Some authorities have suggested that, because of their many primitive anthropoid characteristics, one group of Fayum primates, the parapithecids, may be related to the New World platyrrhines (*14*). A major line of evidence against an African origin for platyrrhines has been the apparent differences in the relative age of the earliest African and South American anthropoids. Earlier correlations placed the earliest platyrrhines at 35 million years ago, nearly 10 million years older than the supposed age of the Fayum primates. However, more recent studies of the geology of the Salla basin in Bolivia (*15*) have shown that the earliest platyrrhines are probably from about 25 million years ago, substantially younger than the Fayum anthropoids. Thus, the new minimum age estimate of the Jebel Qatrani Formation indicates that the Fayum primates are substantially older than the first appearances of all modern anthropoid radiations, a chronology that accords well with morphological analyses that place some species at the base of the higher primate radiation.

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A Critical Period for Macromolecular Synthesis in Long-Term Heterosynaptic Facilitation in *Aplysia*

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Both long-term and short-term sensitization of the gill and siphon withdrawal reflex in *Aplysia* involve facilitation of the monosynaptic connections between the sensory and motor neurons. To analyze the relationship between these two forms of synaptic facilitation at the cellular and molecular level, this monosynaptic sensorimotor component of the gill-withdrawal reflex of *Aplysia* can be reconstituted in dissociated cell culture. Whereas one brief application of 1 μ*M* serotonin produced short-term facilitation in the sensorimotor connection that lasted minutes, five applications over 1.5 hours resulted in long-term facilitation that lasted more than 24 hours. Inhibitors of protein synthesis or RNA synthesis selectively blocked long-term facilitation, but not short-term facilitation, indicating that long-term facilitation requires the expression of gene products not essential for short-term facilitation. Moreover, the inhibitors only blocked long-term facilitation when given during the serotonin applications; the inhibitors did not block the facilitation when given either before or after serotonin application. These results parallel those for behavioral performance in vertebrates and indicate that the critical time window characteristic of the requirement for macromolecular synthesis in long-term heterosynaptic facilitation is not a property of complex circuitry, but an intrinsic characteristic of specific nerve cells and synaptic connections involved in the long-term storage of information.

MEMORY, THE RETENTION OF learned information over time, is thought to have at least two components—short-term memory lasting minutes to hours, and long-term memory lasting for days to weeks or even years (*1*). Behavioral studies in vertebrates suggest that long-term memory requires the synthesis of new protein, whereas short-term memory does not (*2*). Moreover, long-term memory is most sensitive to disruption by inhibitors of protein synthesis when the inhibitors are applied during and immediately after training (*3*). There is no deficit in the retention of the learned behavior when the inhibitors are applied even 1 to 2 hours after training. This time window is remarkably characteristic and has been demonstrated for memory produced by associative as well as nonassociative forms of learning and for various vertebrate species (*3*). Although comparable data are lacking in humans, analogous clinical studies of convulsions and head trauma indicate that human long-term memory is particularly susceptible to disruption during and soon after acquisition (*4*).

The interpretation of earlier experiments, based on the systemic application of protein synthesis inhibitors, was limited, however, in two ways. First, the protein synthesis inhibitors often led to serious side effects such as seizure and sickness (*3*). Second, the studies were based on the examination of behavioral performance rather than on the analysis of the cellular correlates responsible for the neuronal plasticity underlying the altered behavior. Inhibitors of protein synthesis can affect behavioral performance not only through their action on memory processes, but also through their action on other systems that influence performance, such as attention or motivation. As a result, it has not been possible to determine whether new protein synthesis is important for memory and whether the characteristic time window for long-term memory reflects a specific requirement for macromolecular synthesis by particular neurons involved in the storage of long-term information.

To overcome these limitations, we analyzed memory processes at the cellular and molecular level, using the gill-withdrawal reflex of *Aplysia*. The neural circuitry of this reflex behavior has been delineated (*5*), and one important cellular locus has been identified in mediating short-term and long-term

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sensitization (*6*). Both short-term sensitization, lasting minutes to hours, and long-term sensitization, lasting days, involve changes in the strength of the connections that the sensory neurons make on their central target cells, the interneurons and the motor neurons. Short-term sensitization results from the heterosynaptic facilitation of transmitter release from the terminals of the sensory neurons produced by the actions of serotonergic and other modulatory neurons activated by tail or head shock (*7*). Long-term sensitization also leads to an enhancement in the strength of the connections between sensory and motor neurons (*6*). In addition, long-term sensitization results in a growth process leading to an increase in both the number of varicosities of the sensory neurons and in the incidence of active zones for transmitter release (*8*).

As a first step in exploring the molecular mechanisms underlying long-term plasticity, we have further simplified the analysis of the neuronal circuit by reconstituting the monosynaptic sensorimotor component of the gill-withdrawal reflex in dissociated cell culture. We simulated the behavioral training necessary to produce long-term sensitization in the intact animal (repeated trains of head or tail shocks every 30 minutes over 2 hours), by using five repeated applications of serotonin (5-HT). These repeated applications produced long-term facilitation of the connection between the sensory and motor neurons detected 1 day later. This long-term facilitation was blocked, selectively, without interfering with short-term facilitation when a specific inhibitor of either protein or RNA synthesis was applied together with 5-HT. Inhibitors applied either before or after 5-HT application did not block long-term facilitation. These results suggest that long-term synaptic facilitation requires the synthesis of gene products not essential for the expression of short-term facilitation. Protein and RNA synthesis is required during a period of 1 to 3 hours, during and immediately after the application of 5-HT.

A single brief application of 5-HT in dissociated cell culture produced short-term facilitation of the sensorimotor connection comparable in amplitude and time course to the facilitation evoked in the intact nervous system with natural stimulation or with exogenous 5-HT (*9*). We therefore first examined whether these connections can undergo long-term facilitation in vitro when exposed to repeated applications of 5-HT. We co-cultured the gill motor cell L7 to-

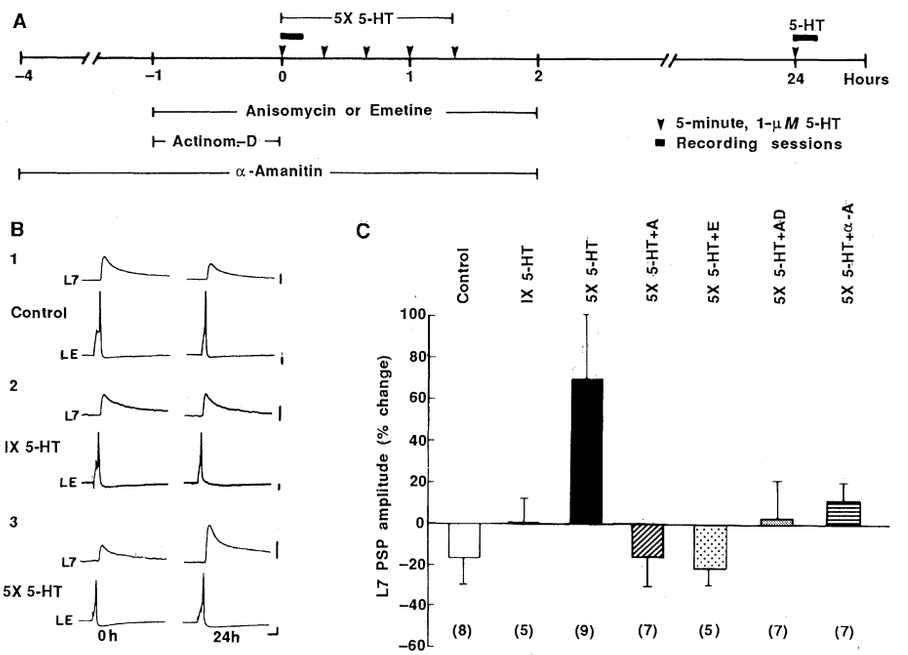

Fig. 1. Long-term facilitation of sensorimotor connections with repeated applications of 5-HT is blocked in the presence of inhibitors of protein or RNA synthesis. (A) Experimental protocol. After 5 days in culture, sensory and motor cells were impaled and the motor cell was hyperpolarized 50 mV below the resting potential to permit accurate measurement of the amplitude of the EPSP. The first recording session (0 hour, dark bar), either in the absence or presence of the inhibitors, consisted of the following. Every 20 seconds, a single spike was evoked in the sensory neurons by injecting a depolarizing pulse. After the fifth action potential, 5-HT was applied in the bath to a final concentration of 1 μM and the extent of facilitation assessed with three additional evoked EPSP's (see Fig. 2). In cultures with two sensory neurons, short-term facilitation was assessed during the recording from the second cell. Four minutes later, the 5-HT was washed out by perfusion with 50% L15 and 50% artificial seawater, *p*H 7.6, at a rate of 1.5 ml per minute (0.5-bath volumes per minute). The electrodes were removed from the cells and four subsequent 5-minute 5-HT applications were given at 15-minute intervals (arrowheads) without further intracellular recording to assure cell viability. After each 5-HT application, 5-HT was washed out at a rate of 1.5 ml per minute. Control cultures received the same number of evoked EPSP's but either no 5-HT or a single application of 5-HT, and were continuously perfused for 2 hours. After 2 hours, both experimental and control cultures were returned to culture medium and replaced in the incubator at 18°C. At 24 hours, both experimental and control cultures were reexamined. The same cells were impaled, and the strength of the connections and short-term facilitation reexamined (24 hour, dark bar) as during the 0-hour recording session. Cultures treated with drugs alone (see Table 1) also received identical stimulation regimens but no 5-HT (*23*). Anisomycin (10 μM) or emetine (100 μM) was added to the cultures 1 hour before the initial recording and was present continuously until 30 minutes after the last 5-HT application. The transcriptional inhibitor α-amanitin (2 μg/ml), which penetrates cells slowly, was added to cultures 4 hours before the initial recording and was present in the perfusion medium throughout the 5-HT applications until 30 minutes after the last 5-HT application. The cultures were then perfused for 15 minutes in drug-free medium. Actinomycin D (50 μg/ml) was added 1 hour before the first recording session, after which the cells were perfused with drug-free medium. Actinomycin D was not perfused continuously because a 1-hour exposure irreversibly blocked total RNA synthesis in *Aplysia* neurons by more than 90%. (B) Sensorimotor connections show long-term facilitation after repeated 5-HT applications. The first evoked EPSP in the motor cell L7 [top traces in (B_1), (B_2), and (B_3), during the 0-hour recording session (left column)] is compared to the first evoked EPSP for the same connection at the 24-hour recording session (right column). When no 5-HT applications were given [control (B_1)] the amplitude of the EPSP shows a slight decline. Twenty-four hours after a single 5-HT application [1× 5-HT (B_2)] the EPSP is unchanged. A significant increase in the amplitude of the EPSP was obtained after five applications of 5-HT [5× 5-HT (B_3)]. For details on recording sessions and protocol, see Fig. 1A. Calibration bars: 10 mV, 20 msec. (C) Summary of long-term facilitation induced by 5-HT and blockade by protein and RNA synthesis inhibitors. A, anisomycin; E, emetine; AD, actinomycin D; α-A, α-amanitin. The height of each bar is the mean ± SEM of the percent change in amplitude of the first evoked EPSP recorded at 0 hours compared to the first evoked EPSP of the same connection reexamined at 24 hours (see Fig. 1B). Each culture (one L7 with one or two LE sensory neuron synaptic connections) was treated as a single sample. The number of samples is indicated in parentheses. In cases where two sensory neurons formed synaptic connections with L7 in a single culture, the percent change was calculated as the mean change for the two synapses. An overall analysis of variance indicated a difference with treatment ($F = 3.02$; df = 6, 41; $P < 0.05$). A comparison of the means (multiple comparison t test) indicated that 5× 5-HT significantly increased EPSP amplitude compared to the other treatments (CR = 57, $P < 0.05$), whereas the other treatments did not differ from each other or control.

gether with one to two sensory neurons from the abdominal ganglion LE cluster (*10*) and examined the strength of the connection on day 5 (Fig. 1A, bar at 0 hour; Fig. 1 B, 0-hour column). We then exposed the cultures to either a single 5-minute application of 5-HT or to five applications of 5-HT (5 minutes each) at 15-minute intervals (Fig. 1A). We measured the response to the first 5-HT application to determine the extent of short-term facilitation (Fig. 1A, arrow at 0 hour). The identical cells and connections were reexamined 24 hours later (Fig. 1A, bar at 24 hours; Fig. 1B, 24-hour column). The cultures treated with five applications of 5-HT showed a significant increase in the strength of their connections of 71 ± 31% (SEM), whereas untreated controls or cultures receiving a single application showed no significant change in their synaptic strength (Fig. 1, B and C).

Because the behavioral expression of long-term memory in vertebrates can be blocked by interfering with protein synthesis during training (*3*), we next determined whether the long-term facilitation of synaptic strength in culture produced by 5-HT can be affected by inhibitors of protein or RNA synthesis present during 5-HT applications. Using the same protocol described in Fig. 1A, we first exposed the cultures to one of two different inhibitors of protein synthesis—10 μ*M* anisomycin or 100 μ*M* emetine (*11*)—or to one of two different inhibitors of RNA synthesis—actinomycin D (50 μg/ml), an inhibitor of total RNA synthesis (*12*), or α-amanitin (2 μg/ml), a specific inhibitor of RNA polymerase II activity (*13*). The presence of either translational or transcriptional inhibitors during the applications of 5-HT blocked the enhancement of synaptic strength produced by 5-HT when retested 24 hours later: −16 ± 14% for anisomycin; −21 ± 10% for emetine; +3 ± 18% for actinomycin D; and +11 ± 9% for α-amanitin (Fig. 1C). By themselves, the inhibitors had no significant effect on synaptic strength, resting potential, or input resistance (Table 1).

Although both the protein and RNA synthesis inhibitors blocked long-term facilitation, none of these inhibitors affected short-term facilitation produced by a single application of 5-HT (Fig. 2). In the same experiments in which the inhibitors blocked long-term facilitation, short-term facilitation was normally expressed, both when the inhibitors were present or 24 hours after the inhibitors had been washed out.

We next examined the critical time course for new protein synthesis required for long-term facilitation by studying in more detail the extent of inhibition by anisomycin and the recovery of protein synthesis after drug washout. *Aplysia* neurons in culture were exposed to anisomycin for 3 hours and pulse labeled with [^{35}S]methionine in the presence of this inhibitor or at various times after its washout (Fig. 3). When anisomycin was present, protein synthesis was inhibited by over 95%, but it recovered rapidly to control levels within 2 to 4 hours after removal of the inhibitor (Fig. 3). This suggests that transient inhibition of protein synthesis, during and immediately after the application of 5-HT, might be sufficient to block the long-term facilitation of the sensorimotor connection.

We tested this idea directly by defining

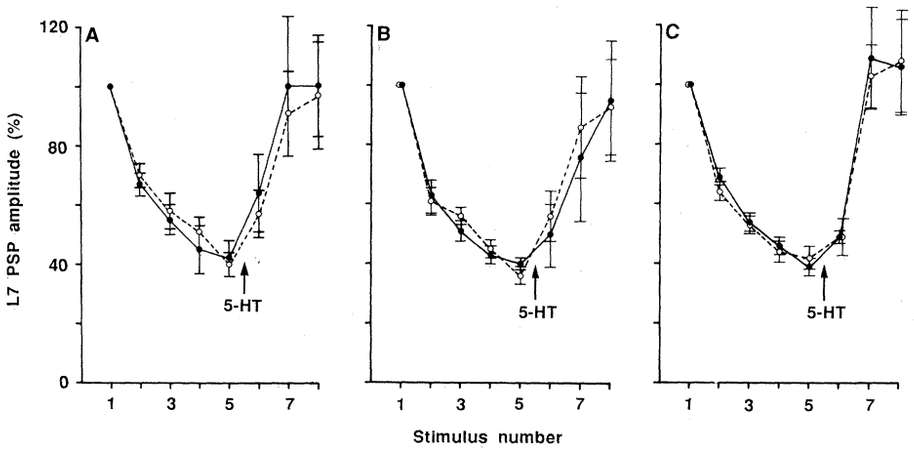

Fig. 2. Protein and RNA synthesis inhibitors do not block short-term facilitation. Summary of homosynaptic depression and 5-HT facilitation of the same connections measured during recording sessions at 0 hours and 24 hours after treatment (see Fig. 1A). The LE-L7 EPSP's were normalized relative to the first evoked EPSP at each time point in each experiment. After five LE evoked EPSP's, which resulted in 60 to 70% depression in EPSP amplitude, 1 μ*M* 5-HT was applied, which produced in all cases an approximately 2.5-fold increase in EPSP amplitude by the seventh stimulus. The facilitation evoked by a single 5-HT application lasted 3 to 5 minutes after starting 5-HT washout. (A) 5-HT facilitation of connections in the absence of drugs during the 0-hour (●) and 24-hour (○) recording sessions ($n = 6$). These cultures subsequently received four additional 5-HT applications that produced long-term facilitation. (B) 5-HT facilitation of the same connections ($n = 6$) in the presence (●) and after washout (○) of anisomycin. (C) 5-HT facilitation of the same connections ($n = 6$) in the presence (●) and after washout (○) of α-amanitin. Similar results were obtained for emetine and actinomycin D. The points are means and SEM.

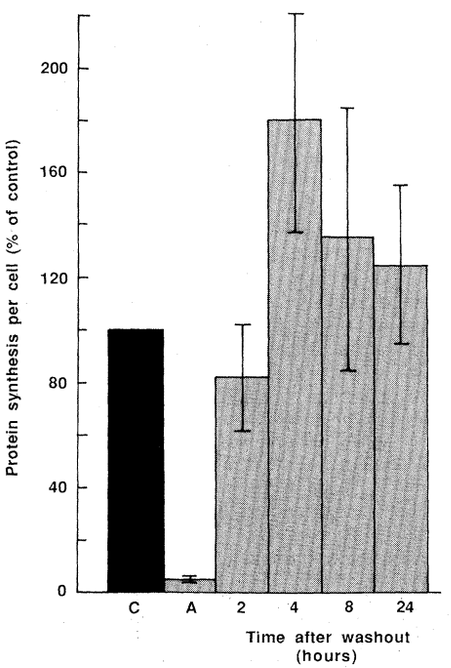

Fig. 3. Anisomycin transiently inhibits protein synthesis by *Aplysia* neurons in culture. Abdominal ganglia neurons derived from ganglia dissected from juvenile animals were dissociated and random populations of cells were plated in culture dishes and maintained for 5 days under the same conditions as the sensory neuron–motor neuron cultures. Each culture dish (25 to 50 cells per dish) was pulse labeled for 2 hours with [^{35}S]methionine (250 μCi; 1000 Ci/m*M*, Amersham) and [^{3}H]uridine (100 μCi; 50 Ci/m*M*, Amersham) and collected (*24*). Control cultures (C) received no exposure to anisomycin. The other cultures were exposed to 10 μ*M* anisomycin for 3 hours and were pulse-labeled either during the last 2 hours in anisomycin (A) or 2, 4, 8, and 24 hours after the anisomycin was washed out with ten bath volumes of L15–artificial seawater and returned to growth medium. Incorporation of [^{3}H]uridine into total RNA (TCA-precipitable counts) was assayed to determine cell viability. In each case, [^{3}H]uridine incorporation per cell was not significantly altered by the presence of anisomycin compared to controls. The TCA-precipitable [^{35}S] per cell recovers to control levels after anisomycin exposure. The height of each bar is the mean ± SEM from four separate experiments, each normalized to the control value in each experiment. By 4 hours, there is recovery and overshoot of protein synthesis levels. The increased level of protein synthesis following the removal of anisomycin could be a result of translation of mRNA that accumulated during the period of protein synthesis inhibition. At 8 and 24 hours, protein synthesis levels are not different from control levels.

more precisely the exact time period during which new protein synthesis is required for the expression of long-term facilitation (Fig. 4A). We applied anisomycin for a 3-hour period at various times before (12 hours) and after (0.5 hour and 4 hours) the applications of 5-HT. Whereas anisomycin blocked the facilitation when given at −1 hour [−16 ± 14% (SEM)], exposure to anisomycin at other times did not interfere with the long-term facilitation of the sensorimotor connections (Fig. 4A). There was little or no effect of anisomycin given at −12 hours (65 ± 10%), at 0.5 hour (51 ± 19%), or at 4 hours (90 ± 32%). Thus, expression of long-term facilitation requires new protein synthesis only transiently, during and immediately after 5-HT applications.

We also explored whether an additional requirement for new macromolecular synthesis might be revealed at periods other than during the 5-HT applications by exposing the cultures to inhibitors of macromolecular synthesis for the entire 22-hour period following the 5-HT applications. Cultures were exposed to anisomycin (10 μ*M*) or the transcriptional inhibitor α-amanitin (2 μg/ml) for a 22-hour period, extending from 0.5 hour after the last 5-HT application until the onset of the recording session at 24 hours. Neither inhibitor significantly interfered with the 5-HT–induced long-term facilitation (Fig. 4B). The long period of inhibition affected neither the strength of the connections nor their capacity to undergo short-term facilitation with a single application of 5-HT.

Our findings indicate that the monosynaptic component of the *Aplysia* gill-withdrawal reflex can show both short-term and long-term facilitation in dissociated cell culture with applications of 5-HT. As is the case with sensitizing stimuli to the tail or head, a single exposure to 5-HT produced facilitation that lasted minutes (*9*), repeated 5-HT exposures produced facilitation that lasted more than 24 hours. This result suggests that enhancement in the connections between sensory and motor neurons in long-term sensitized animals can be produced by repeated actions of a single modulating neurotransmitter (such as 5-HT) that is released with each sensitizing stimulus.

We do not know the intracellular signaling systems whereby repeated actions of 5-HT (or other modulatory transmitters) produce long-term enhancement of the connection. Short-term facilitation of transmitter release by sensory neurons produced by a single sensitizing stimulus is associated with an increase in cyclic adenosine monophosphate synthesis and protein phosphorylation that produces closure of a serotonin-sensitive potassium channel and altered handling of calcium (*14*). Long-term facilitation could work through an independent and parallel signaling system. Alternatively, the two types of systems could be in series so that some or all of the steps involved in the short-term process may also participate in the long-term process initiated by repeated 5-HT exposures. The structural changes described by Bailey and Chen (*8*) suggest that at least some additional steps are likely to be involved in the long-term facilitation of sensorimotor connections. The long-term change may also involve alterations in the postsynaptic motor neuron, perhaps in the density or distribution of transmitter receptor proteins (*15*).

As is the case with vertebrates, long-term behavioral sensitization in *Aplysia* also requires new protein synthesis. In a partially intact animal preparation, consisting of all the components of the behavior, long-term sensitization of gill-withdrawal was blocked when repeated tail shocks were given while the abdominal ganglion was exposed to anisomycin, whereas short-term sensitization was not affected (*16*). In dissociated culture, we now have been able to examine a significant component of the cellular substrate of this long-term memory for sensitization—the changes that occur in the monosynaptic component of the gill-withdrawal reflex—at the level of a single identified and behaviorally relevant synaptic connection. As is the case with the intact behavior, the long-term facilitation of this connection shows a dependence on protein and RNA synthesis that parallels the memory. By contrast, both the short-term memory and the short-term facilitation are not dependent on new macromolecular synthesis. This result is consistent with the findings of studies on invertebrates such as *Aplysia, Hermissenda*, and *Drosophila* that short-term memory is

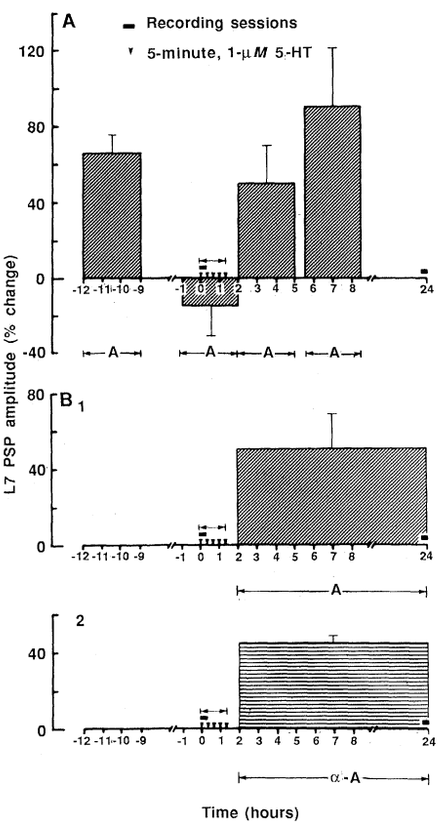

Fig. 4. Transient suppression of protein synthesis during and immediately after 5-HT application blocks long-term facilitation of sensorimotor connections formed in vitro. The protocol used to induce long-term facilitation of the connections with 5-HT applications is described in Fig. 1A. (A) Cultures were exposed to 10 μ*M* anisomycin (A) for 3 hours at four different times: starting 12 hours before the first recording session (bar at −12 to −9 hours) ($n = 4$); 1 hour before 5-HT application until 0.5 hour after the last 5-HT application (bar at −1 to 2 hours) ($n = 7$); starting either 0.5 hour ($n = 4$) or 4 hours ($n = 4$) after the last 5-HT application (bars at 2 to 5 hours and 5.5 to 8.5 hours, respectively). The height of each bar is the mean ± SEM of the percent change in amplitude of the first evoked EPSP recorded at 0 hour, compared to the first evoked EPSP of the same connection reexamined at 24 hours. Each culture was treated as a single sample. An overall analysis of variance indicated a difference with treatment ($F = 6.09$; df = 3, 15; $P < 0.05$). A comparison of the means (multiple comparison t test) indicated that the increases in EPSP amplitude with 5-HT, when anisomycin was applied either before or after the 5-HT applications, were significantly greater than that measured in cultures receiving 5-HT applications in the presence of anisomycin (CR = 61, $P < 0.05$). Cultures exposed to anisomycin at these same time points, but receiving no 5-HT applications, showed no significant change in EPSP amplitude when reexamined 24 hours later. (B) Inhibition of macromolecular synthesis for the entire period following 5-HT application does not block long-term facilitation of sensorimotor connection. Anisomycin (A in B_1) or α-amanitin (α-A in B_2) was added to cultures 0.5 hour after the last 5-HT application. Control cultures received the same stimulation regimen and drug treatment, but no 5-HT applications. (B_1) Mean ± SEM of the percent change in amplitude of the first evoked EPSP recorded before 5-HT applications compared to the first evoked EPSP of the same connection following anisomycin treatment ($n = 4$). Anisomycin application alone produced a slight decline in EPSP amplitude (−6 ± 12%, $n = 3$). A two-tailed t test comparison of the means indicated that the increase in EPSP in cultures treated with 5-HT was significantly greater ($P < 0.02$) than in cultures treated with anisomycin alone. (B_2) Mean ± SEM of the percent change in the first evoked EPSP recorded before 5-HT applications compared to the first evoked EPSP of the same connections following α-amanitin ($n = 4$). Application of α-amanitin alone resulted in a small decline in EPSP amplitude (−9 ± 11%, $n = 3$). A two-tailed t test comparison of the means indicated that the increase in the EPSP in 5-HT treated cultures was significantly greater ($P < 0.02$) than in cultures treated with α-amanitin alone.

Table 1. Effects of inhibitors on physiological properties of sensory and motor cells. The values (mean ± SEM) were measured on cells as in Fig. 1A. Drug treatments alone had no significant effect on initial amplitudes of the excitatory postsynaptic potential (EPSP) evoked in L7. Percent change in EPSP amplitude was measured as in Fig. 1C. The drugs had no significant effect on sensory or motor cell resting potentials (V_m) and L7 input resistance (R_m).

Drug treatment	n	Connections	Percent change			
			EPSP amplitude	LE V_m	L7 V_m	L7 R_m
None	8	13	−14 ± 12	+1 ± 6	−2 ± 4	+4 ± 12
5× 5-HT	9	16	+71 ± 31	+4 ± 7	+3 ± 5	−3 ± 15
Anisomycin (10 μ*M*)	12	18	+12 ± 19	−5 ± 6	+4 ± 4	+6 ± 10
Emetine (100 μ*M*)	4	5	−19 ± 14	−6 ± 4	−6 ± 5	−3 ± 7
Actinomycin D (50 μg/ml)	5	9	+2 ± 11	+10 ± 6	−10 ± 7	+7 ± 10
α-Amanitin (2 μg/ml)	6	7	−6 ± 21	−8 ± 7	−9 ± 8	+5 ± 8

evoked by modification of preexisting proteins (*17*).

Moreover, our findings indicate that the narrow time window of 1 to 3 hours during which protein and messenger RNA (mRNA) must be synthesized for the expression of long-term facilitation of sensorimotor connections in vitro—a feature characteristic of long-term behavioral memory for many forms of learning in vertebrates—is not a systems property but reflects a fundamental property in the storage of information by specific neurons and their connections. At this storage site, long-term facilitation differs from short-term facilitation in requiring the synthesis of gene products not required for short-term facilitation. Thus, whereas the proteins and mRNA's critical for short-term facilitation already exist and turn over slowly (*17*), certain proteins and mRNA's necessary for long-term facilitation must either be induced or, if constitutively expressed, are only transiently accessible to modification (*18*).

Although our data indicate that the synthesis of gene products is necessary for long-term facilitation, we do not know the nature of the gene products or how their expression affects the phenotypic properties of the sensory or motor cells. We also do not know how the synthesis of these gene products is regulated. Exposures to 5-HT could lead, via the appropriate activation of second messenger systems within sensory neurons (*19*), to long-lived modifications of constitutively expressed gene products, or to direct induction of effector genes (*20*), resulting in synaptic enhancement. However, the time window for macromolecular synthesis in long-term facilitation resembles that found in certain developmental processes (*21*) and in the initiation of lordosis behavior in rats by steroid hormones (*22*), both of which appear to involve sequential gene activation. In these cases an external signal (such as transmitter or hormone) triggers the transient expression of early regulatory genes that have products that are thought to regulate the expression of effector genes responsible for the persistent phenotypic changes observed. The long-term facilitation of sensorimotor connections may thus involve a sequential induction mechanism. The second messengers might first activate early regulatory genes, which act on later effector genes to bring about maintained changes in the synthesis of gene products responsible for synaptic enhancement (*18*).

Reconstitution of a portion of the neural circuit of the gill-withdrawal reflex in culture has allowed replication of a component of the long-term change in two identified cells and their connections, and has identified a single transmitter as the extracellular signal necessary for inducing this long-term change. In addition, it has been possible to constrain the critical expression of gene products underlying this long-term change to a specific 1- to 3-hour time period. These simplifications should facilitate the search for the macromolecules required for the retention of long-term behavioral sensitization in *Aplysia* and allow study of the mechanisms regulating the synthesis of these macromolecules. Application of these methods for studying long-term changes in other systems might elucidate the general nature of these mechanisms.

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Lactate Transporter Defect: A New Disease of Muscle

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New methods were used to identify the abnormality in a patient who showed evidence of neuromuscular dysfunction on extensive clinical examination. The methods revealed that the lactate content of the patient's skeletal muscle does not decline normally after exercise and that his red cells are defective in lactate transport. These results suggest that skeletal muscle and erythrocyte membranes share the same genetic lactate transporter (or a common subunit), which is deficient in this patient. This defect may be a common cause of elevated serum creatine kinase levels, as seen in the patient described here and of unexplained episodes of rhabdomyolysis and myoglobinuria.

STRIATED MUSCLE HAS A GREATER DYnamic range than any other tissue. Upon strenuous exercise its oxidative demands may increase a hundredfold, and its glycolytic rate, a thousandfold (*1–3*). Moreover, it is the inherent paradox of the contractile machinery that attainment of maximal force by the recruitment of all fiber groups, with the greatest demand for oxidative energy provision, simultaneously produces the greatest blockade of capillary blood flow and hence the most anoxic state. It is particularly for this tissue that the distinction between conditioning and disease becomes blurred, since poor conditioning may produce more functional compromise in muscle than many diseases produce in other target organs. Muscle is thus a prime candidate for functional or metabolic diseases, that is, enzyme or carrier defects that limit maximal performance, but may be unaccompanied by anatomic pathology. Examples of such defects have thus far been limited to enzyme deficiencies, such as those involved in glycolysis and glycogenolysis (*4–6*) and myoadenylate deaminase (*7*). I have previously referred to some of these enzymes as "perquisitory" catalysts, because, although not essential for muscular activity, they provide important perquisites for maximal performance (*8*). One may anticipate, therefore, that defects in such catalysts may produce "diseases of healthy people."

Since most patients whose major complaint is of muscle pain or weakness are never identified with a specific disease or pathologic diagnosis (*9*), it occurred to me that a logical candidate for the pathogenesis of some of these cases would be the lactate transporter of the cell membrane. This transporter has been known for about a dozen years. It was first demonstrated in bacterial cells, then later studied in detail in mammalian tumor cells (*10*) and human red blood cells (*11*). It is now clear that it is present in most animal cells and tissues that produce lactate above their utilization level, which include erythrocytes and skeletal muscle.

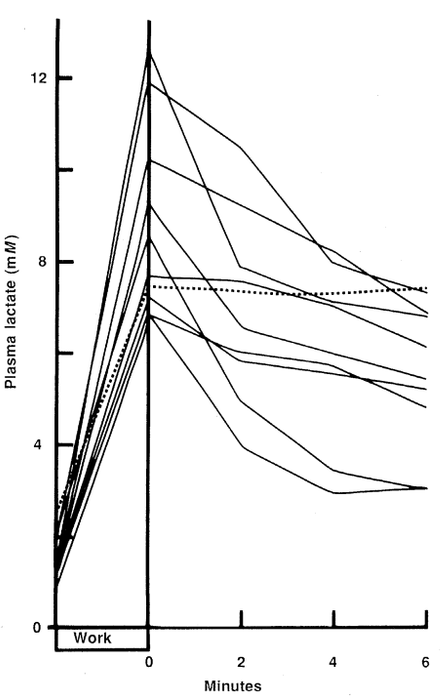

Fig. 1. Rise and decline of plasma lactate in nine control subjects and the affected patient (dotted line) after 2 minutes of ischemic forearm exercise. All cases show a peak lactate immediately after exercise, and all, except the patient, show a considerable decline during 6 minutes of reperfusion.

Often referred to as the organic anion or substituted monocarboxylate transporter because of its generic carrier properties (*12*), this transporter's main function is to export lactate, the major organic acid that accumulates intracellularly. Although, to my knowledge, there have been no definitive studies on its isolation and characterization, the transporter seems to be a membrane protein that cotransports a lactate anion and a proton externally (OH^- uptake has not been excluded), without a requirement for adenosine triphosphate (ATP), to reduce and prevent intracellular metabolic acidosis (*10–12*). The carrier must contain one or more essential sulfhydryl groups, since micromolar concentrations of organic mercurial compounds inhibit the transporter, and permit one to conclude that it is responsible for about 90% of lactate efflux; the remainder is carried by diffusion and by the chloride/carbonate exchanger (*13*). The transport rate is sensitive to the *p*H gradient across the cell membrane, and increases markedly as the intracellular *p*H falls with accumulation of lactic acid (*10–12*).

The human red blood cell, which has no aerobic energy-yielding pathway, is almost completely dependent upon glycolysis for its energy stores, and therefore upon rapid lactate export. Lactate efflux is even more critical for muscle, which produces more lactate than it uses under all conditions, even at rest. Although aerobic electron transport is a much more efficient energy producer, glycolysis is much faster, and muscle metabolism is designed to maintain an adequate supply of pyruvate (and hence lactate) for all oxidative capabilities. If the excess lactic acid is not exported rapidly, the developing acidosis will render inefficient both the contractile apparatus and the energy-yielding pathways.

Over the past year we have developed a clinical assay for the human erythrocyte lactate transporter and an ischemic exercise test suitable for evaluating muscle lactate transport. These have been applied to normal subjects and to patients with unexplained muscle dysfunction. I report here the first case of what may prove to be a common muscle disease.

The patient was a 26-year-old military drill instructor in superb physical condition. He had had three brief episodes of severe, diffuse anterior chest pain over the past 5

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