80 and -90 mV (the reversal potential for K^+), and is mimicked by the GABA_B receptor agonist baclofen /184/. GABA receptor antagonists have proven to be only weakly effective in blocking the postsynaptic response, although GABA_B receptor antagonists have had the most consistent effect /228/, and the K^+ channel blocker TEA completely eliminates the response /24,184/. Consistent with its apparent similarity to GABA_B receptors, the inhibitory response of the B cell to micro application of GABA or to hair cell stimulation is attenuated by intracellular injections of nonhydrolyzable forms of GDP /228/, strongly suggesting that the receptor affects postsynaptic channels via a G-protein coupling mechanism.

At the level of the central visual interneurons, hair cell stimulation can result in either excitation or inhibition depending on the target cell /5/. While the functional significance of these synaptic interactions has not been determined, the stimulation of interneurons by the hair cells is a potential source of polysynaptic serotonergic projections onto the soma membrane of the B cell. Serotonergic fibers have been reported to pass in close proximity to the eye /25,151/, and pressure micro application of 5-HT indicates that receptors are present on the soma of the B photoreceptors, but not on terminal fibers /229/. Rogers and Matzel /229/ reported that 5-HT micro application to the soma induces two distinct depolarizing responses. A strong depolarization (often +10 mV or more when measured at resting potentials) peaks rapidly (1-2 sec) and dissipates

within several seconds. A distinct smaller depolarization appears as the initial depolarization wanes, and dissipates at a slower rate. The ionic bases of these responses are complex, apparently being carried by both decreases in basal K^+ currents, an increase in a Ca^{2+} current, and an increase in one or more unidentified current [4,104,229,275]. A clear understanding of the integrative contribution of these currents to the depolarizing response has not emerged, in part owing to their separate interactions with membrane voltage and their complex temporal dynamics [229]. This characterization has proven particularly difficult in that the (presumed) serotonergic neurons which project onto the eye have not yet been identified, precluding any examination of serotonergic responses in the photoreceptor under physiological conditions. It is important to note, however, that both fast and slow depolarizing voltage responses of the B cell to 5-HT micro application are eliminated by intracellular injection of nonhydrolyzable GDP, indicating their G-protein dependence. Since the voltage response of the B cells to 5-HT micro application has only recently been described [229], systematic studies of the effectiveness of 5-HT receptor antagonists have not been conducted. Based on its G-protein dependence and electrophysiological properties, Rogers and Matzel [229] suggested that this receptor was most analogous to the 5-HT₂ receptor subtype.

Transduction of visual (CS) and vestibular (US) stimulation in the B photoreceptors. Light induces a depolarization in the B photoreceptors through a somewhat unusual molecular cascade. Unlike common photo transduction cascades which require the coupling of rhodopsin to a G-protein [105,214], stimulation of rhodopsin in the B photoreceptor appears to directly stimulate phospholipase C (PLC) [234] which in turn hydrolyses PIP_2 . This is demonstrated by the observations that injections of GTP or nonhydrolyzable analogs of GDP [234], as well as incubation with the G-protein activator aluminum fluoride [186], have no effect on the depolarizing light response, while phosphoinositide turnover inhibitors eliminate the light response [234,261]. As illustrated in Figure 4, light stimulates the degradation of PIP_2 into DAG and IP_3 [205]. Although these second messengers can play important regulatory roles, Sakakibara *et al.* [234]

reported that PIP_2 directly interacts with Na^+ selective channels, suggesting no role for IP_3 or DAG on the early light-induced depolarization.

The depolarizing response to light induces a large increase in intracellular Ca^{2+} concentration [72,260]. This increase in free Ca^{2+} derives in small part from a voltage-dependent activation of inward Ca^{2+} currents, but is more heavily attributable to the IP_3 -dependent release of Ca^{2+} from intracellular stores [199,260]. This liberation of Ca^{2+} from cytosolic sequestration contributes to the maintenance of the generator potential and acts to slow the repolarization to resting membrane potentials after light offset. Moreover, the liberation of DAG concomitant with the IP_3 -induced rise in intracellular Ca^{2+} is likely to transiently activate protein kinase C (PKC) [205,209]. Since PKC phosphorylates voltage- and Ca^{2+} -dependent K^+ channels in these cells [15,101], the combined generation of these second messengers is likely to contribute to a transient reduction of K^+ conductance that occurs during the light response [12,14,18], thus minimizing the opposition to the light-induced depolarization that these K^+ currents would otherwise exert. The transduction cascade underlying the light response in the B cell is summarized in Figure 4A. It should be explicitly stated that most of our understanding of the light transduction cascade has come from electrophysiological studies of the effects of various pharmacological agents on the light-induced depolarization. Although such techniques are valuable in that they permit the assessment of responses in the intact nervous system, the precise nature of these cascades is in part speculative owing to the poor selectivity of pharmacological agents. Thus a complete elucidation of these cascades awaits the necessary biochemical analyses.

The transduction cascade that mediates GABAergic responses in the B cell is illustrated in Figure 4B. In contrast to the independence of the light response on G-proteins, both GABAergic and serotonergic responses in the B cell require the stimulation of a receptor-coupled G-protein [148, 228]. Given that serotonergic cells that innervate the eye have not been identified, little is known about the transduction of indigenous serotonergic responses. It appears though that the 5-HT and GABA_B receptors on the B cells may stimulate a similar

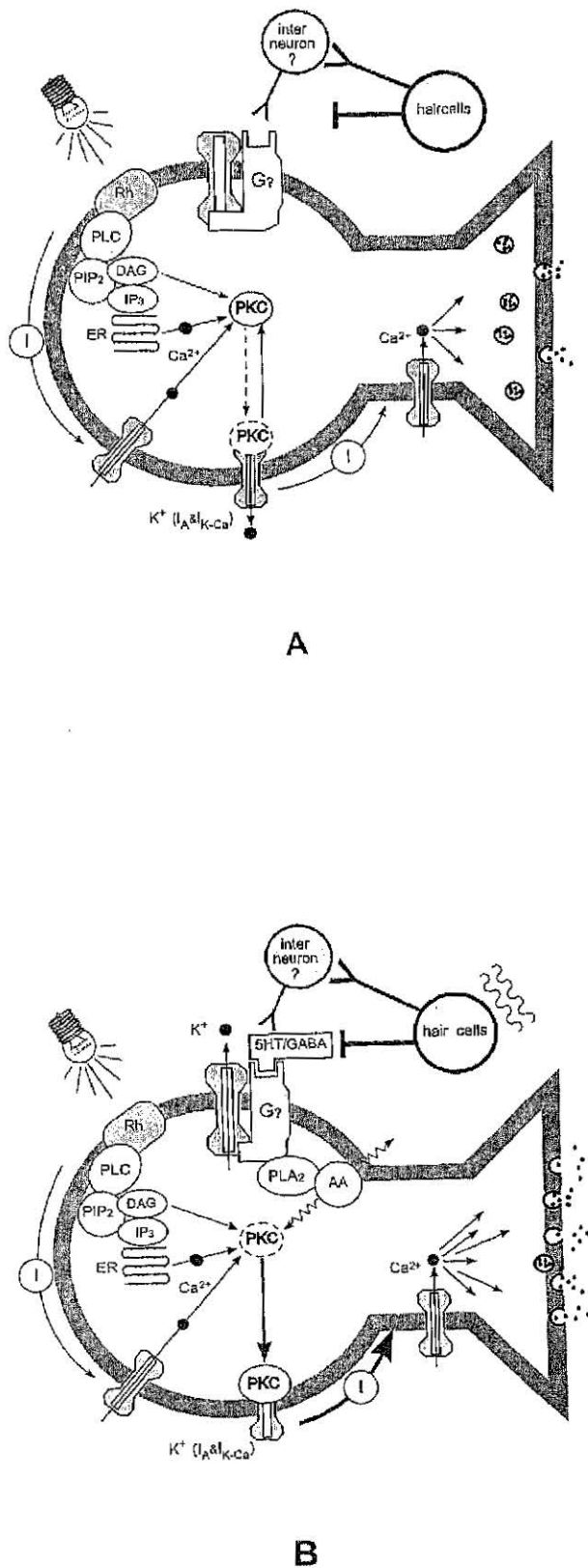


Fig. 4: Molecular constituents of activity-dependent facilitation in *Hermissenda* B cells. **A.** Presentation of light stimulates a transduction cascade which ultimately cleaves PIP_2 into IP_3 and DAG. Light-induced depolarization of the B cell is initiated by a direct interaction of PIP_2 with nonvoltage-sensitive Na^{2+} channels (not shown), and this depolarization stimulates voltage-gated Ca^{2+} channels, resulting in an influx of Ca^{2+} . In addition, a larger (several fold) intracellular Ca^{2+} signal arises from an IP_3 -mediated release from intracellular stores (assumed, though not confirmed, to be rough endoplasmic reticulum, ER). The combined action of high Ca^{2+} concentration and free DAG activates PKC which forms a weak and transient association with the neural membrane where it phosphorylates and reduces conductance through two classes of K^+ channels. This reduction in K^+ conductance returns to normal levels shortly after light offset, and is likely to occur so as to reduce opposition of the light-induced depolarization. **B.** Hair cell activity stimulates the release of GABA and 5-HT onto G-protein coupled receptors. (These receptors are not colocalized as depicted here; see Figure 3 for relative locations. Moreover, 5-HT and GABA appear to modulate different classes of K^+ channels, shown here as one channel for simplicity.) Both the synaptic responses to GABA and 5-HT binding are dependent on unknown classes of G-proteins, and the response to GABA is known to stimulate PLA_2 and a resultant generation of free AA. In these cells, Ca^{2+} , DAG, and AA act synergistically to produce a more effective activation of PKC (or of unique PKC isoforms) which is likely (though not yet demonstrated in this cell type) to bind to the neural membrane until undergoing degradation. As a result of this potentiated activation and translocation of PKC to the membrane, K^+ conductance is further reduced relative to that caused by light alone, and the reduction persists long after light offset. Consequently, the B cell is more responsive to subsequent light exposure, and action potentials are prolonged, resulting in an increase in the release of transmitter and the strength of the synaptic interaction with effector cells. Thus as in *Aplysia*, the facilitation of synaptic responses during associative learning in *Hermissenda* is a synergistic consequence of a contiguous rise in intracellular Ca^{2+} (CS induced) and stimulation of G-protein-coupled receptors (US induced). Note that normal transmission between the hair cell and the B cell is not affected by associative training as the class of K^+ channel that mediates that interaction is not phosphorylated by PKC.

class of G-proteins /229/, as they sometimes do in other cell types /21/. In contrast to the 5-HT response, hair cell-induced hyperpolarization of the eye has been relatively well characterized. Interestingly, in mammalian cells, GABA_B receptors stimulate a slow hyperpolarization via a G-protein-induced stimulation of phospholipase A₂ (PLA₂) and a resultant stimulation of K⁺ channels /87,111/. We have suggested /228/ that the GABA-induced hyperpolarization was sufficiently fast (peaking in less than 50 msec) that the G-protein might be directly coupled to the postsynaptic K⁺ channels (see /49,63/ for a review of similar metabotropic receptors). More recently, it has been reported that the inhibition of PLA₂ with quinacrine has no effect on the hair cell-induced hyperpolarization of the B cell /261/, further suggesting that the G-protein might directly modulate K⁺ channels without an intermediary phosphorylation event. Although not required for the GABA-induced hyperpolarization in these cells, PLA₂ may contribute to the small reduction of voltage-dependent Ca²⁺ currents induced by both GABA /275/ and baclofen /184/, as well as the 5-HT-induced depolarization that we have observed. These latter suppositions have not been tested, although it does appear that GABA binding generates PLA₂ (discussion below; see /231/). It should also be noted that pertussis toxin, which in other cell types inactivates certain G-proteins (e.g., G_o and G_i) including that which couples the GABA_B receptor to its effectors /21,88/, has no effect on GABA responses or hair cell-induced hyperpolarization in the B cell, whether microinjected for 14 hours (unpublished observations) or incubated for up to 26 hours /228/. Similarly, cholera toxin (which commonly inhibits G_s and G_{transducin}) was without effect on these responses (Rogers and Matzel, unpublished observations). Given the rather unique properties of this receptor, it is perhaps not surprising that GABA_B receptor antagonists are only weakly effective in attenuating the GABA-induced hyperpolarization.

3.3 Stimulus convergence and learning-induced neuronal facilitation in the B photoreceptor

Following associative learning, *Hermisenda* B photoreceptors exhibit a general increase in excit-

ability that is characterized by an increase in input resistance /181,272/, an increase in the amplitude of the light-induced generator potential as well as the amplitude and duration of the long-lasting depolarization after the offset of light /75,80,100,272/, an increase in evoked spike frequency /230/, and an increase in the synaptic efficacy between the B cell and its efferent targets /110,239/. It has recently been found that associative learning is also accompanied by an increase in the height and width of evoked action potentials in the B cell /157/. All of these indices of facilitation are believed to arise from a reduction of voltage- and Ca²⁺-activated K⁺ currents /17,69,97/ in the soma membrane, although Clark and Schuman /66,239/ have suggested that some component of synaptic facilitation may be synapse specific. Nevertheless, the modifications of K⁺ currents as well as synaptic facilitation appear to be induced by the sustained activation of PKC, in that bath application or microinjection of PKC and its activators into the B cell causes a similar reduction of currents /15,101,239/, and the inhibition or down-regulation of PKC blocks the training-induced increases in excitability /76,103,183/. Moreover, a training-induced translocation of PKC from its inactive state in the cytosol to its active state in the membrane has been directly observed using autoradiographic techniques /191/, and we have recently reported that Ca²⁺-dependent PKC isoforms are incrementally translocated to the neuronal membrane with successive *in vitro* training trials. In this latter case, the translocation of PKC was extremely well correlated with an incremental increase in several indices of neuronal excitability /198/.

It had been previously suggested that the neuronal facilitation that accompanies learning in *Hermisenda* arose from network interactions that produced a progressive depolarization of the B cell that accumulates with successive pairings of light and rotation /9/. This "cumulative" depolarization was thought to result in a sustained increase in free Ca²⁺ levels, which in turn was proposed to activate PKC and/or other Ca²⁺ sensitive kinases /9,10,100/. While the requirement for increases in intracellular Ca²⁺ concentration has been confirmed /95,185,260/, we have demonstrated that the cumulative depolarization in the B cell that accompanies learning does not contribute to the induction of

facilitation in that cell. In fact, the cumulative depolarization tends to interfere with the induction of the biophysical memory trace due to the sustained inactivation of voltage-dependent Ca^{2+} currents /185,231/ and/or the preferential stimulation of protein phosphatases /197/. Since pre-synaptic stimulation of the hair cells coincident with a light-induced rise in intracellular Ca^{2+} in the B cell is necessary for the induction of a sustained increase in excitability, we have more recently suggested that the interaction of postsynaptic activity concomitant with stimulation of a metabotropic receptor underlies the induction of learning-induced facilitation in the B cells.

Available evidence strongly suggests that learning-induced modifications of the B photoreceptor result from the concomitant binding of GABA and/or 5-HT (US signal) to the B cell during periods of light-induced Ca^{2+} elevation (the CS signal). The convergence of these two signals from the CS and US pathways must induce a long-lasting modification of K^+ conductance across the B cell membrane that neither signal alone can account for, because neither repeated presentations of the CS and US alone, nor their unpaired presentation, results in any long-lasting modification of the B cell. A transduction cascade which satisfies this requirement and which is consistent with the data reviewed here is illustrated in Figure 4B. This model requires no assumptions about complicated network interactions (e.g., cumulative depolarization) and is consistent with signaling pathways described in this and many other cell types. As described above, light alone is sufficient to activate PKC in the B cell via the simultaneous liberation of DAG and IP_3 , a common route of activation which results in a relatively loose and short-lived association of the enzyme with the neuronal membrane /205/. This role of PKC is consistent with the observation that voltage- and Ca^{2+} -activated K^+ channels are transiently suppressed during the light response, but quickly return to baseline after light offset. Clearly, such a mechanism is not sufficient for the sustained reduction of K^+ currents associated with memory storage in these cells.

Since the activation of PKC has been strongly implicated as mediating the long-lasting reduction of K^+ currents that underlie memory storage in *Hermisenda*, these results have been viewed as

somewhat perplexing. A 1991 report shed some light on this anomaly /156/. Lester *et al.* reported that analogs of DAG were only weakly effective in inducing a reduction of K^+ currents across the B cell membrane. However, the combined application of DAG analogs and the fatty acid arachidonic acid (AA) caused a far greater reduction of the same currents. Interestingly, this signaling mechanism had recently been delineated by Nishizuka, who has demonstrated that the combined stimulation of PKC by DAG and AA induces a more stable bond between the kinase and the neuronal membrane which long outlasts the initial signals, possibly persisting until the kinase undergoes normal degradation /205/.

At the same time that Nishizuka had observed the synergistic effects of DAG and AA on PKC activation, some of the general characteristics of GABA-activated G-protein coupling mechanisms were becoming apparent. Importantly, Axelrod and his colleagues /26,50/ had reported that the G-protein that coupled the GABA_B receptor to its postsynaptic effectors stimulated the activation of PLA_2 , which through hydrolysis releases AA from its chemical linkage in the phospholipid bilayer. In the *Hermisenda* B cell and other cell types, AA is a weak or ineffective activator of PKC, though, as stated above, it can act synergistically with DAG to activate the kinase. Consequently, it appears that the strong and persistent activation of PKC by the convergence of Ca^{2+} , DAG, and AA during contiguous presentations of light and rotation might be sufficient to account for activity-dependent neuronal facilitation.

In support of this proposal, it has been reported that inhibition of the PLC signaling pathway eliminated light-induced depolarization in the B cell /234,261/. However, consistent with our assumption that the receptors which mediate hair cell-induced hyperpolarization stimulate a G-protein that is directly coupled to a K^+ channel, inhibition of PLC or PLA_2 had no effect on the hyperpolarizing response. Nevertheless, inhibition of both PLA_2 and AA breakdown blocked the increase in excitability that normally accompanies *in vitro* pairings of light and mechanical stimulation of the hair cells. This leads to the conclusion that although the G-protein that affects the postsynaptic hyperpolarization is directly coupled to a K^+ ionophore, it activates

PLA₂ as it does in other cell types, probably as a first step in other signaling pathways as described above. While these electrophysiological studies allow us to infer characteristics of this cascade, recently begun studies in our laboratory have confirmed that PLA₂ and GABA do indeed induce a rise in levels of free AA as indexed by fluorescent labeling (Ghandi, Talk, Muzzio, and Matzel, unpublished observations), providing further support for this hypothesis.

Although the above results are all consistent with the assertion that the convergence of AA, DAG, and Ca²⁺ prolongs the insertion of PKC into the lipid membrane (and thus its phosphorylation of K⁺ channels), these results do not provide unique support for this hypothesis. It has become clear in recent years that "PKC" is not a single enzyme, but instead represents a family of distinct isoforms /200/. The various isoforms in the PKC family exhibit distinct sensitivities to various combinations of each of these activators, and the route of activation as well as the isoform stimulated is a critical determinant of the enzyme's activity. Thus there are numerous routes by which the convergence of AA (as provided by rotation) and Ca²⁺/DAG (as provided by light) might influence the sustained activity of PKC, depending on the isoenzyme expressed in the target cell, and further work will be required before this signaling pathway is fully elucidated in *Hermisenda* B photoreceptors. A detailed discussion of the family of PKC isoforms and their routes of activation is provided below in Section 4.4.

It should be noted that associative learning in *Hermisenda* has been found to cause a two-fold increase in the expression of a 20 kDa G-protein with characteristics similar to V-ras oncoproteins /201-203/. Isolation of this protein and micro-injection into B photoreceptors of untreated animals results in a reduction of the same K⁺ currents reduced by associative training /202/. While the exact function of this protein is uncertain, it is interesting to note that oncogene activity is often associated with increased levels of unbound DAG and AA in other cell types /93,94/, and thus the expression of this protein induced by associative conditioning may serve to maintain the facilitation of the B cell for prolonged periods.

Although we have alluded to the role of both GABA and 5-HT in the induction of facilitation in the B photoreceptor, we should explicitly state that we are uncertain as to whether one or both of these transmitters is critical to this process. At present, little is known about the 5-HT signaling cascade (although it is G-protein dependent), and we have added it to our model for illustrative purposes. It should be noted that several reports indicate that 5-HT receptor antagonists (but also *agonists*) may attenuate the induction of facilitation in the B cell /118/, and 5-HT paired with light can induce an increase in several indices of excitability /75,77/, but so too can 5-HT application in the absence of light /118/. Based on these data, at least two molecular descriptions of neuronal facilitation in *Hermisenda* have been proposed that rely exclusively on 5-HT's role in that process /66,74/. Although GABA paired with depolarization can induce short-term increases in B cell excitability /179/, effective antagonism of this GABA receptor has not yet been accomplished, so an assessment of its role in facilitation under physiological conditions is not yet certain. Since both 5-HT and GABA may stimulate a similar class of receptor-coupled G-proteins, it is possible that these transmitters have an additive effect, given that it has been demonstrated that direct stimulation of G-proteins in combination with an increase in intracellular Ca²⁺ reduces K⁺ conductance in a manner analogous to classical conditioning /186/. Although our description of the events underlying learning in *Hermisenda* are limited by these caveats, they are not critical to our primary objective, which is to illustrate the functional similarity of learning-induced modulation of *Hermisenda* B photoreceptors to other activity-dependent forms of facilitation, a comparison to be made explicit in the conclusion of this paper.

4. HIPPOCAMPAL LONG-TERM POTENTIATION

Following a collaboration with Per Anderson, long-term potentiation (LTP) was first formally described by Bliss and Lomo in 1973 /37,38/. LTP is typically expressed as an increase in synaptic efficacy lasting from hours to days following brief tetanic (high-frequency) stimulation of an afferent pathway. Thus, following LTP induction, a constant

amount of presynaptic stimulation induces a "potentiated" post-synaptic response, e.g., an increase in amplitude of evoked EPSPs. Although LTP is commonly referred to as a likely mechanism of memory storage, direct evidence in support of this claim is not available and available evidence even contradicts this assertion (e.g., /30,121,206,236/), leading some to conclude that LTP is poorly suited for this role /190,245/. This failure to find conclusive evidence in support of a role for LTP in memory stems in part from the inability to identify single neurons in the mammalian brain, in combination with the extreme complexity of mammalian memory, difficulties not encountered by researchers studying invertebrates. Since the data which link LTP to memory formation are correlational and are subject to multiple interpretations /30,236/, we will not attempt to review the evidence here (for recent reviews, see /177,245/). Nevertheless, LTP in the hippocampal formation provides a convenient preparation to study neuronal or synaptic facilitation in the mammalian brain, and thus provides some point of comparison to the invertebrate models described above.

Long-term potentiation, in various forms, can be induced at the three major synaptic connections of the hippocampus, a brain region considered critical to the acquisition or processing of some forms of memory /242/. LTP has been observed in dentate gyrus granule cells following strong stimulation of the perforant path as originally described by Bliss and Lomo /38/, in CA3 pyramidal cells following stimulation of the mossy fibers /7,274/, and in CA1 pyramidal cells by stimulation of the Schaffer collateral branches of the CA3 neurons /20,241/. In addition to its presence in the hippocampus, LTP in various forms has been observed in a wide range of other subcortical as well as cortical brain areas (see /245/ for review) and can thus potentially play a widespread role in brain plasticity.

Consistent with a point made in the introduction of the present paper, the cellular and molecular events that underlie LTP induction at different synapses within the hippocampal formation and throughout the brain are tremendously diverse, and no single mechanism is representative of the general phenomenon. Thus the term "LTP" has been suggested to have little descriptive value /245/. This however illustrates our premise that the specific

molecular constituents of any single mechanism are not likely to represent *the* mechanism underlying the storage of associative (or other) memories. Rather, it may be beneficial to identify a *class* of features which is conserved across each of these various mechanisms.

4.1 Postsynaptic Ca^{2+} and LTP induction

We focus here on two mechanistically similar and historically prototypical forms of hippocampal LTP which are observed in the dentate gyrus granule cells and CA1 pyramidal cells. For simplicity, we often refer to these forms of LTP as "hippocampal LTP", although it is clear that LTP in CA3 pyramidal cells is in some ways dissimilar /133,134/. The forms of LTP that we describe (as well as most characterized forms) share a common requirement for a transient elevation of postsynaptic Ca^{2+} (but see /235/).

Much of the evidence implicating biochemical processes in LTP induction has been inferred from pharmacological manipulations of the phenomenon itself as opposed to having been directly observed biochemically (as was also the case for *Hermissenda*), leading to many conflicting accounts of the molecular constituents of LTP. Although the exact role of Ca^{2+} in LTP induction continues to be a matter of some debate, elevation of postsynaptic Ca^{2+} is clearly necessary and may in some instances be sufficient for its induction. For example, induction of LTP between Schaffer collaterals and CA1 pyramidal cells is prevented by a pretetanus injection of Ca^{2+} chelators into the postsynaptic cell /165,171/, and induction occurs when the postsynaptic cell is artificially loaded with the ion /171/. A great deal of evidence (e.g., /70,123/) indicates that one source of the intracellular Ca^{2+} signal during the induction of hippocampal LTP arises from influx through an ion channel coupled to the NMDA subtype of glutamate receptor. This receptor is unique in that stimulation of the channel ionophore requires glutamate binding as well as a moderate level of depolarization. At normal resting potentials (≈ -70 mV), the channel is blocked by magnesium ions, and glutamate binding is insufficient to open it. However, at depolarized membrane potentials (> -40 mV), magnesium is expelled from the pore, allowing glutamate to open the channel,

which is primarily permeable to Ca^{2+} ions. Thus, the NMDA receptor complex is said to be dually regulated by two factors: ligand and voltage. These cofactors can be recruited through several means. First, a relatively long, high intensity presynaptic burst of activity (such as a high-frequency train of stimulation) can induce LTP by releasing glutamate onto the postsynaptic receptor while simultaneously depolarizing the postsynaptic cell through stimulation of the non-NMDA type of glutamate receptors (AMPA) which conducts Na^{2+} . Second, and more physiologically relevant, shorter and less intense levels of presynaptic activity can induce hippocampal LTP if the postsynaptic cell is depolarized via an alternative means such as an input from a second afferent pathway. As alluded above, LTP can be induced without the participation of NMDA receptors, provided that the tetanus (or postsynaptic depolarization) is of an intensity to induce a sufficient Ca^{2+} load via influx through voltage-dependent Ca^{2+} channels /119,148/. Thus, while activation of the NMDA receptor may be critical to these (but not other) forms of LTP, even in these cases it may not be necessary. The consequence of NMDA receptor stimulation, a rise in intracellular Ca^{2+} , appears to be the critical element necessary for initiation of most forms of LTP.

4.2 Ca^{2+} -dependent biochemical processes underlying potentiation

A generic model of LTP induction in the hippocampus (excluding CA3 pyramidal cells) is depicted in Figure 5. Since a rise in postsynaptic Ca^{2+} is presumed necessary for the induction of LTP, much emphasis has been placed on the elucidation of the underlying Ca^{2+} -dependent processes. Several different approaches have been taken to achieve this goal, including the application of inhibitors of Ca^{2+} -dependent enzymes, the direct activation of Ca^{2+} -dependent enzymes in the postsynaptic cells, biochemical assays of enzyme activity and distribution, and most recently, the ablation of genes that encode specific Ca^{2+} -dependent enzymes. In such a complicated system, each of these approaches are subject to interpretative difficulties (see reviews by /85,245/), but collectively they strongly implicate a role for Ca^{2+} -dependent

enzymes in the induction of LTP. For instance, it has been demonstrated that introduction of activated Ca^{2+} /calmodulin-dependent kinase (CaMK) into the postsynaptic cell induces LTP and blocks LTP induction by afferent stimulation (i.e., acts as a competitive antagonist /216/), and that peptidergic inhibition of calmodulin blocks LTP induction /170/. Moreover, mice deficient in the gene that codes CaMK are similarly deficient in hippocampal LTP as well as the capacity to acquire new (hippocampal dependent) spatial memories /189,247,248/.

Though suggestive, any conclusion based on the above observations is complicated by similar experiments that indicate an analogous role for cAMP-dependent kinases /62,108,219/ and for PKC /1,172,174,224/, as well as other kinases /207,280/. Since these kinases contribute in many ways to regulatory functions, signal transduction cascades, and normal synaptic transmission, it cannot be said with certainty that their inhibition does not affect processes that are only indirectly relevant to LTP. In this regard, it is important to note that it has been demonstrated that induction of hippocampal LTP is accompanied by a translocation of PKC from the cytosol to the membrane of postsynaptic cells /6, 22,142/. PKC (among other kinases) is an allosteric enzyme, and in the typical isoforms, its activation is dependent on (and indicated by) its interaction with membrane lipids /205/. Similarly, it has been observed that PKA activity increases during the induction phase of LTP /227/. These observations suggest that PKC and/or PKA are activated by stimulation protocols sufficient for the induction of LTP, and are consistent with a specific role for these Ca^{2+} -dependent kinases in this form of facilitation. At present it is not clear whether these kinases act on similar or independent substrates, although it has been suggested that they may play separate roles in the induction and maintenance phases of LTP /131,204/.

4.3 Stimulus convergence and LTP induction

Forms of LTP that are normally induced by prolonged stimulation of the NMDA receptor do not necessarily require the convergence of multiple inputs onto a single target cell for induction. In fact, in their first full report on LTP, Bliss and Lomo /38/ demonstrated that high frequency stimulation

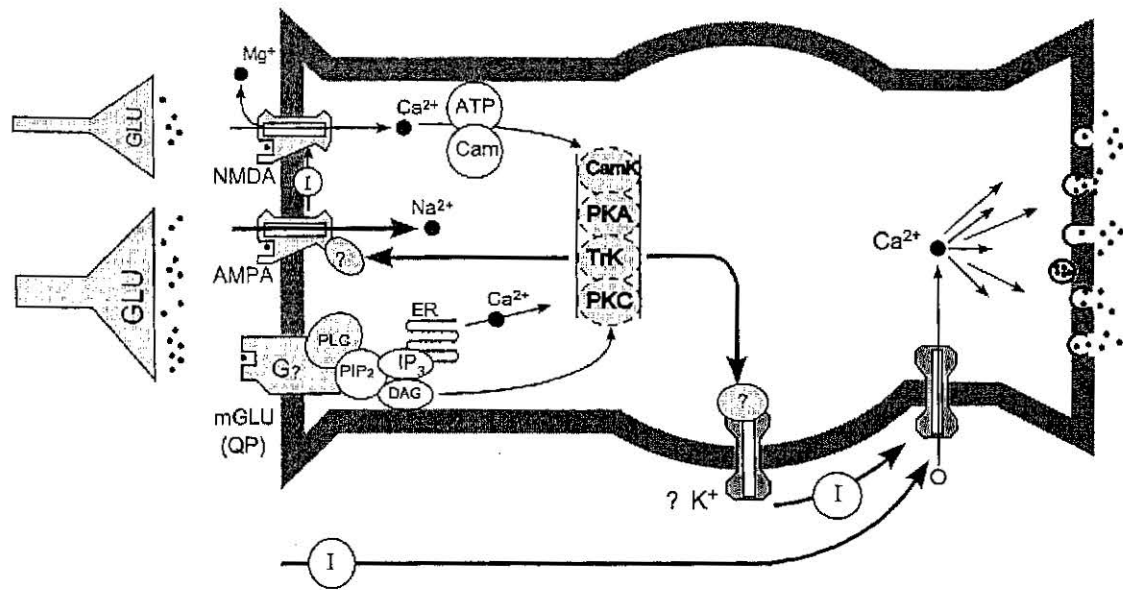


Fig. 5: Molecular constituents of hippocampal long-term potentiation (LTP). Glutamate (GLU) binds to three classes of receptors which tend to be colocalized on these cells. Ionotropic AMPA receptors are directly coupled to a Na⁺ channel which mediates fast synaptic response and induces postsynaptic depolarization via the resultant EPSP. Likewise, the ionotropic NMDA receptor is stimulated directly by glutamate, but will not conduct current unless the postsynaptic membrane is also depolarized, which induces a voltage-dependent expulsion of Mg²⁺ ions which otherwise clog the channel. When open, the channel conducts a large Ca²⁺ current. The metabotropic glutamate receptor (mGlu) contributes little to postsynaptic voltage responses but stimulates a PLC transduction cascade which cleaves IP₃ and DAG from PIP₂, generating a release of Ca²⁺ from intracellular pools as well as the potential activation of PKC (by DAG and Ca²⁺). At present, the relative contribution of these receptors to LTP is uncertain, and stimulation of either the NMDA or mGlu receptor in isolation can induce LTP, although probably through independent mechanisms. Regardless, LTP induction requires the interaction of Ca²⁺ (and probably DAG) with several classes of protein kinases, and the activity of at least two of those kinases (PKC and PKA) is increased by the induction protocol. Moreover, although it is well established that the expression of LTP involves an increase in conductance through the AMPA receptor, other modifications (not depicted) of both pre- and postsynaptic function have been reported to contribute to the potentiation of the EPSP indicative of LTP. Conceptually, associative LTP can be viewed as the sufficient stimulation of any two receptor classes to induce a large rise in intracellular Ca²⁺ concentration. This can also occur through the weak stimulation of the NMDA receptor combined with postsynaptic depolarization arising from an independent pathway. However, even levels of synaptic transmission sufficient to initiate Ca²⁺ flux through the NMDA channel may be insufficient under normal conditions to induce LTP *in vivo*, suggesting a potentially critical role for the generation of interactive second messengers by conjoint stimulation of the mGlu receptor. Since these receptors are colocalized and may be stimulated by a single input, associative LTP is more representative of the additive effects of an increased level of stimulation, as opposed to the synergistic mechanisms suggested to underlie associative neuronal facilitation in *Aplysia* and *Hermisenda*. Still, one way to induce or enhance the induction of LTP, is through the conjoint actions of Ca²⁺ influx and stimulation of a G-protein-dependent transduction cascades. Such an interaction of these two receptor classes may represent the most common and/or efficient induction mechanism *in vivo*. In this regard, it should be noted that high levels of intracellular Ca²⁺ is in itself sufficient to induce a reduced form of facilitation in both *Aplysia* and *Hermisenda*, but sufficiently high levels are not likely to occur under physiologically relevant conditions.

of an afferent pathway was in itself sufficient for LTP induction. As described above, this potentiation arises from a combined influx of Ca²⁺ through NMDA and voltage-dependent channels, and the resultant activation of Ca²⁺-dependent kinases.

However, the afferent volley necessary to induce potentiation may not commonly occur under physiological conditions, or may be insufficient to induce sustained potentiation /152,168,169/. Consequently, it is useful to think of tetanus-induced

LTP as an extreme version of a mechanism that may be recruited under different conditions in the behaving animal. These conditions may take one of several forms. Commonly, LTP is described as being induced "associatively", a term borrowed from the Pavlovian conditioning literature. When discussing LTP, associativity refers to the observation that roughly contiguous, low intensity stimulation of two pathways, or higher intensity stimulation of convergent weak inputs are sometimes sufficient for the induction of LTP when stimulation of neither pathway alone is sufficient /32,159/. In some instances, this associative property may be a reflection of the same underlying mechanism as the strictly Ca^{2+} -dependent form of LTP described above, that is, converging inputs onto a single postsynaptic target may generate a sufficiently large intracellular Ca^{2+} signal (often through the combined contribution of depolarization-induced Ca^{2+} influx and activation of the NMDA receptor) to activate postsynaptic kinases. The existence of this associative feature of LTP suggests that LTP may be unlikely to result from normal synaptic transmission, but rather, might be reserved for detection of spatially and temporally contiguous events (i.e., as in associative learning).

Although associative LTP can arise solely as a result of the summation of postsynaptic Ca^{2+} signals, it may in some instances reflect a mechanism similar to those described above underlying learning in *Hermisenda* and *Aplysia*. Regarding this point, it is useful to note that allosteric protein kinases may have varying "degrees" of activity. As described above, for instance, PKC activation by DAG and Ca^{2+} is accompanied by a relatively weak association with the neuronal membrane that is enhanced and prolonged by AA /205/. Since DAG and AA generation occur in response to stimulation of receptor-coupled G-proteins, these cofactors for the activation of PKC might act in combination with NMDA-dependent Ca^{2+} influx to synergistically activate PKC during associative LTP induction. Numerous studies provide indirect support for such a hypothesis, including the observation that LTP in CA1 pyramidal cells can be induced or enhanced by direct stimulation of metabotropic glutamate receptors with the agonist ACPD /43,44/, and that ACPD-induced long-lasting potentiation occurs in the absence of the short-term potentiation that normally

accompanies tetanus-induced LTP. Moreover, ACPD-induced potentiation occurs in the presence of NMDA receptor antagonists /42,43/, and in the presence of antagonists of the metabotropic glutamate receptor; tetanizing stimulation of the afferents to CA1 pyramidal cells induces short-term potentiation but no persistent potentiation. These results suggest that although the NMDA receptor is necessary for short-term potentiation and contributes to LTP through the generation of a postsynaptic Ca^{2+} signal, persistent LTP requires the coactivation of metabotropic glutamate receptors and the resultant generation of a second messenger signal.

Consistent with the proposed role of metabotropic receptors in LTP, it has been demonstrated that a complex of aluminum and fluoride ions (AlF_4^-), which activates G-proteins by simulating the terminal phosphate of GTP, induces LTP in hippocampal slices /218/, and that this induction is not dependent on Ca^{2+} flux through the NMDA receptor activator but does require the presence of Ca^{2+} in the bathing medium as well as the stimulation of phosphoinositide signaling pathways /48/. It should be noted that in the case of AlF_4^- and ACPD-induced potentiation, LTP can be induced either through a G-protein mediated release of Ca^{2+} from intracellular storage pools /36,192/ or through the G-protein mediated generation of second messengers which in combination with Ca^{2+} synergistically activate or prolong the activation of a protein kinase. In the case of ACPD, it is known that it induces a release of Ca^{2+} from intracellular stores /107/. However, this Ca^{2+} signal alone is unlikely to be solely responsible for the induction of LTP, in that it is not sufficient to induce transient short-term potentiation. More likely, the phosphoinositide signaling pathway responsible for the stimulation of intracellular Ca^{2+} pools also generates the release of Ca^{2+} -sensitive second messengers such as DAG, which in turn could activate kinases such as PKC. The possibility that the metabotropic glutamate receptor contributes to the induction of LTP through the activation of PKC has previously been suggested by Collingridge and colleagues (e.g., /41,43/). The potential role of allosteric activation of protein kinases by Ca^{2+} and phospholipase-derived second messengers in LTP induction was previously proposed by Lester and Bramham /155/,

and is consistent with the observation that inhibition of PLA₂ effectively blocks LTP induction /178,210/. Although the exact contribution of such an allosteric mechanism to LTP induction is unknown, analogous signaling pathways are relatively common /140/ and play a central role in a number of phosphorylation events that may be critical to LTP induction (e.g., /67/).

4.4 Multiple roles of PKC in LTP

Although at present it is not entirely clear how the various protein kinases implicated in LTP induction interact to induce or maintain LTP, the role of PKC has been particularly well characterized in recent years and provides a representative framework for considering the contribution of various biochemical cascades to enzyme activity. Initial pharmacological evidence for the role of PKC in LTP induction or maintenance was somewhat perplexing, and contradictory results were often obtained with different inhibitors of the enzyme and with different modes of drug application. Although these discrepancies were often attributed to the lack of specificity of some inhibitors, they also raised the possibility that different isoenzymes of PKC, with distinct pharmacological sensitivities, might be activated during various LTP induction protocols, and moreover, might contribute to different stages of LTP maintenance /23,147/.

The various isoforms which comprise the PKC family vary in structure, and these structural variations impart each isoform with the requirement for specific combinations of cofactors for activation /205/. At present, identified isoforms of PKC can be divided into three groups, described as conventional (cPKC), novel (nPKC) and atypical (aPKC). All the subspecies require phosphatidylserine (PS) for their activation but display differential requirements for lipids and Ca²⁺. The cPKC family requires Ca²⁺ and diacylglycerol (DAG) for activation, and activation can be enhanced by cis-unsaturated fatty acids (UFAs; e.g., AA), such that in the presence of UFAs, this group of isoenzymes can be activated at basal levels of Ca²⁺ /244/. The nPKC family does not require Ca²⁺ for activation and different isoforms respond differently to UFAs. Activation of isoforms in the aPKC group is independent of both Ca²⁺ and DAG, but is uniquely dependent on UFAs

/139/. This diversity in the modes of activation reflects the heterogeneity of the structures of these enzymes, and is suggestive of many potential routes of activation during an LTP induction protocol.

Recently, direct measurements of PKC using specific antisera /232/ and assays of enzyme activity using specific PKC substrates /141/ began to elucidate the inconsistencies suggested by earlier work based on the pharmacological manipulation of PKC. In these studies, sustained PKC activity has been observed during the maintenance phase of LTP that persists in the absence of Ca²⁺ or other PKC activators, suggesting that the enzyme becomes autonomously active during this phase. Moreover, it has been suggested that the proteolytic cleavage of the regulatory domain of one PKC isoform (zeta) could result in a persistently active catalytic subunit. These latter results might explain earlier observations that drugs which inhibit the enzyme at its catalytic domain block the maintenance of LTP whereas regulatory inhibition is typically ineffective at this stage. Consistent with this possibility, it has been reported /212/ that the degree of potentiation observed during early maintenance of LTP correlates with an increase in PKCzeta. As noted above, the various isoforms of PKC are stimulated by different combinations and subsets of activators. In total, these results suggest that in addition to its role in LTP induction, various forms of LTP expression and maintenance may result from the combinatorial effects of different converging biochemical pathways. It should be recalled that a similar conclusion was arrived at in our previous descriptions of learning in *Hermissenda* and *Aplysia*.

In total, studies directed at the elucidation of the mechanisms underlying associative LTP suggest the possibility that the combined action of Ca²⁺ and receptor-generated second messengers may serve to induce or prolong the expression of LTP. Although a general class of phenomena referred to as LTP appear to be induced by many different protocols, "associative" LTP may be induced most readily and under physiological conditions if an elevation of intracellular Ca²⁺ and stimulation of G-protein-coupled receptors co-occur in the same post-synaptic cell. Such an induction protocol may more efficiently activate a single class of protein kinase, or alternatively (and as suggested by several

studies) may activate protein kinases that are not activated by either signal in isolation. In this regard, LTP is conceptually similar to the forms of activity-dependent facilitation described in *Aplysia* and *Hermissenda*.

4.5 Biophysical mechanisms underlying the expression of LTP

In both *Hermissenda* and *Aplysia*, activity-dependent facilitation arises in large part from the kinase-induced phosphorylation of ion channel proteins which modulate excitability and/or action potential duration. To this point, we have intentionally avoided any discussion of the substrates for the maintenance of potentiation following stimulation protocols that induce LTP. The expression of LTP reflects both enhanced conductance at specific synaptic terminals that are active during the induction protocol, as well as a more general increase in excitability across the postsynaptic membrane /38/. These two components of LTP are likely to interact and thus may complicate any attempt to assess the biophysical substrates of LTP. Moreover, LTP is measured in a number of ways, including (but not limited to) the amplitude of the postsynaptic population spike, the population EPSP, and EPSPs recorded intracellularly in single postsynaptic cell bodies or axons, all of which may reflect varying combinations of interactive mechanisms. Adding to these concerns, biophysical analysis of single synaptic terminals is technically difficult and has only recently become practical. As a result, a consensus view regarding the biophysical substrates of LTP expression has not emerged. It has been suggested that LTP expression is: 1) an entirely presynaptic phenomenon that results from an increase in the number or quantal content of transmitter vesicles released upon stimulation /255/ (but see /132/); 2) an entirely postsynaptic phenomenon resulting from an increase in number or kinetics of transmission in postsynaptic AMPA (ionotropic glutamate) receptors /19,160,252/, or 3) a combination of pre- and postsynaptic mechanisms that contribute to both an increase in transmitter release as well as enhanced receptivity at the postsynaptic terminal /254/ (for review, see /173/).

In addition to specific increases in synaptic efficacy, LTP induction is accompanied by an

increase in excitability that is not limited to synaptic regions. This was first demonstrated by Bliss and Llomó /38/, who reported that a decrease in the threshold for the elicitation of a spike was observed even when the amplitude of the synaptic response was held constant. In fact, although this widespread increase in excitability always accompanies synaptic enhancement, it can be observed in the complete absence of synaptic enhancement /20,61,262/ and is accompanied by a decrease in spike-rate accommodation /39/. These observations are reminiscent of the increases in excitability observed in both *Aplysia* and *Hermissenda*, and suggest the possibility that LTP is associated with a decrease in K^+ conductance across the postsynaptic membrane, although no direct evidence has been reported (cf. /60/). In summary, it appears that the expression of LTP is composed of at least two components, and while the increase in synaptic efficacy may in part reflect a more diffuse increase in excitability, the diffuse increase in excitability can clearly occur independently /262/.

At this point, no singular description of the substrates of LTP maintenance has emerged, and we will not attempt to review further the current status of this literature. However, we will assume that LTP requires a transient rise in postsynaptic Ca^{2+} concentration, a concomitant generation of receptor-coupled second messengers, and a resultant activation of one or a class of protein kinases. Moreover, LTP induction and possibly its maintenance is accompanied by a postsynaptic phosphorylation event that results in increased transmission through AMPA-coupled receptors as well as a more general increase in excitability that may involve the reduction of K^+ conductances.

5. UBIQUITOUS MECHANISMS UNDERLYING ACTIVITY-DEPENDENT FACILITATION

A number of similarities in the cellular mechanisms of learning-related forms of synaptic and/or neuronal facilitation have become apparent. As a heuristic convenience, features of activity-dependent facilitation can be categorized into several distinct facets, including the nature of the network interactions necessary for facilitation, the biochemical and molecular events that induce

facilitation, and the biophysical targets of facilitation. We briefly review the degree to which the examples of facilitation described here are analogous with regard to each of these characteristics.

5.1 Network interactions underlying activity-dependent facilitation

Activity-dependent facilitation, as first delineated in *Aplysia*, is in many respects similar to the mechanisms underlying neuronal plasticity in other models of associative learning (i.e., *Hermisenda* and some forms of hippocampal LTP). Importantly, in all systems, a transient increase in intracellular Ca^{2+} in combination with stimulation of G-protein coupled surface receptors results in the synergistic modification of membrane proteins which enhances synaptic efficacy, increases cellular excitability, reduces the threshold for the elicitation of an action potential, or modifies the voltage kinetics of the action potential. Although these various forms of facilitation arise from similar network interactions, the exact nature and locus of the modification appears dependent on the cell type, available second messenger cascades, and particular substrate proteins. Somewhat ironically, one principle that emerges from this review is that the network interactions that give rise to memory expression in various systems and between cell types are quite diverse and sometimes even complimentary within a single system. Consequently, the elucidation of a singular mechanism of memory *expression* at the level of single neurons appears unlikely (see also /51/).

While the network interactions underlying induction of facilitation in the systems reviewed here share common features, the different mechanisms of expression have important implications regarding the function of a neural network. For instance, LTP is commonly lauded as a potential memory mechanism because it is "synapse specific" and thus can encode complex yet subtle changes in a neural network that are considered necessary to represent a memory. Likewise, synapse-specific modifications could support a greater potential for plasticity than would modifications that occur more diffusely across a cell body. These supposed features of LTP clearly distinguish it from learning in *Hermisenda*, for which there is no evidence of

synapse specificity, as well as in *Aplysia* for which there are both cell-wide and synapse specific components of facilitation /65/. However, even in the case of LTP, the plastic modifications that accompany an induction protocol are not limited to synapses and are accompanied by enhanced somatic excitability (see discussion above). Moreover, LTP can spread to synapses in adjacent cells not directly stimulated during the induction protocol /40,240/, and unilateral LTP induction can induce bilateral changes in mRNA expression that may alter cell excitability /47,249/. Thus, although LTP is often cited as a synapse-specific mechanism, direct evidence indicates that LTP expression involves a more pervasive modulation of cellular excitability. Consequently, it may be premature to assume that memory storage devices will be limited to synapse-specific modifications of neural transmission. It is interesting to note that no direct evidence indicates that memories are encoded exclusively as alterations in synaptic efficacy (cf. /211/), and an explicit feature of some network models is that the modulation of excitability along somatic membranes may be sufficient to encode memories /263/.

The form, site, or combination of sites that express the plastic modification associated with memory storage have effects on the function of a neural network that go beyond their implications for synapse specificity. For instance, a decrease in spike elicitation threshold or accommodation that results from the reduction of some classes of K^+ currents will result in an increase in frequency of postsynaptic potentials but not an increase in their amplitude. However, the reduction of a voltage-dependent K^+ current that opposes an action potential might result in both a decrease in threshold and accommodation (as a result of an increase in membrane resistance) while *also* prolonging the action potential. In this latter case, both the frequency and amplitude of postsynaptic responses might be enhanced. In contrast, an increase in transmission specific to the synapse, or an increase in postsynaptic receptivity, would result in an increase in amplitude of the postsynaptic response with no increase in the frequency of responses. Functionally, the effect of these plastic modifications will depend on the integration of changes in spike frequency, width, and accommodation, as well as the resultant change in amplitude and frequency

of potentials in the effector cells. Since some combination of these modifications occurs in each of the systems reviewed here, proposed descriptions of their combined effects in a complex network of cells is in large part conjecture. Even in the invertebrate models in which input and output pathways are reasonably well defined, it is generally conceded that the various "learning networks" that have been proposed should be considered as simplified illustrations /230/ as opposed to literal descriptions. Given the number of cells and synapses in a functional network of cells, and the number of permutations of each of the potential sources of plasticity, network variations that account for the *expression* of a stored memory may prove to be as intractable as some have suggested (e.g., /163, 176/). Likewise, hypothetical neural networks based solely on synaptic efficacy which ignore the dynamics of the cells within the network are most certainly inadequate as heuristic devices.

In total, although the network interactions which underlie the *induction* of various forms of facilitation share important similarities, the mechanisms which contribute to the expression of that facilitation are more idiosyncratic. This lack of concordance is not surprising given the diversity of second-messenger cascades and protein substrates within the nervous system. It does however complicate our understanding of memory, and in part reflects on our limited understanding of the true nature of memory. It should be recognized that descriptions of memory expression, even in the relatively well defined invertebrate nervous systems considered here, are in fact descriptions of the modification of a behavioral *response*, and although indicative of memory, are not in themselves descriptive of the psychological process of *remembering*. (It must be noted that a behavioral change can be *indicative* of memory, but a memory does not require any associated behavioral change, and a behavioral change in and of itself is not memory /194,222/.) A similar argument could be made in regard to the well delineated cerebellar network believed to subserve acquisition of the classically conditioned nictating membrane response in rabbits /153,253/. Although that network accounts for the generation of the conditioned nictating membrane response, it is clear that the "memory" of the predictive relationship between the CS and US does

not necessarily reside in the cerebellar circuit responsible for the generation of that response /237/. In short, the expression of memory and the psychological process of remembering is complex, and the elucidation of these processes will require a better understanding of the dynamics of functional neural networks.

5.2 Biochemical and molecular events that underlie facilitation

In spite of our failure to understand the emergent properties of neural networks that account for memory, a critical network interaction appears to play a central and ubiquitous role in the induction of activity-dependent facilitation. Specifically, in each of the systems that we have described, conjoint pre- and postsynaptic activity either induces or augments neuronal/synaptic facilitation. While the specific biochemical and molecular substrates of this stimulus convergence differ in each system, the dissimilarities are eclipsed by the more general concordance of the three systems. Inherent to each of these systems is the necessity for a transient rise in intracellular Ca^{2+} in the cell or synapse that expresses the plastic modification. Based on the sufficiency of postsynaptic Ca^{2+} to induce a reduced form of facilitation, the modifications in *Aplysia* sensory neurons, *Hermisenda* photoreceptors, and hippocampal CA1 pyramidal cells or dentate granule cells can be classified into different forms of facilitation. In each case, a strong Ca^{2+} load in the target cell may be sufficient to induce a reduced form of facilitation even in the absence of any converging input onto that target (an *in vitro* analog of nonassociative learning). For instance, in *Hermisenda*, strong depolarization of the B photoreceptor (in the absence of input from the presynaptic hair cells) is sufficient to induce a transient reduction of neuronal K^+ conductance /12,16/, an increase in neuronal excitability and responsiveness to light /102,183/, as well as a modification of light-elicited behaviors in the intact animal /102/. Likewise, in *Aplysia*, high frequency activity and a resultant Ca^{2+} load in the siphon sensory neuron is sufficient to induce a transient broadening of evoked action potentials /91/ of a magnitude similar to that which results from sensitization training. In the case of

LTP, the "nonassociative" form of potentiation was described prior to any reports of the associative induction protocol. For instance, a presynaptic volley sufficient in itself to strongly depolarize the postsynaptic target induces LTP [38,165], as does strong depolarization of the target cell independent of presynaptic stimulation [120], and both of these effects are dependent on a rise in postsynaptic intracellular Ca^{2+} . As described above, all of these "nonassociative" forms of facilitation have been attributed to a Ca^{2+} -dependent activation of various protein kinases and a resultant phosphorylation of ion or receptor channels (or their precursors).

Each of the nonassociative forms of facilitation described in the preceding paragraph can be enhanced, prolonged, or the Ca^{2+} requirement reduced, if the Ca^{2+} signal coincides with the stimulation of G-protein-coupled receptors, a second ubiquitous feature of these model systems. In the case of both *Hermisenda* and *Aplysia*, the enhancement of the Ca^{2+} signal by contiguous stimulation of a G-protein-coupled receptor is attributable to a conceptually similar mechanism. In *Aplysia*, stimulation of the tail activates "facilitatory" interneurons that project serotonergic fibers to sensory neurons (see Figure 1). Serotonergic receptors are coupled to the catalytic subunit of adenylate cyclase via an intermediary G-protein. Binding of the transmitter stimulates the cyclase to produce cAMP and cleaves DAG from PLC, each of which activate protein kinases (PKA and PKC, respectively) that phosphorylate two classes of K^+ channels. In a resting sensory neuron, the resultant prolongation and decreased threshold for the elicitation of action potentials tends to be short-lived (e.g., up to 24 h, depending on the stimulation protocol) and the effects are rather modest. However, if the sensory neuron is active at the time of 5-HT binding, the activity is accompanied by an influx of Ca^{2+} through voltage-dependent channels. This conjoint influx of Ca^{2+} with the stimulation of the receptor-coupled G-protein potentiates the activity of cAMP and DAG. The increased activity of cAMP and stimulation of multiple PKC isoforms results in a prolonged and enhanced facilitation of the sensory neuron (see Figure 2).

Unlike in *Aplysia*, in *Hermisenda* no facilitation arises as a result of presynaptic stimulation alone. Rather, presynaptic stimulation of vestibular hair

cells results in the release of GABA onto the terminal branches of the B cell which in turn increases K^+ conductance across the B cell membrane via the stimulation of an intermediary G-protein. (Any effect of hair cell activity-induced 5-HT binding on K^+ conductance is comparatively small and cannot be directly observed under physiological conditions, i.e., during costimulation of GABA receptors [229].) A typical effect of the stimulation of the receptor-coupled G-proteins is the release of AA from its chemical linkage in the bilayer. When the sensory neuron (B cell) is active, as during light-induced depolarization, the activity is accompanied by a rise in intracellular Ca^{2+} (arising from both voltage-dependent influx and intracellular release) as well as a cleavage of PIP_2 into IP_3 (augmenting the Ca^{2+} signal by release from intracellular stores) and DAG. High Ca^{2+} levels alone may cause a weak and transient reduction of several voltage-sensitive K^+ channels on the cell membrane; however, Ca^{2+} in combination with DAG and AA serves as a potent activator of PKC. This route of activation, unlike the activation induced by light alone, is presumed to result in the activation of unique PKC isoforms and/or a more prolonged association of the kinase with the neuronal membrane. In the membrane, the phosphorylation of K^+ channels results in an increase in action potential amplitude and rate of discharge, as well as a decreased threshold for elicitation (see Figure 4).

It is apparent that in both *Hermisenda* and *Aplysia*, activity-dependent facilitation represents an amplification of a molecular process which can be initiated nonassociatively, and that this amplification arises from the synergistic action of G-protein stimulated second messengers and high intracellular Ca^{2+} levels. Interestingly, in both intact *Aplysia* and *Hermisenda* it is the CS that induces activity in the sensory neuron which underlies the rise in intracellular Ca^{2+} . However, in the intact *Hermisenda* the CS (light) induces a transient facilitation of the B cell, while in *Aplysia*, it is the binding of transmitter to the sensory neuron (arising from the tail shock US) that induces short-term non-associative facilitation (but see [91]). This illustrates the arbitrary nature of terms like "CS" and "US" and suggests that neither possess specific cellular properties. Rather, our use of the terms CS and US reflects a convenience based on the laboratory

paradigm and the parameters of a particular experiment. This same logic has been applied to theoretical accounts of learning in which the terms "CS" and "US" have been suggested to have no inherent meaning (/86,222/; see /182/ and /238/ for empirical analogs). Rather than imagine that a cellular process is based on properties of a CS or a US, it may be more instructive to assume that behaviors of the organism indicative of the properties of the CS and US arise from higher-order processes that emerge from the animal's attempt to represent its environment in combination with the specific architecture of its neural network /163,230/.

As in both *Aplysia* and *Hermisenda*, hippocampal LTP results from a Ca^{2+} -dependent molecular cascade which culminates in the activation of protein kinases and the phosphorylation of cytosolic and/or membrane proteins (see Figure 5). Unlike *Hermisenda* and *Aplysia*, however, the synaptic facilitation that is indicative of LTP may not require the phosphorylation of K^+ channel proteins, although at present the mechanism responsible for the increase in somatic excitability that accompanies LTP is unknown. Moreover, unlike the facilitation observed in *Aplysia* and *Hermisenda* sensory neurons, it appears that the synaptic facilitation indicative of LTP can be induced in its most robust form homosynaptically, that is, strong afferent stimulation *can* induce a form of potentiation that is as persistent as the potentiation that might follow an associative LTP protocol. As described above, this conclusion must be qualified, given that a typical induction protocol involves the simultaneous stimulation of both ionotropic (NMDA sensitive) and metabotropic (ACPD sensitive) glutamate receptors. In *Aplysia* and *Hermisenda*, ionotropic receptors stimulated by the CS provide a Ca^{2+} signal while G-protein coupled receptors stimulated by the US generate the production of second messengers that interact with the Ca^{2+} signal to activate protein kinases. In the case of LTP, this relationship is less well delineated. Not only has LTP been induced by stimulation of either receptor in isolation, it is not yet clear whether the stimulation of the metabotropic glutamate receptor contributes second messengers which interact with the Ca^{2+} signal (an associative process), or whether the metabotropic glutamate receptors merely augment the Ca^{2+} signal (via stimulation of cytosolic storage pools) that

arises from stimulation of the NMDA channel. In either case, since both ionotropic and metabotropic receptors can be stimulated by a single afferent signal, even "associative" LTP can be induced without the convergence of independent signals as is required in the other models of activity-dependent facilitation described above. For these reasons, hippocampal LTP, often cited as a neurophysiological model of association formation, may not be well suited to serve such a function. It is clear, however, that levels of activity in single afferent fibers that occur *in vivo* may be insufficient in many instances to induce LTP. Thus, associative LTP, which *can* arise from the independent stimulation of ionotropic and metabotropic receptors via two convergent pathways, may be better suited for the induction of potentiation *in vivo*.

6.0 CONCLUSION AND SUMMARY OF UBIQUITOUS MECHANISMS

Table 1 lists a number of general characteristics of activity-dependent facilitation, and compares these qualities for the forms of facilitation described in *Aplysia*, *Hermisenda*, and hippocampal LTP. As was clear from the above descriptions, mechanistic details vary tremendously between these three models, as they often do between various forms of "LTP". It should be apparent from Table 1 that in spite of this variability, each of the model systems share striking global similarities. The single most ubiquitous feature of each of the molecular cascades is the requirement for a rise in intracellular Ca^{2+} well above resting levels. Not only is this true of the types of facilitation reviewed here, it is true of many other forms of neuronal and/or synaptic facilitation as well as other neuronal growth processes /113, 250/. Moreover, in each of the three systems reviewed, the rise in intracellular Ca^{2+} contributes to the activation of a serine/threonine protein kinase and a resultant phosphorylation of receptor channels or neuronal ion channels.

A second ubiquitous feature which emerges from this comparison is the dependence of these various systems on the conjoint stimulation of G-protein coupled receptors. While ionotropic receptors are better suited for the fast transmission of signals between cells, the dependence on and generation of

TABLE 1
Common features of activity-dependent facilitation

	<i>Aplysia</i>	<i>Hermisenda</i>	LTP
postsynaptic Ca ²⁺ required	yes	yes	yes
depolarization (Ca ²⁺ load) alone sufficient	yes ¹	yes ¹	yes ¹
costimulation of G-protein-coupled receptor enhances requirement for Ca ²⁺ -dependent protein kinases	yes	yes	yes ²
	yes ³	yes ³	yes ³
PKC	yes ⁴	yes ⁴	yes ⁴
PKA	yes ⁴	yes ⁵	yes ⁴
CaMK	yes ⁴	no	yes ⁵
phosphorylation of ion channels	yes	yes	yes
phosphorylation of K ⁺ channels	yes	yes	?
decrease in spike rate accommodation	yes	yes	yes
spike broadening	yes	yes	no
excitability increases limited to synapse	no	no	no
synapse-specific component	yes	?	yes
site of synaptic change	pre/post(?) ⁶	post	pre(?)/post ⁷

¹ Sufficient to induce a reduced form of facilitation, the effects of which are relatively transient.

May augment Ca²⁺ signal rather than acting synergistically as in *Hermisenda* and *Aplysia*.

³ Targets of action vary in each system. The attenuation of facilitation by a kinase inhibitor is not sufficient to assess a kinase's role in the process. Minimally, it is necessary to demonstrate also that the kinase is activated by the induction protocol. These requirements have been met to varying degrees for PKC, PKA, and CaMK in each of the systems reviewed here.

⁴ Activated by induction protocol and inhibition prevents induction of facilitation. Distinct isoforms play specific roles in induction and maintenance of facilitation.

⁵ Inhibition prevents some aspect of facilitation; activation by induction of facilitation not adequately demonstrated.

⁶ Presynaptic modification is probably sufficient.

⁷ Postsynaptic modification is probably sufficient.

second messenger cascades by metabotropic surface receptors makes them uniquely suited for the integration and amplification of signals. In the learning-related neuronal/synaptic facilitation exhibited by both *Aplysia* and *Hermisenda*, G-protein coupled receptors play a crucial role as coincidence detectors, modifying the cellular response to otherwise benign Ca²⁺ signals. In LTP, it appears likely that metabotropic glutamate receptors may serve a similar function, although their contribution re-

quires further elucidation. In total, the convergence of two signals, a rise in intracellular Ca²⁺ and stimulation of G-protein-coupled surface receptors, serves as a cellular device for contiguity detection. Given the similar role that this mechanism plays in each of the systems reviewed, we conclude that the contiguous occurrence of environmental events is a principal determinant of the cellular modifications underlying learning (see also /182,193,213/). The common assertion that a "contingent" relationship

between stimuli is the more critical determinant of learning /221,222/ is not supported at this mechanistic level. Rather, the effects of contingency in behaving animals are likely to reflect the expression of an integrative process of the larger nervous system.

We propose that a general class of neuronal facilitation can be described as requiring a post-synaptic Ca^{2+} rise in conjunction with the stimulation of surface receptor-coupled G-proteins and the resultant activation of allosteric serine/threonine protein kinases. Features of these models which are of only incidental relevance appear to be the class of kinase activated by the facilitation protocol, as well as the site of the neuronal modulation relative to the effector targets. Moreover, although it is often stated that the specificity of memory could be accomplished by confining the necessary Ca^{2+} rise to dendritic spines /167/, such a requirement does not appear *necessary* for memory storage. First, invertebrate neurons do not contain spines, yet invertebrates clearly acquire and retain memories. In addition, although the facilitation that accompanies learning in *Aplysia* and LTP does appear to have a synapse-specific component (not yet determined in *Hermisenda*), each of the models that we have described exhibit excitability increases that are not limited to the synapse. Even more strikingly, a once popular notion that a "learning transmitter" would emerge as a common feature of different model systems (see /59/ for discussion) finds no support from the comparisons made here; learning in *Aplysia* is dependent on 5-HT, in *Hermisenda* GABA and 5-HT may serve similar functions, and in hippocampal LTP (though not necessarily other forms) glutamate is critical. Finally, there is no evidence forthcoming from the review of these model systems which would suggest that the *de novo* synthesis of new pathways (or even new synapses) is necessary for the establishment of an associative memory. It should be noted that in the case of LTP, some evidence would suggest that functionally "silent" synapses become active during the induction regimen /132/, and in *Aplysia*, sensitization training may enhance dendritic arborization /28,29/. Nevertheless, ample evidence suggests that changes in the flow of current through *existing* neural pathways may be sufficient for the establishment of a new memory.

We have proposed that increased intracellular Ca^{2+} and stimulation of second messengers by G-protein coupled surface receptors are two elemental and universal cellular events from which neuronal properties are modified and from which memories emerge. It should be noted that events such as muscle contraction /220,243/, cell development /270/ and even some forms of cancer /81/ are mechanistically similar to our description of the elemental storage of memory. The observation that similar cascades serve a number of distinct functions speaks to the universality of processes in cell biology, but also raises a question as to how similar processes are ultimately expressed so differently. This paradox might be resolved by noting the distinct protein substrates expressed in neurons that process information, e.g., multiple classes of K^+ channels and AMPA receptors. However, the diversity of substrate proteins in the brain is likely greater than in all other organ systems combined, suggesting that several unique substrates are not solely responsible for the differences in the expression of memory-related neuronal facilitation and these other process. A second and more complete solution to this paradox is similarly weak for its imprecision. It is likely (although empirically intractable) that memory is unique owing to the complexity of the neural network that comprises the brain in combination with (or as a consequence of) the unique substrates contained there. Of particular relevance is the observation that brain cells do not act in unison as do the cells which comprise a muscle; rather, to a very large extent, brain cells can respond independently of one another while still in communication. While the elemental cascades that underlie neuronal/synaptic facilitation may play roles in other processes, the psychological process of memory is an emergent phenomenon. Thus it is critically important to distinguish between the elemental units of memory storage and the psychological perception of remembering. While we are largely ignorant with regard to the latter process, the former process is well described as a product of the synergistic action of an activity-induced rise in intracellular Ca^{2+} and stimulation of receptor-coupled G-proteins on the serine/threonine protein kinases.

In reviewing the three mechanisms of facilitation described here, one might recognize another appar-

ent contradiction. In *Aplysia* sensory cells and possibly in associative LTP, it was suggested that the combined action of intracellular Ca^{2+} and DAG might be sufficient to induce a persistent phosphorylation of substrate proteins, while the same signals were suggested to induce only a weak and transient phosphorylation of substrates in *Hermissenda* B cells. This disparity may only be indicative of normal diversities in the expression of PKC isoforms in various cell types based on their respective functions. For instance, the B photoreceptor is somewhat unusual in that PKC activation is presumed to occur during routine signal transduction, i.e., during a light response in which the activated enzyme contributes to the maintenance of light-induced generator potentials (through a transient reduction of K^+ conductance). Thus the B cell may employ a unique class of protein phosphatases to reverse this phosphorylation and/or may express isoforms of the enzyme that are uniquely sensitive to this combination of activators. Thus in these cells, AA may be required for the prolonged translocation and activation of PKC, whereas in other cell types it is not. Likewise, it has been established in both *Aplysia* sensory cells as well as in hippocampal neurons that different isoforms of PKC are stimulated concurrently during a typical facilitation protocol, and these isoforms may contribute differentially to different stages of maintenance [147,251]. It is similarly possible that different isoforms of PKC are activated with and without the contribution of AA in *Hermissenda* B cells, and that the AA-dependent isoforms bind more effectively and persistently to the neuronal membrane. No direct evidence in support of this hypothesis is presently available, although preliminary data suggest that multiple isoforms of PKC are present in the B cell [198], and antibody binding studies in our laboratory have identified three distinct isoforms of PKC in B cell extracts (Muzzio and Matzel, unpublished observations).

Contemporary reviews of the induction of learning-related neuronal facilitation have often concluded that the dissimilarities between various systems were too great to warrant the suggestion of any general model of underlying molecular substrates [51,56]. The recent delineation of the molecular constituents of facilitation in *Hermissenda* and the recognition of a contribution of metabotropic

receptors to hippocampal LTP have made possible a more meaningful comparison of several of these systems. In conclusion, although many of the details of these molecular cascades differ, important similarities have begun to emerge, and are likely to provide clues about ubiquitous characteristics of memory formation. Although in the present article we considered only induction mechanisms of short-term memory storage, the elucidation of long-term maintenance mechanisms is likely to illuminate still greater similarities, particularly in that genetic modifications and substrates are often stimulated by the same molecular cascades responsible for these more transient modifications (e.g., [27,113,250,277]). An important future consideration will be the elucidation of the signaling pathways by which genetically programmed modifications of membrane/receptor proteins are directed toward the same substrates that are involved in the expression of short-term facilitation (e.g., [109,162,256]). Since many types of facilitation appear to utilize some or all of the same modifications to influence both short- and long-term facilitation, the identification of such a feedback mechanism may represent the single most important point of convergence between these different model systems of cellular "memory".

ACKNOWLEDGEMENTS

This work was supported by the US Public Health Service (NIMH Grants MH48387 and MH 52314), the Charles and Johanna Busch Memorial Fund, and a Hoechst-Celanese Young Faculty Award (all to LDM), and an American Psychological Association Predoctoral Minority Fellowship (to IAM). We thank Chet Gandhi and Tracey Shors for their comments on an earlier version of the manuscript.

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