Cell, Vol. 91, 927–938, December 26, 1997, Copyright ©1997 by Cell Press

Synapse-Specific, Long-Term Facilitation of Aplysia Sensory to Motor Synapses: A Function for Local Protein Synthesis in Memory Storage

Kelsey C. Martin,† Andrea Casadio,† Huixiang Zhu, Yaping E, Jack C. Rose, Mary Chen, Craig H. Bailey, and Eric R. Kandel*

Howard Hughes Medical Institute
Center for Neurobiology and Behavior
College of Physicians and Surgeons of Columbia University
722 West 168th Street
New York, New York 10032

Summary

The requirement for transcription during long-lasting synaptic plasticity has raised the question of whether the cellular unit of synaptic plasticity is the soma and its nucleus or the synapse. To address this question, we cultured a single bifurcated Apylsia sensory neuron making synapses with two spatially separated motor neurons. By perfusing serotonin onto the synapses made onto one motor neuron, we found that a single axonal branch can undergo long-term branch-specific facilitation. This branch-specific facilitation depends on CREB-mediated transcription and involves the growth of new synaptic connections exclusively at the treated branch. Branch-specific long-term facilitation requires local protein synthesis in the presynaptic but not the postsynaptic cell. In fact, presynaptic sensory neuron axons deprived of their cell bodies are capable of protein synthesis, and this protein synthesis is stimulated 3-fold by exposure to serotonin.

Introduction

Synaptic plasticity, the capacity of neurons to modulate the strength of their synaptic connections in response to their cellular experience and environment, appears to contribute to a variety of physiological and pathological processes in the adult brain, including learning, memory storage, age-related memory loss, tolerance to and dependence on drugs of abuse, and epilepsy (Bliss and Collinridge, 1993; Martin and Kandel, 1996; Nestler and Aghajanian, 1997). However, the role of synaptic plasticity is best understood in the context of memory storage. Like behavioral memory, synaptic plasticity can exist in both short-term and long-term forms. Short-term synaptic plasticity requires only covalent modifications of preexisting proteins, whereas long-term synaptic plasticity requires alterations in gene expression, new protein synthesis, and the establishment of new synaptic connections (Goelet et al., 1986; Bailey et al., 1996).

The finding that long-term memory requires alterations in gene expression and thus the nucleus—a resource shared by all the synapses within a cell—posed a cell biological paradox in the study of memory. Does the requirement of transcription for long-lasting forms of synaptic plasticity mean that long-lasting memory needs to be cell-wide, or can the strength of individual synaptic connections be modified independently? Each of the approximately 10^{11} neurons in the brain makes, on average, one thousand synaptic connections on its population of target cells. To maximize information processing, it has been thought that the critical cellular unit of synaptic plasticity is the individual synapse. Although attractive, this idea has never been tested directly on the level of individual neurons because there have not been appropriate cellular systems for exploring long-term changes in the synaptic strength of different terminals of an individual neuron.

Specifically, one would like to know: How can the products of gene expression be differentially targeted to alter synaptic strength at some synapses made by a given presynaptic neuron but not at others? How can one reconcile the possibility of branch-specific modifications in synaptic connections, postulated to be characteristic of certain types of memory storage, with a genetic program that would appear to be neuron-wide? In addressing this problem, two alternative mechanisms have been considered (Goelet et al., 1986; Martin and Kandel 1996; Sossin, 1996; Frey and Morris, 1997; Schuman, 1997). One possibility is that proteins might be targeted specifically down one branch rather than another of an axonal arbor. Given that a single neuron has many branches and synaptic terminals, this type of targeting mechanism has seemed unlikely. An alternative possibility is that synapses are "tagged" by synaptic stimulation to capture products of gene expression that are exported throughout the cell but are only functionally incorporated at synapses tagged by previous activity.

Experimental support for possible synaptic tagging during learning-related plasticity in the adult recently emerged from studies of the late phase of long-term potentiation in the hippocampus (Frey and Morris, 1997). These studies were based on populations of neurons and synapses and indicated that, with appropriate timing of activity, the products of the gene expression induced by strong stimulation of one set of synaptic connections can be "captured" by another set of synaptic connections that have been tagged by stimuli that normally produce transient LTP.

To examine whether an individual neuron can demonstrate synapse specificity, we turned to Aplysia sensory-motor neuron cultures. When Aplysia sensory to motor synapses are reconstituted in culture, they manifest both short- and long-lasting forms of plasticity characteristic of the intact animal (Montarolo et al., 1986; Rayport and Schacher, 1986). One brief application of serotonin, a neurotransmitter released by interneurons during sensitization of the gill-withdrawal reflex, produces a short-term facilitation that lasts for minutes and that involves covalent modification of preexisting proteins. By contrast, five spaced applications of serotonin produce a long-term facilitation that lasts for days and that involves transcription, translation, and the growth of new synaptic connections. This long-term facilitation depends on activation of both PKA and MAP

*To whom correspondence should be addressed.
†These authors contributed equally to this work.

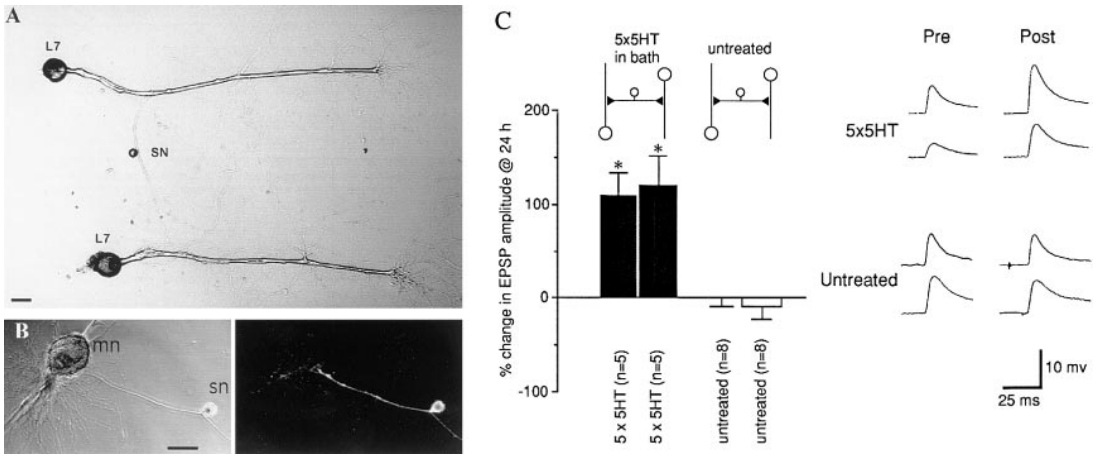

Figure 1. Bifurcated Aplysia Sensory Neurons Form Synapses with Two Spatially Separated L7 Motor Neurons in Culture

(A) Bifurcated sensory neurons were plated to make connections with two spatially separated L7 motor neurons.

(B) Immunoreactivity for sensorin, the peptide neurotransmitter specific to sensory neurons, indicates that the bifurcated neurons are indeed sensory neurons.

(C) The EPSPs formed by the sensory cell onto each motor neuron did not change in amplitude between days 5 and 6 in untreated cultures, indicating that the synaptic connection formed between the sensory cell and each of the motor neurons was stable during this time. Bath application of five spaced pulses of serotonin caused an increase in the amplitude of the EPSPs recorded in both motor neurons 24 hr later, indicating that these synapses can undergo serotonin-induced long-term facilitation (*$p < 0.01$, change in EPSP amplitude in serotonin-treated versus untreated cultures, Student's t-test). Shown are representative recordings of EPSPs and a histogram of the mean change in EPSP amplitude $\pm$ SE. Scale bar in A and B is 100 μm. Abbreviations: SN, sensory neuron; L7, motor neuron; mn, motor neuron.

kinase, which leads to CREB1-mediated transcription, derepression of the transcription factor CREB2, and the induction of a set of immediate-early genes, including C/EBP and C-terminal ubiquitin hydrolase (Dash et al., 1990; Alberini et al., 1994; Bartsch et al., 1995; Hegde et al., 1997; Martin et al., 1997). In addition, long-term facilitation involves the growth of new synaptic connections (Bailey and Chen, 1983, 1988).

We have modified the standard Aplysia sensory-motor culture system by plating a single bifurcated sensory neuron in culture with two spatially separated follower motor neurons to determine whether a single neuron can undergo synaptic plasticity that is both dependent on transcription and branch-specific, and to explore the molecular mechanisms underlying this specificity. Using perfusion microelectrodes, we applied serotonin selectively to the synapses made onto one motor neuron and not the other. With this system, we have found that a single cell can undergo long-lasting synaptic plasticity that is both branch-specific and dependent on CREB-mediated transcription. This branch-specific long-term facilitation is dependent on local translation of synaptically localized mRNAs in the presynaptic, but not the postsynaptic, cell. We therefore examined protein synthesis in the presynaptic axons deprived of their cell bodies and found that basal protein synthesis was stimulated 3-fold by exposure to serotonin. In contrast to the initial establishment of branch-specific long-term facilitation, synapse capture—the ability to recruit the long-term process at a second branch after it has been established at another branch in the same cell—does not require local protein synthesis.

Results

Single Sensory Neurons Show Branch-Specific Long-Term Facilitation

The standard Aplysia sensory to motor neuron culture system consists of one or two sensory neurons making synaptic contact with a single postsynaptic target neuron (Rayport and Schacher, 1986). To establish a system for exploring the elementary mechanisms of synapse specificity, we modified the traditional system by plating a single bifurcated sensory neuron and having it make synaptic contact with two spatially separated motor neurons (Figure 1A). We found that 1%–5% of the sensory neurons in the pleural ganglion sensory cell cluster contained bifurcated axonal processes; we verified that these were truly sensory neurons by showing that they were immunoreactive for sensorin, the peptide neurotransmitter specific to sensory cells (Figure 1B). Simultaneous recording of the excitatory postsynaptic potentials (EPSPs) evoked in the two motor neurons by extracellular stimulation of the single sensory neuron revealed that the cells formed synapses in culture and that these synapses were stable between days 5 and 6 in vitro (Figure 1C). Notably, synaptic connections are only formed between the sensory and motor neurons; sensory cells do not synapse with other sensory cells, nor do motor neurons form synapses with one another (Rayport and Schacher, 1986; Glanzman et al., 1989). While we use the EPSP as a measure of the effectiveness of the synapse formed between the sensory and motor neuron, the synaptic connection is actually composed of multiple morphologically discrete synaptic boutons or varicosities made by the sensory cell onto the motor

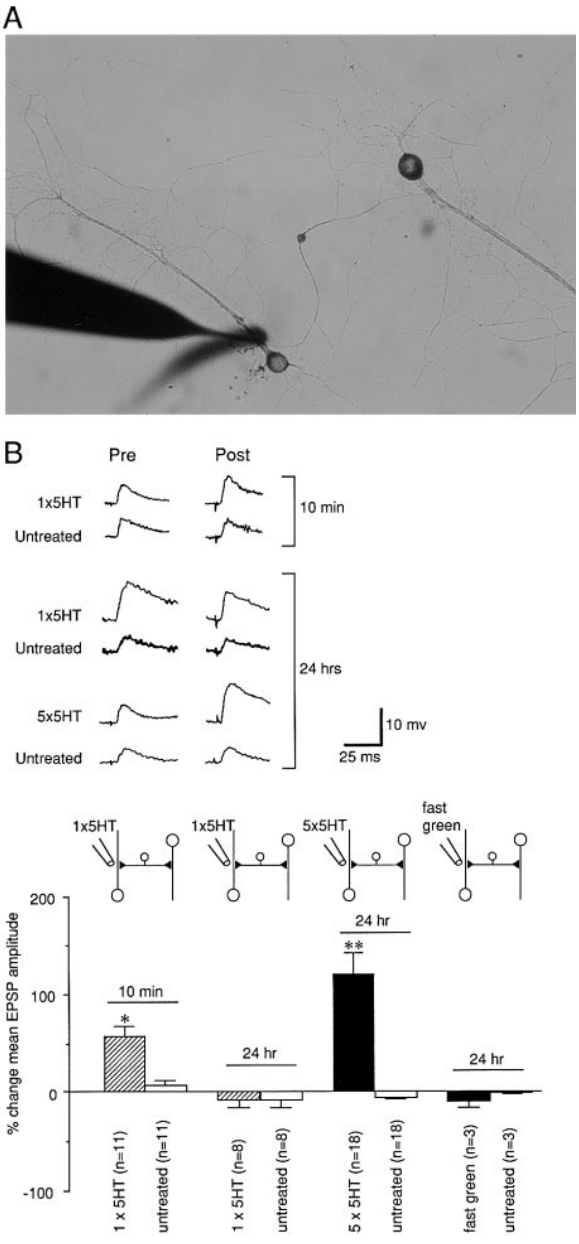

Figure 2. Synapse Specific Short-Term and Long-Term Facilitation

(A) Serotonin was applied locally to the synaptic connections formed onto one motor neuron using a perfusion microelectrode. Bulk flow of the perfusate prevented the cell body of the sensory neuron or the synaptic connections made onto the other motor neuron from being exposed to the serotonin. A dye (0.05% fast green) was included to visualize the serotonin perfusion.

(B) Application of a single pulse of serotonin increased the amplitude of the EPSP at the serotonin-treated branch 10 min after serotonin application without changing the EPSP at the untreated branch (*$p < 0.05$, change in EPSP amplitude in serotonin-treated versus untreated branch, Student's t-test). This facilitation was transient: there was no increase in the EPSP at 24 hr at either the serotonin or the untreated branch. In contrast, local perfusion of five spaced pulses of serotonin onto one set of synapses produced long-term facilitation of the EPSP at the serotonin-treated branch without any change at the untreated branch (**$p < 0.01$, change in EPSP amplitude in serotonin-treated versus untreated branch, Student's t-test). In control experiments, perfusion of the dye alone (fast green) did not change the EPSP at the perfused or the nonperfused branch. Shown are representative recordings of EPSPs and a histogram of the mean change in EPSP amplitude ± SE.

cell; the EPSP therefore reflects the activity of a population of individual synaptic contacts.

As has previously been shown for the standard Aplysia sensory-motor culture (Montarolo et al., 1986), five brief, spaced applications of serotonin in the bath produced a comparable facilitation of the EPSP at both synapses (Figure 1C). In the animal, serotonergic interneurons form synapses onto the sensory cell presynaptic terminal, and the release of serotonin from these interneurons produces facilitation of the sensory-motor neuron synapse. To mimic this geometry, we used perfusion microelectrodes to apply serotonin locally onto the synapses made by only one branch of the sensory cell onto one motor neuron. Bulk flow of the perfusate protected the synapses formed onto the second motor neuron from exposure to the serotonin (see Figure 2A). To visualize the perfusion, a dye (0.05% fast green) was included in the serotonin solution. Very low pressure (approximately 1 lb/in^2) was used to deliver the serotonin, and the bulk flow was adjusted such that the serotonin produced a cloud that covered much of the initial axon segment. Since the initial segment is where most synapses made by the sensory neurons on the motor neurons are found (Glanzman et al., 1990; Bailey et al., 1992), the serotonin perfusion most likely affected many of these synapses, as well as the distal segment of the sensory neuron branch. The serotonin perfusion did not, however, contact the proximal axon or the cell body of the sensory cell.

Local application of a single, transient pulse of serotonin onto one set of synaptic connections produced a facilitation of 61.8 ± 11.2% at that connection (n = 11) when the EPSP was measured 10 min after serotonin application (Figure 2B). A single application of serotonin to the synapse did not produce changes in excitability in the sensory cell body (data not shown), indicating that the serotonin application was indeed local. The facilitation following one pulse was transient and did not persist: 24 hr later, the EPSP amplitude was −10.3 + 7.1 % (n = 8). By contrast, the contralateral synapse that was not exposed to serotonin showed neither short-term nor long-term facilitation (+9.2 ± 7.4% change in mean EPSP amplitude 10 min after 5-HT application, and −9.4 ± 7.3% change at 24 hr, n = 11). Thus, one local application of 5-HT produced synapse-specific short-term facilitation, as has previously been demonstrated in the intact ganglion (Clark and Kandel, 1984).

To examine long-term facilitation, we applied five spaced pulses of serotonin to one of the branches. The treated branch showed long-term facilitation: when measured 24 hr later, repeated serotonin application was found to increase the mean EPSP amplitude of the treated synapse by 122.3 ± 22.1% (n = 18, Figure 2B) without having any effect on the untreated synapse (mean change in EPSP amplitude of −7.4 ± 2.8%, n = 18). A single presynaptic cell thus can undergo long lasting facilitation that is branch-specific.

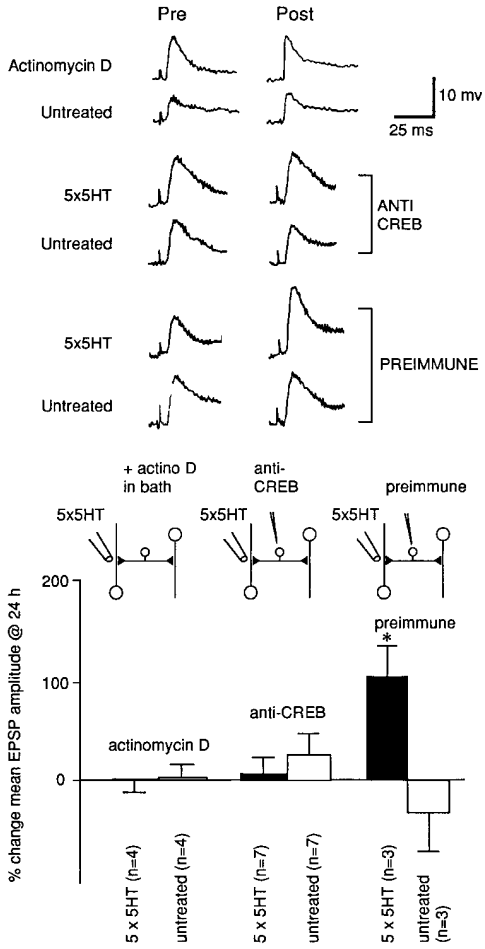

Figure 3. Synapse Specific Long-Term Facilitation Requires Transcription

Bath application of 50 μM actinomycin D, a transcriptional inhibitor, for 30 min prior to, during, and 30 min after local application of five pulses of serotonin blocks synapse-specific long-term facilitation. Microinjection of anti-CREB antibodies into the presynaptic sensory neuron 1 hr prior to local application of five pulses of serotonin also blocks synapse specific long-term facilitation, while microinjection of preimmune serum has no effect. (*$p < 0.5$, change in EPSP amplitude in cells injected with preimmune serum and receiving local 5HT application versus cells injected with anti-CREB antibody or receiving actinomycin D in bath, ANOVA and Neuman-Keul's multiple range test). Shown are representative recordings of EPSPs and a histogram of the mean change in EPSP amplitude ± SE.

Branch-Specific Facilitation Requires Transcription Mediated by CREB

To test whether this branch-specific facilitation is dependent on transcription, and thus on the nucleus, we bath-applied actinomycin D, an inhibitor of transcription, and found that this blocked long-term synapse-specific facilitation (mean change in EPSP at 24 hr of −8.3 ± 11.7% in the 5-HT treated branch and +7.2 ± 13.2% in untreated branch, n = 4 each, Figure 3). Long-term facilitation in the traditional Aplysia sensory to motor culture system requires transcription that is mediated specifically by CREB. To test the role of CREB in synapse-specific long-term facilitation, we microinjected anti-CREB antibodies into the cell body of the sensory cell and found that they blocked synapse long-term facilitation (mean change in EPSP amplitude at 24 hr of 9.4 ± 16.5% in the 5-HT-treated branch and 25.9 ± 21.3% in the untreated branch, n = 7 each), whereas injection of preimmune serum had no effect (mean change in EPSP amplitude at 24 hr of 105.1 ± 31.4% in the 5-HT-treated branch and −33.3 ± 39.3% in the untreated branch, n = 3 each, Figure 3). Taken together, these findings indicate that a single cell can undergo synaptic plasticity that is both synapse-specific and dependent on transcription, and that this transcription is specifically mediated by CREB.

Branch-Specific Long-Term Facilitation Involves the Growth of New Synapses

One of the hallmarks of long-term facilitation is that it involves the growth of new synapses, which can be visualized in culture by light microscopic imaging of carboxyfluorescein-injected sensory neurons. When our cultures were imaged before and after serotonin treatment using a blind protocol, we found that only the branch that had received five applications of serotonin displayed evidence of synaptic growth (Figure 4). The branches treated with 5-HT underwent a mean change in EPSP amplitude of 141.5 ± 52.3% and had a mean increase in varicosity number of 106.2 ± 31.8 (n = 6). The untreated branch had a mean change in EPSP amplitude of −11.7 ± 12.6% and a mean change in varicosity number of 2.3 ± 2.3 (n = 6). Thus, both physiological and structural changes underlying branch-specific long-term facilitation (both of which are long lasting) are synapse-specific.

Branch-Specific Facilitation Requires Local Protein Synthesis in the Presynaptic Cell

Given that branch-specific facilitation requires the nucleus but is synapse-specific, what are the possible underlying mechanisms? One possibility we explored was that marking the synapse for synapse-specific long-term facilitation required local translation of mRNAs. Support for this possibility comes from two sets of findings. First, many studies have shown that mRNAs, ribosomes, and components of the translational machinery are present not only in the perinuclear cell soma of neurons but also in distal neuronal processes in the postsynaptic spine and in the presynaptic terminals (Scheller et al., 1982; Garner et al., 1988; Brunet et al., 1991; Mayford et al., 1996; Van Minnen et al., 1997; for review, see Steward, 1997). Second, an intermediate form of synaptic facilitation, which is dependent on translation and not on transcription, has been described in Aplysia sensory to motor neurons (Ghirardi et al., 1995).

To test whether local protein synthesis was required for synapse-specific long-term facilitation, we applied the protein synthesis inhibitor emetine (100 μM) at the synapse for 20 min prior to, during, and 20 min after the application of five pulses of serotonin. To control for the spatial restriction of locally applied emetine and to assure that it does not spread to the cell body, we applied emetine at one branch and serotonin at the other. To control for the effect of emetine alone, we

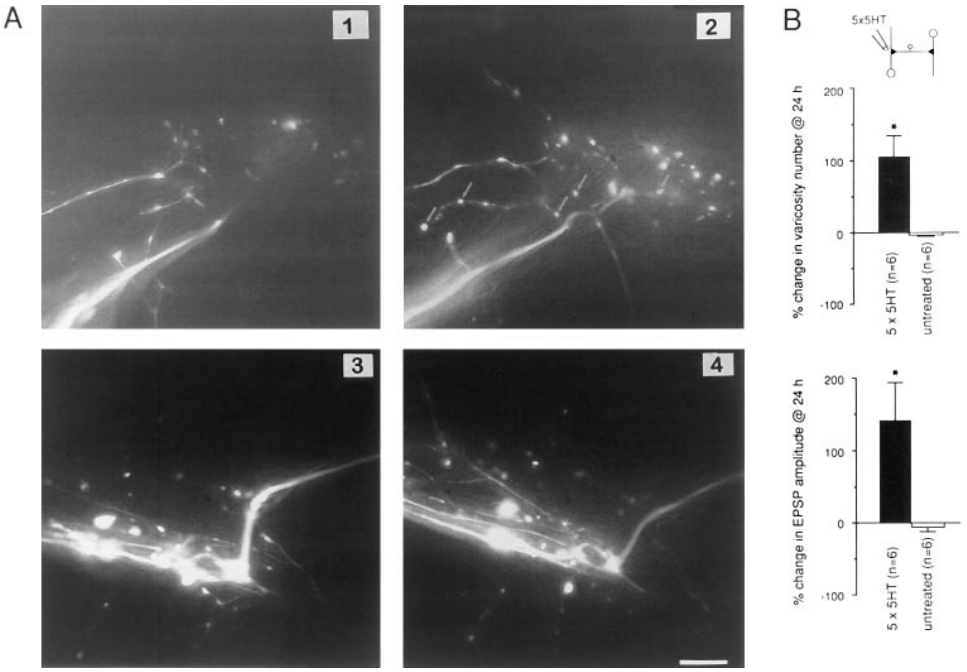

Figure 4. Synapse-Specific Long-Term Facilitation Involves Synapse-Specific Growth

Carboxyfluorescein-filled sensory neurons were imaged at the region of contact with the axon hillock of the L7 motor neuron. Five pulses of serotonin were locally perfused onto one of the sets of synaptic connections by a separate individual. 24 hr later, the EPSPs were again recorded, and the sensory neuron was injected with carboxyfluorescein and imaged at the same region of contact with the axon hillock of the L7 motor neuron. The number of varicosities was counted using a blind procedure.

(A) Representative micrographs of the varicosities formed by a single sensory cell onto two separate motor neurons before and after local application of five pulses of serotonin. 1 and 2, branch treated with serotonin at time 0 and 24 hr, respectively; 3 and 4, untreated branch at time 0 and 24 hr, respectively. The arrows point to examples of new varicosities present at the branch treated with serotonin. All micrographs are composed of superimpositions of labeled sensory neurite images taken from all focal planes of the view area. As a result, the shape of individual varicosities may be obscured. Scale bar is 20 μm.

(B) Histogram of the mean change in EPSP amplitude $\pm$ SE and of the change in varicosity number $\pm$ SE. *$p < 0.01$, change in EPSP amplitude in serotonin treated versus untreated branch, Student's t-test, and change in varicosity number in serotonin-treated versus untreated branch, Student's t-test.

applied emetine in the absence of serotonin. When emetine was applied to one set of synaptic connections and serotonin to the other, the serotonin-treated synapse underwent normal synapse-specific facilitation (mean change in EPSP amplitude at 24 hr of 123.8 $\pm$ 48.9%, n = 5) and the emetine-treated synapse remained stable (mean change in EPSP amplitude at 24 hr of $-15.9 \pm 10\%$, n = 5, Figure 5). By contrast, inhibition of protein synthesis at the branch receiving serotonin blocked branch-specific long-term facilitation: the mean change in EPSP amplitude at 24 hr at the branch receiving both serotonin and emetine was 2.7 $\pm$ 6.4% (n = 7) while that in the untreated branch was $-1.2 \pm 13.5\%$ (n = 7, Figure 5). This indicates that local protein synthesis was required for the establishment of branch-specific long-term facilitation. Emetine alone had no effect on the synaptic connection (Figure 5). Inhibition of synapse-specific long-term facilitation was also obtained with local application of the protein synthesis inhibitors anisomycin (100 μM) and cycloheximide (30 μM) (data not shown). Emetine application had no effect on short-term synapse-specific facilitation: the mean change in EPSP amplitude 10 min after one pulse of serotonin to a branch also receiving emetine for 20 min before, during, and 20 min after the serotonin treatment was 44.9 $\pm$ 10.8% (n = 3).

Our perfusion system does not allow us to discriminate between a pre- or postsynaptic requirement for local protein synthesis. We therefore injected the membrane-impermeant protein synthesis inhibitor gelonin (Stirpe et al., 1980) into the pre- or postsynaptic neuron prior to local serotonin application. Gelonin has previously been found to inhibit protein synthesis in Aplysia neurons (Trudeau and Castellucci, 1995). Whereas presynaptic injection of gelonin completely blocked synapse-specific long-term facilitation (mean change in EPSP amplitude at 24 hr of $-5.8 \pm 25.6\%$ in the serotonin-treated branch and $-2.3 \pm 12.8\%$ in the untreated branch, n = 4 each), postsynaptic gelonin injection had no effect (mean change in EPSP amplitude at 24 hr of 84.6 $\pm$ 14.5% in the serotonin-treated branch and $-7 \pm 7.7\%$ in the untreated branch, n = 5 each, Figure 5). Thus, local protein synthesis appears to be required in the presynaptic sensory neuron but not in the postsynaptic motor neuron.

Translation Can Be Induced in Isolated Presynaptic Sensory Cell Neurites by 5-HT

To test directly the ability of the neurites of the presynaptic sensory neurons to synthesize proteins, we cultured 30–40 sensory cells in a dish, cut off their cell bodies,

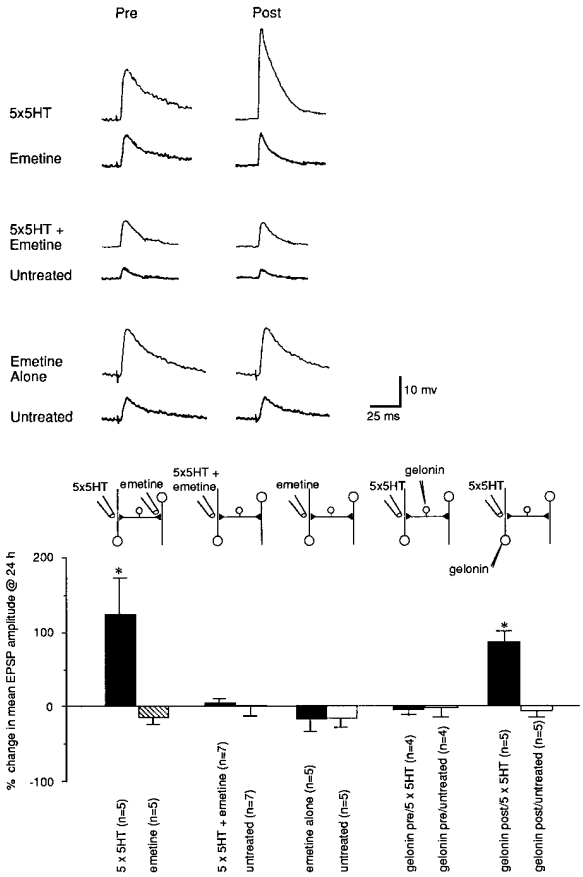

Figure 5. Synapse-Specific Long-Term Facilitation Requires Local Protein Synthesis in the Presynaptic Neuron

The protein synthesis inhibitor emetine (100 μM) was perfused locally for 20 min prior to, during, and 20 min after local perfusion of five pulses of serotonin. When emetine and serotonin were applied at opposite branches, the serotonin-treated branch underwent long-term facilitation. When emetine was applied at the same branch as serotonin, synapse specific long-term facilitation was blocked (*$p < .05$, emetine plus serotonin at same branch versus emetine and serotonin on opposite branches, Student's t-test). In control experiments, emetine alone was applied to one branch, without any treatment of the other branch, and did not have any effect on the EPSP amplitude. To differentiate between a requirement for protein synthesis in the pre- or postsynaptic neuron, the protein synthesis inhibitor gelonin (25 μM) was injected into either the presynaptic sensory neuron or the postsynaptic motor neuron 1 hr prior to local application of five pulses of serotonin. Whereas synapse specific long-term facilitation was completely blocked by presynaptic injection of gelonin, postsynaptic injection had no effect. *$p < .05$, gelonin presynaptic plus serotonin, versus gelonin postsynaptic plus serotonin. Shown are representative recordings and a histogram of the mean changes in EPSP amplitude plus SE.

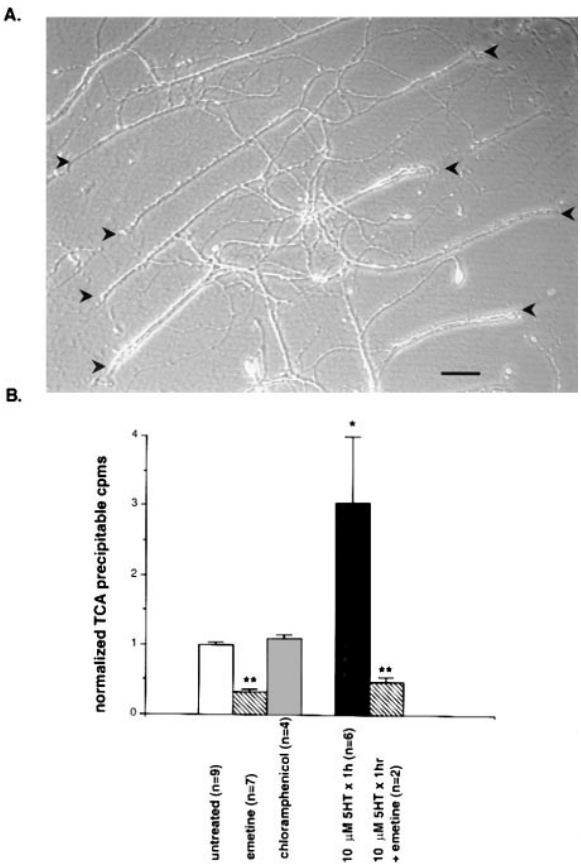

Figure 6. Translation in Isolated Presynaptic Sensory Cell Neurites is Induced by Serotonin

(A and B) Sensory cells (30–40) were cultured for five days. The cell body of each neuron was removed and the processes were labeled with ^{25}S methionine. Emetine (200 μM) and chloramphenicol (100 μM) were present for 30 min prior to and during the metabolic labeling, and serotonin (10 μM) was present during the metabolic labeling. The cells were then TCA precipitated, and the TCA insoluble counts were counted on a scintillation counter. Total cpm were divided by the number of cells in each dish. Shown is a histogram of the mean counts plus SE, normalized to the counts in the untreated dishes. The mean cpm/untreated cell was 5.922 ± 1.541. *$p < .05$, serotonin treated versus untreated processes; **$p < .01$, emetine treated versus untreated and chloramphenicol treated processes, ANOVA followed by Neuman-Keul's multiple range test.

allowed them to rest for 1–2 hr, and carefully washed the dishes. We confirmed that no glia or neuronal cell somata were present by examination with a dissecting microscope and then incubated the processes with ^{35}S-methionine (Figure 6). We then TCA-precipitated the cell processes and counted the incorporation of the labeled methionine into protein. We found that sensory cell processes were able to synthesize TCA-precipitable ^{35}S-methionine-labeled proteins and that this synthesis was inhibited 67% by the protein synthesis inhibitor emetine (200 μM, Figure 6). By contrast, the mitochondrial protein synthesis inhibitor chloramphenicol (100 μM) had no effect. Application of serotonin (10 μM) during the 1 hr ^{35}S-methionine labeling increased the TCA-precipitable counts 3-fold, and this serotonin-induced translation was also inhibited by the protein synthesis inhibitor emetine. These metabolic labeling experiments provide strong evidence that the presynaptic sensory cell processes are capable of protein synthesis.

Unlike the Initial Establishment of Synapse-Specific Long-Term Facilitation, Synaptic Capture Does Not Require Local Protein Synthesis

In their experiments, Frey and Morris (1997) examined synaptic tagging using a protocol based on synaptic

capture. Their protocol involved the induction of long-lasting LTP at one pathway, followed by a weaker stimulus that usually produces transient LTP at a second pathway. If given within a 3 hr time window, the weaker stimulus produced long-lasting LTP at the second pathway. In contrast to our findings in synapse-specific facilitation, the synaptic capture they examined was protein synthesis independent. We performed a similar synaptic capture experiment in our system by giving a single application of serotonin to one synapse, which produces only transient, short-term facilitation (see Figure 2), immediately following five pulses of serotonin to the other synapse, and we found that this protocol produced long-lasting facilitation at both branches (mean change in EPSP amplitude at 24 hr of 121 $\pm$ 50% in the branch receiving five pulses of 5-HT and 69 $\pm$ 23% in the branch receiving one pulse of serotonin, n = 5 each, Figure 7). Thus, as in the Frey and Morris experiments (1997), a single pulse of serotonin appeared to mark the second set of synapses so that they could capture the products of gene expression induced by five pulses of serotonin at the first set of synapses. Notably, the amplitude of the facilitation produced at the synapse receiving a single pulse of serotonin was consistently lower than that produced at the synapse receiving five pulses, although this difference was not statistically significant.

To determine whether synaptic capture was also dependent on local translation, we locally perfused the protein synthesis inhibitor emetine onto the second branch 20 min prior to, during, and 20 min following the single application of serotonin. Inhibition of protein synthesis did not block long-term facilitation at the second branch (Figure 7): the mean change in EPSP amplitude at 24 hr was 120.7 $\pm$ 48.1% in the branch receiving five pulses of 5-HT, and 61 $\pm$ 16.5% in the branch receiving one pulse of serotonin and emetine (n = 5). Thus, unlike the establishment of synapse-specific long-term facilitation at a primary site, the capture of long-term facilitation at a second site after it has been initiated or established at a primary site does not require local protein synthesis. However, if protein synthesis were inhibited at the branch receiving five pulses of serotonin, no facilitation was observed at the branch receiving a single application of serotonin: mean change in EPSP amplitude at 24 hr of 1.1 $\pm$ .1% in the branch receiving five pulses of serotonin and emetine, and of .83 $\pm$ .1% in the branch receiving a single pulse (n = 6 each, Figure 7). This suggests that the retrograde signal to the nucleus from the initial site of synapse-specific long-term facilitation may itself require new protein synthesis.

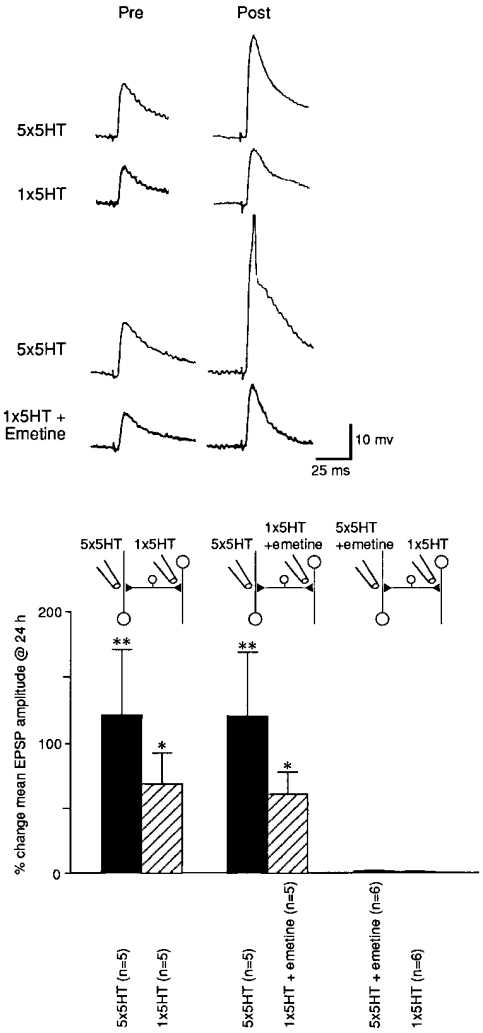

Figure 7. Synaptic Capture

A single pulse of serotonin, which usually only produces transient facilitation, was given to one branch immediately after five pulses of serotonin were given to the opposite branch. When the EPSPs were measured 24 hr later, long-term facilitation was observed at both branches. Synaptic capture—the capture of the long-term process at a second synapse after it has been established at another synapse in the same cell—does not require protein synthesis. When emetine is applied 20 min prior to, during, and 20 min after the application of one pulse of serotonin, long-term facilitation is still observed at both branches. Inhibition of protein synthesis at the branch receiving five pulses of serotonin, however, blocks synaptic capture. Shown are representative recordings and a histogram of the mean changes in EPSP amplitude plus SE. *$p < .05$; **$p < .01$ versus branches receiving five pulses of serotonin plus emetine.

Discussion

The requirement for transcription during long-lasting forms of synaptic plasticity has raised the question of whether the critical cellular unit of synaptic plasticity is the cell and its nucleus or the synapse. If it is the synapse, then there must be a set of mechanisms that allows the products of gene expression to alter synaptic strength at some synapses independently of others made by the same cell. By culturing a single, bifurcated presynaptic neuron with two spatially separated target neurons and by restricting the application of serotonin to the connections made onto only one of these target neurons, we have found that a single presynaptic cell is able to undergo plasticity that is synapse-specific. In a parallel study, Greg Clark and colleagues have also found—much as we have seen in dissociated cell culture—that a single sensory neuron in the intact Aplysia ganglion can undergo synapse-specific long-term facilitation (G. Clark, personal communication). The establishment of synapse-specific long-term facilitation

is dependent both on transcription and local protein synthesis. By contrast, capture of this long-term process at a second site does not require local protein synthesis.

A Function for Local Protein Synthesis: Establishing Synapse-Specific Long-Term Facilitation

Our studies provide evidence for a specific role for local protein synthesis in the establishment of synapse-specific synaptic plasticity. Although it has long been known that there are mRNAs and ribosomes in dendrites and specifically at the base of dendritic spines (for review, see Steward, 1997), the precise function of this protein synthesis machinery was not known. More recently, several groups have found that the mRNAs in these distal processes can actually be translated (Feig and Lipton, 1993; Weiler and Greenough, 1993; Crino and Eberwine, 1996; Crispino et al., 1997; for review, see Steward, 1997). This finding led to the suggestion that the local translation of these mRNAs might somehow be involved in synaptic plasticity (Steward, 1997; Schuman, 1997). Consistent with this suggestion, recent studies by Kang and Schuman (1996) demonstrated that application of BDNF or NT-3 to hippocampal slices caused a rapid, long-lasting synaptic potentiation and that brief pretreatment of slices with a protein synthesis inhibitor blocked this potentiation. The immediate dependence of this neurotrophin-induced potentiation on protein synthesis suggested local rather than somatic synthesis, and this was confirmed by showing that the potentiation occurred in slices in which the cell bodies had been severed from their processes.

The regulation of translation offers specific advantages as a mechanism for regulating protein expression—it allows rapid responses to external stimuli without requiring pathways for retrograde signaling to the nucleus, mRNA synthesis, and return anterograde transport from the nucleus to the synapse. Rapid response is crucial in neurons, where distances between synapse and nucleus can be extremely large. Local, regulated translation of dendritic mRNAs would allow a neuron to alter rapidly the synaptic efficacy and even the local structure of individual, specifically stimulated synaptic sites.

Our experiments indicate that in Aplysia, local protein synthesis is specifically required in the presynaptic sensory neuron process, a finding that is consistent with the presence of various mRNAs in the processes of Aplysia neurons (Scheller et al., 1982; Brunet et al., 1991). This appears inconsistent with the mammalian case, where mRNAs and ribosomes are located primarily in dendrites (Steward, 1997). However, unlike vertebrate neurons, invertebrate neurons are not polarized into axons and dendrites, but transmissive and receptive surfaces can be found adjacent to one another in any process. Furthermore, while the sensory neuron is presynaptic to the motor cell in Aplysia, it is in fact "postsynaptic" to the serotonin-containing perfusion pipette, just as it is postsynaptic to serotonergic interneurons in the animal (see Figure 8). Thus, regulated protein synthesis in Aplysia sensory neuron processes may occur at postsynaptic sites that are analogous to the dendritic spines of vertebrate neurons.

A New Class of Synaptic Actions: Synaptic Regulation of Local Protein Synthesis

The finding that translation is activated by repeated application of 5-HT to neuronal processes adds one more dimension to the three known signaling functions that conventional synaptic transmitters are capable of initiating. In 1951, Fatt and Katz first described the properties of ionotropic receptors that produce fast synaptic actions lasting milliseconds by regulating ion flux directly through conformational changes in transmitter-gated ion channels (Fatt and Katz, 1951). In the 1960's, Sutherland and Greengard and their colleagues described a second class of receptors that activate second messenger pathways and produce slow synaptic activity lasting minutes mediated by covalent modification of ion channels and other target proteins (Robison et al., 1968; Nestler and Greengard, 1984). In the 1980's, a third type of even more persistent synaptic actions lasting days was characterized, whereby repeated applications of a transmitter lead to the translocation of a second messenger kinase to the nucleus, where it activates a cascade of gene actions and leads to the growth of new synaptic connection, a persistent synaptic action lasting days (Montarolo et al., 1986). The data we present here describes a novel, fourth function mediated by chemical transmitters: the regulation of local protein synthesis to establish synapse-specific changes in synaptic strength.

The Initial Establishment of Synapse-Specific Long-Term Facilitation Differs from Synaptic Capture in Requiring Local Protein Synthesis

Our data indicate that the establishment of synapse-specific long-term plasticity requires two separate events: first, the generation of a retrograde signal to recruit transcription in the nucleus, and second, the marking of the synapse to recruit the products of gene expression back to that synapse and not to other synapses made by the same cell. In contrast, synaptic capture does not require retrograde signaling to initiate transcription in the nucleus, but rather simply the marking of the synapse so that the products of gene expression are used to alter synaptic strength at that synapse. The finding that synapse-specific long-term facilitation requires protein synthesis while the capture of the long-term process at the second site does not indicates that these two processes are mechanistically distinct.

What is the protein synthesis–dependent step that distinguishes synapse-specific facilitation from capture? One possibility is that proteins synthesized locally contribute to the retrograde signal that goes back to the nucleus to recruit transcription. That synaptic capture is blocked if emetine is applied to the branch receiving five pulses of serotonin but is not blocked by emetine applied to the branch receiving a single pulse of serotonin, is consistent with this possibility (Figure 6). Another possibility is that local protein synthesis actually is required to mark a synapse when the stimulus precedes the nuclear events. This could occur in one of two ways. First, the mechanism used to mark the initially stimulated synapse may be distinct from that used to mark synapses during synaptic capture. Alternatively, it is possible that there are constitutive inhibitory constraints on

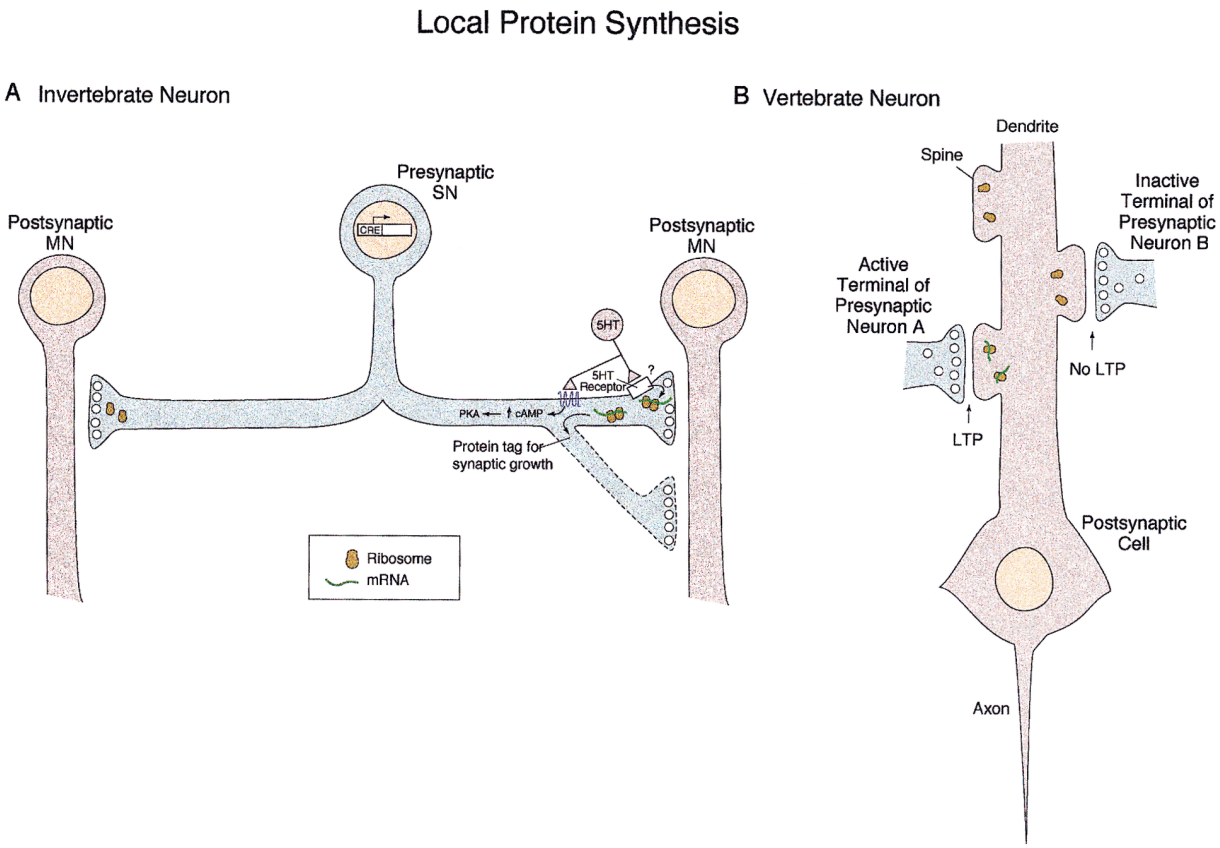

Figure 8. Locus of Local Protein Synthesis during Long-Term Synaptic Plasticity in Invertebrate and Vertebrate Neurons

Five pulses of 5HT trigger protein synthesis in the terminals of Aplysia sensory neurons, indicating that local protein synthesis is regulated in the presynaptic cell. However, although the sensory neuron is presynaptic to the motor neuron, it is in fact postsynaptic to the serotonergic interneuron. Furthermore, invertebrate neurons are not polarized, and receptive and transmissive surfaces exist adjacent to one another. In contrast, vertebrate neurons are rigidly polarized into receptive domains (dendrites) and transmissive domains (axons). As ribosomes and mRNAs are found primarily in dendrites (frequently in dendritic spines), it is likely that local protein synthesis is regulated in the postsynaptic cell in vertebrates.

growth as there are on transcription and that multiple pulses of serotonin and local protein synthesis are required to relieve these inhibitory constraints. If the relief is cell-wide, then the neuron might undergo a period of time during which it is primed for long-lasting synaptic plasticity. During this time, synapse-specific local protein synthesis would not be required, and a single pulse of serotonin would be sufficient to mark a synapse for long-lasting modulation.

One of the remarkable insights to emerge from the recent attempts to relate synaptic plasticity to memory storage is the delineation of the various ways in which the total synaptic output of a single neuron can be modulated. The opportunity to examine single bifurcating neurons has allowed us to expand investigation of the plastic possibilities of neurons by seeing the degree to which different synaptic terminals of the same cell can be specifically and locally modulated. The difference between synaptic tagging and synaptic capture illustrates that there will likely be multiple ways to modulate synaptic strength at different sites within a single neuron and that different signaling pathways may be recruited and combined to produce enduring changes. At one extreme, previous studies have shown that long-term facilitation can occur in a cell-wide manner when serotonin is applied to the cell body of sensory neurons in the ganglion (Clark and Kandel, 1993; Emptage and Carew, 1993). Similarly, we have found in our culture system that application of serotonin to the cell body also produces long-term facilitation at both branches (unpublished data). On the other hand, while application of serotonin to specific branches produces branch-specific long-term facilitation, it is possible that serotonin application to individual boutons might produce bouton-specific plasticity within a single branch. In fact, it now becomes conceivable that synapses can undergo separate phases of long-term facilitation that require only translation and not transcription. Finally, it may be possible for some terminals of a neuron to be facilitated while others are depressed.

Experimental Procedures

Aplysia Cell Culture

Culture dishes and medium were prepared as previously described (Schacher and Proshansky, 1983; Montarolo et al., 1986). To prepare the cultures, the abdominal ganglion was removed from 0.9–1.5 g juvenile Aplysia and incubated in Type IX protease (10 mg/ml, Sigma) for 1 hr 40 min at 34.5°C. The pedal-pleural ganglia were removed from 80–100 g Aplysia and incubated in Type IX protease (10 mg/

ml, Sigma) for 1 hr 50 min at 34.5°C. Two L7 motor neurons were identified in the abdominal ganglia, removed using sharp glass electrodes as previously described (Schacher and Proshansky, 1983), and plated in a culture dish at a distance of approximately 1 mm from one another. Bifurcated sensory neurons were then removed from the pleural ganglion using sharp glass electrodes. Approximately 1%–5% of sensory neurons were found to be bifurcated, and the majority of these were found at the periphery of the cluster. Sensory neurons were removed, and when a bifurcated neuron was found, it was transferred to the culture dish containing the two L7 motor neurons. The two axons of the sensory cell were carefully teased apart using glass microelectrodes, and the distal end of one axonal branch was brought into contact with the initial axon segment of one motor neuron, while the distal end of the other axonal branch was brought into contact with the initial axon segment of the other motor neuron. Cultures were left on the microscope stage overnight and then transferred to an 18°C incubator.

Electrophysiology

After five days in culture, the strength of the synaptic connection between the sensory neuron and each of the motor neurons was tested. Each motor cell was impaled with a recording microelectrode (8–10 mΩ) containing 2.5 M KCl and held at a potential of −30 mV below its resting potential. EPSPs were evoked in both L7 motor neurons by stimulating the sensory cell with a brief depolarizing pulse using an extracellular microelectrode. The EPSPs were recorded on two channels of a 4-channel tape recorder (Raclan) and printed on a chart recorder. The mean initial EPSP amplitude on day 5 in untreated cultures was 11.8 ± 2.2 mV. There was no significant difference in the initial EPSP amplitude between treated and untreated branches; for example, in Figure 2, the initial EPSP amplitude of untreated branches was 7.7 ± 1.7 mV, and the initial EPSP amplitude of the treated branch was 9.1 ± 1.8 mV. Cultures in which the initial EPSP amplitude was less than 4 mV were not used. When the posttreatment EPSP was a spike, a value of 60 mV was used for quantitation.

Bath application of serotonin was done as previously described (Montarolo et al., 1986). To produce synapse specific short-term facilitation, 100 μM 5HT (Sigma) in L15 containing 0.05% fast green was applied to the contact made by one sensory neuron branch onto one L7 motor neuron using a perfusion microelectrode (approximately 1 MΩ resistance). Very low pressure (approximately 1 lb/in^2) was applied using a picospritzer (World Precision Instruments) and five 5 s pulses were delivered at 10 s intervals. The bath was perfused with 50% L15/50% artifical sea water (Instant Ocean) at a rate of approximately 0.5 ml/min. The serotonin and bath perfusions were adjusted such that a cloud of serotonin (visualized by the fast green) covered much of the L7 motor neuron initial axon section without contacting the sensory cell body or the synapse made onto the other motor neuron. To produce synapse-specific long-term facilitation, five separate episodes of 5-HT perfusion (each episode consisting of five 5 s pulses at 10 s intervals) were given at 10 min intervals. Thus, the total number of pulses was 25, the total time of perfusion was 2 min 5 sec, and the total time of treatment (including 5-HT application and inter-5-HT intervals) was 45 min 25 sec. To produce synaptic capture, five episodes of 5-HT application were applied to one synaptic connection, then immediately after the end of the last 5HT pulse, the serotonin perfusion electrode was moved to the second synaptic connection, where a single episode of 5-HT was given. After serotonin perfusion, all cultures were incubated in normal culture medium (50% L15/50% hemolymph). To measure long-term facilitation, EPSPs were recorded in both L7 motor neurons 24 hr after 5-HT treatment.

In experiments using actinomycin D, the drug was included in the perfusion medium at a concentration of 50 μg/ml for 30 min before, during, and 30 min after serotonin treatment. In experiments in which protein synthesis inhibitors were applied locally, emetine (100 μM), anisomycin (10 μM), or cycloheximide (30 μM) were applied to the indicated synaptic connection using a second perfusion microelectrode connected to a second picospritzer. The inhibitors were diluted in 0.05% fast green in L15 and were continuously delivered using very low pressure (<1 lb/in^2) for 20 min before, during, and 20 min after serotonin application. When the protein synthesis inhibitor was applied to the branch opposite that receiving five pulses of serotonin, or when it was given alone, it was applied for 90 min. It is worth noting that, while all three inhibitors blocked synapse-specific long-term facilitation when given with 5-HT to the same branch, anisomycin given at the opposite branch or anisomycin given alone also produced a long-term increase in the EPSP amplitude. We believe that this may be due to the ability of anisomycin to activate the MAP kinase pathway (Zinck et al., 1995) and are currently investigating this possibility.

Gelonin (25 μM), anti-CREB antibodies (described in Bartsch et al., 1995), and preimmune serum were diluted in 0.5 M potassium chloride, 10 μM Tris (pH 7.6) and injected into sensory or motor neurons with microinjection electrodes (15–20 MΩ) using brief pressure pulses. All microinjections were done 1 hr prior to serotonin treatment.

All data are presented as mean percentage change ± SEM in the EPSP amplitude measured after treatment, as compared with its initial pretreatment amplitude. The significance of the EPSP changes was determined using either an unpaired Student's t-test or a one-way analysis of variance and Newman-Keuls multiple range test, as specified in the figure legends.

Immunocytochemistry

Immunocytochemistry of cultured Aplysia neurons was performed as previously described (Martin et al., 1997). Affinity-purified rabbit antisensor in antibodies (described in Brunet et al., 1991) were diluted 1:100 (approximately 10 μg/ml).

Dye Injection, Cell Imaging, and Quantification of Structural Changes

Dye injection, imaging, and analysis were done as previously described (Bartsch et al., 1995).

Metabolic Labeling

Thirty to forty sensory neurons (with single axons) were plated in a culture dish. On day 4 or 5, the number of cells was counted, and the cell body of each neuron was removed by cutting at a distance of approximately 50 microns distal to the cell soma/initial axon junction using a glass microelectrode. The cell bodies were removed by aspiration. There were no obvious qualitative differences in the extent of process outgrowth between cells or between dishes. The cultures were allowed to rest for 1–2 hr at 18°C and then washed gently several times with methionine-free medium (made using MEM select-amine kit from Gibco and supplemented with salts as previously described; Schacher and Proshansky, 1983). After a 1 hr incubation in methionine-free medium at 1°C, 50 μCi of ^{35}S-labeled methionine (Dupont-NEN, specific activity of >1000 mCi/μmol) were added to the dish and incubated for 1 hr at 18°C. The cultures were then gently washed with artificial sea water (Instant Ocean), placed on ice, and TCA precipitated. To do this, all but 100 μl of artificial sea water was removed from the dish, 100 μl of 50% ice-cold TCA was added, and the dishes were incubated on ice for 1 hr. Cells were scraped off the dish, rinsed twice with 25% TCA, and spotted onto Whatman GF-C filter paper in a vacuum manifold. The filters were washed with 5% TCA in the vacuum manifold, air dried, and counted on a liquid scintillation counter (United Technologies Packard). The total number of cpm was divided by the number of cells in each dish to obtain a value for the TCA-insoluble counts per cell. To normalize for differences in the incorporation of radioactive label from experiment to experiment, results were normalized to the mean cpm/cell in untreated control dishes. In each experiment, there were 2–3 dishes per condition. The mean cpm per untreated dish did not vary more than 30% within an individual experiment, the mean value for cpm per untreated dish for all experiments was 209,200 ± 55,241 cpm, and the mean value for cpm per untreated cell for all experiments was 5,922 ± 1,541 cpm. The significance of the differences in cpm per cell was determined by a one-way analysis of variance and Newman-Keuls multiple range test performed separately on the untreated, emetine- and chloramphenicol-treated samples, and on the untreated, serotonin-treated and serotonin-plus-emetine-treated samples.

Acknowledgments

We thank Ted Abel, Mark Barad, and Dusan Bartsch for critical reading of the manuscript and for helpful discussions throughout the work; Harriet Ayers and Millie Pellan for preparation of the manuscript; and Charles Lam for assistance with the art work. This work was supported by grants from HHMI (to E. R. K.), NIH grants MH37134 and GM32099 to C. H. B., NARSAD (to K. C. M.), and the Burroughs Wellcome Fund (to K. C. M.).

Received November 4, 1997; revised November 26, 1997.

References

Alberini, C., Ghirardi, M., Metz, R., and Kandel, E.R. (1994). C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. Cell *76*, 1099–1114.

Bailey, C.H., and Chen, M. (1983). Morphological basis of long-term habituation and sensitization in *Aplysia*. Science *220*, 91–93.

Bailey, C.H., and Chen, M. (1988). Long-term memory in *Aplysia* modulates the total number of varicosities of single identified sensory neurons. Proc. Natl. Acad. Sci. USA *85*, 2373–2377.

Bailey, C.H., Montarolo, P.G., Chen, M., Kandel, E.R., and Schacher, S. (1992). Inhibitors of protein and RNA synthesis block structural changes that accompany long-term heterosynaptic plasticity in Aplysia. Neuron *9*, 749–758.

Bailey, C.H., Bartsch, D., and Kandel, E.R. (1996). Toward a molecular definition of long-term memory storage. Proc. Natl. Acad. Sci. USA *93*, 13445–13452.

Bartsch, D., Ghirardi, M., Skehel, P.A., Karl, K.A., Herder, S.P., Chen, M., Bailey, C.H., and Kandel, E.R. (1995). Aplysia CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. Cell *83*, 979–992.

Bliss, T.V.P., and Collinridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31–39.

Brunet, J.F., Shapiro, E., Foster, S.A., Kandel, E.R., and Iino, Y. (1991). Identification of a peptide specific for *Aplysia* sensory neurons by PCR-based differential screening. Science *252*, 856–859.

Clark, G.A., and Kandel, E.R. (1984). Branch-specific heterosynaptic facilitation in *Aplysia* siphon sensory cells. Proc. Natl. Acad. Sci. USA *81*, 2577–2581.

Clark, G.A., and Kandel, E.R. (1993). Induction of long-term facilitation in *Aplysia* sensory neurons by local application of serotonin to remote synapses. Proc. Natl. Acad. Sci. USA *90*, 11411–11415.

Crino, P.B., and Eberwine, J. (1996). Molecular characterization of the dendritic growth cone: regulated mRNA transport and local protein synthesis. Neuron *17*, 1173–1187.

Crispino, M., Kaplan, B.B., Martin, R., Alvarez, J., Chun, J.T., Benech, J.C., and Giuditta, A. (1997). Active polysomes are present in the large presynaptic endings of the synaptosomal fraction from squid brain. J. Neurosci. *17*, 7694–7702.

Dash, P.K., Hochner, B., and Kandel, E.R. (1990). Injection of cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation. Nature *345*, 718–721.

Emptage, N.J., and Carew, T.J. (1993). Long-term synaptic facilitation in the absence of short-term facilitation in *Aplysia* neurons. Science *262*, 253–256.

Fatt, P., and Katz, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. J. Physiol. *115*, 320–370.

Feig, S., and Lipton, P. (1993). Pairing the cholinergic agonist carbachol with patterned Schaffer collateral stimulation initiates protein synthesis in hippocampal CA1 pyramidal cell dendrites via a muscarinic, NMDA-dependent mechanism. J. Neurosci. *13*, 1010–1021.

Frey, U., and Morris, R.G.M. (1997). Synaptic tagging and long-term potentiation. Nature *385*, 533–536.

Garner, C.C., Tucker, R.P., and Matus, A. (1988). Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. Science *336*, 674–677.

Ghirardi, M., Montarolo, P.G., and Kandel, E.R. (1995). A novel intermediate stage in the transition between short- and long-term facilitation in the sensory to motor neuron synapse of Aplysia. Neuron *14*, 413–420.

Glanzman, D.L., Kandel, E.R., and Schacher, S. (1989). Identified target motor neuron regulates neurite outgrowth and synapse formation of Aplysia sensory neurons in vitro. Neuron *3*, 441–450.

Glanzman, D.L., Kandel, E.R., and Schacher, S. (1990). Target-dependent structural changes accompanying long-term synaptic facilitation in *Aplysia* neurons. Science *249*, 799–802.

Goelet, P., Castellucci, V.F., Schacher, S., and Kandel, E.R. (1986). The long and the short of long-term memory—a molecular framework. Nature *322*, 419–422.

Hegde, A.N., Inokuchi, K., Pei, W., Casadio, A., Ghirardi, M., Chain, D.G., Martin, K.C., Kandel, E.R., and Schwartz, J.H. (1997). Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in Aplysia. Cell *89*, 115–126.

Kang, H., and Schuman, E.M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science *273*, 1402–1406.

Martin, K.C., and Kandel, E.R., (1996). Cell adhesion molecules, CREB, and the formation of new synaptic connections. Neuron *17*, 567–570.

Martin, K.C., Michael, D., Rose, J.C., Barad, M.G., Casadio, A., Zhu, H., and Kandel, E.R. (1997). MAP kinase tranlocates into the nucleus of the presynaptic cell and is required for long-term facilitation in Aplysia. Neuron *18*, 899–912.

Mayford, M., Baranes, D., Podsypanina, K., and Kandel, E.R. (1996). The 3′-untranslated region of CaMKII-α is a cis-acting signal for the localization and translation of mRNA in dendrites. Proc. Natl. Acad. Sci. USA *93*, 13250–13255.

Montarolo, P.G., Goelet, P., Castellucci, V.F., Morgan, J., Kandel, E.R., and Schacher, S. (1986). A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia*. Science *234*, 1249–1254.

Nestler, E.J., and Greengard, P. (1984) Protein Phosphorylation in the Nervous System (New York: John Wiley & Sons).

Nestler, E.J., and Aghajanian, G.K. (1997). Molecular and cellular basis of addiction. Science *278*, 58–63.

Rayport, S.G., and Schacher, S. (1986). Synaptic plasticity in vitro: cell culture of identified neurons mediating short-term habituation and sensitization. J. Neurosci. *6*, 759–763.

Robison, G.A., Butcher, R.W., and Sutherland, E.W. (1968). Cyclic AMP. Annu. Rev. Biochem. *37*, 149–174.

Schacher, S., and Proshansky, E. (1983). Neurite regeneration by *Aplysia* neurons in dissociated cell culture: modulation by *Aplysia* hemolymph and the presence of the initial axonal segment. J. Neurosci. *3*, 2403–2413.

Scheller, R.H., Jackson, J.F., McAllister, L.B., Schwartz, J.H., Dandel, E.R., and Axel, R. (1982). A family of genes that codes for ELH, a neuropeptide eliciting a sterotyped pattern of behavior in Aplysia. Cell *28*, 707–719.

Schuman, E.M. (1997). Synapse specificity and long-term information storage. Neuron *18*, 339–342.

Sossin, W.S. (1996). Mechanisms for the generation of synapse specificity in long-term memory: the implications of a requirement for transcription. Trends Neurosci. *19*, 215–218.

Steward, O. (1997). mRNA localization in neurons: a multipurpose mechanism? Neuron *18*, 9–12.

Stirpe, F., Olsnes, S., and Pihl, A. (1980). Gelonin, a new inhibitor of protein synthesis, nontoxic to intact cells. J. Biol. Chem. *255*, 6947–6953.

Trudeau, L.E., and Castellucci, V.F. (1995). Postsynaptic modifications in long-term facilitation in *Aplysia*: upregulation of excitatory amino acid receptors. J. Neurosci. *15*, 1275–1284.

Van Minnen, J., Bergman, J.J., Van Kesteren, E.R., Smit, A.B., Geraerts, W.P.M., Lukowiak, K., Hasan, S.U., and Syed, N.I. (1997).

De novo protein synthesis in isolated axons of identified neurons. Neuroscience *80*, 1–7.

Weiler, I.J., and Greenough, W.T. (1993). Metabotropic glutamate receptors trigger postsynaptic protein synthesis. Proc. Natl. Acad. Sci. USA *90*, 7168–7171.

Zinck, R., Cahill, M.A., Kracht, M., Sachsenmaier, C., Hipskind, R.A. and Nordheim, A. (1995). Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. Mol. Cell. Biol. *15*, 4930–4938.