# Biochemistry of Information Storage in the Nervous System

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**The use of molecular biological approaches has defined new mechanisms that store information in the mammalian nervous system. Environmental stimuli alter steady-state levels of messenger RNA species encoding neurotransmitters, thereby altering synaptic, neuronal, and network function over time. External or internal stimuli alter impulse activity, which alters membrane depolarization and selectively changes the expression of specific transmitter genes. These processes occur in diverse peripheral and central neurons, suggesting that information storage is widespread in the neuraxis. The temporal profile of any particular molecular mnemonic process is determined by specific kinetics of turnover and by the geometry of the neuron resulting in axonal transport of molecules to different synaptic arrays at different times. Generally, transmitters, the agents of millisecond-to-millisecond communication, are subject to relatively long-lasting changes in expression, ensuring that ongoing physiological function is translated into information storage.**

THE RECENT APPLICATION OF MOLECULAR APPROACHES TO the study of neural function has yielded a number of notable advances. For example, contrary to traditional views, it is now apparent that single neurons use multiple transmitter signals, that different transmitters are independently expressed and regulated in the same neuron, and that experience alters gene expression in the nervous system. These and related insights are prompting alteration of long-held concepts of brain function. The new approaches are also encouraging a reformulation of some classic problems in the study of cognition. One particularly active area concerns the molecular basis of memory mechanisms.

The theoretical work of Hebb (*1*), nearly half a century ago, focused attention on the synapse, the communicative junction between neurons, as the physical locus of learning and memory. Subsequently, animal psychology has provided a behavioral framework, which has defined stimulus-response relationships and the nature and importance of timing in learning, as well as generating critical insights concerning the meaning of experimental paradigms themselves (*2*). Another school has provided seminal discoveries by using relatively simple model systems to analyze process and mechanism. Elegant work in the invertebrates, *Aplysia* and *Hermissenda*, has elucidated cellular mechanisms involved in habituation and memory (*3*).

Complementary studies in mammals that define the mechanisms associated with long-term potentiation in the hippocampus (*4*), a brain area long associated with mnemonic function, and examine the pathways underlying conditioning of simple reflexes (*5*) have broadened concepts of learning and memory. Simultaneously, the study of human memory in patients with different brain lesions has led to the formulation of new taxonomies of mnemonic function (*6*). Finally, the recent use of computer simulation to analyze the processes of learning and memory has refocused attention on "connectionist" issues, the centrality of functional connections among neuronal arrays in the genesis of information storage (*7*).

However, heretofore, the reigning model of memory and the nervous system has been based on a static conception of individual neurons, small neuronal populations, and molecules within neurons. In contrast, current work, focusing on molecular transformations within neurons, is providing a different, dynamic picture. Critical molecular processes appear to be in constant flux, influenced by environmental stimuli and conditions. Indeed, environmentally induced molecular change appears to constitute one mechanism by which external information is translated into neural language. This plasticity occurs at a fundamental level of neuronal function, alteration of gene expression.

Definition of information storage at the molecular level is yielding new insights about the nature of information processing itself; the new approaches are not simply sketching the characteristics of psychologic memory at another level of function in the nervous system. For example, functionally critical molecules in the peripheral and central nervous systems encode and store environmental information over time, indicating that the potential for mnemonic function is widespread. Consequently, the notion that memory is localized exclusively to restricted populations in the brain appears to be ill-framed. Rather, information storage occurs in diverse neural subsystems that subserve entirely different neurophysiologic roles. For example, storage over time occurs in the peripheral sympathetic system, which governs cardiovascular function, and in the brain locus coeruleus system, which appears to play a key role in arousal and attention.

In this article we examine a number of practical questions of critical importance. In fact, what relevant biochemical processes actually do transpire in the nervous system? Can molecular biochemistry contribute to the understanding of information storage in a dynamically organized nerve network? Specifically, can we detect biochemical processes that receive, transduce, encode, store, and transmit environmental information? In turn, how might such

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biochemical changes translate into long-lasting, altered function of the synapse?

Although a variety of modifications may potentially alter synaptic function, we focus on a single specific category, transmitter plasticity. Emerging evidence indicates that neurotransmitters, the prime agents of synaptic communication, also store information. Simply stated, the same molecules that govern millisecond-to-millisecond physiologic neural function also represent environmental information over time. The molecules that mediate physiologic actions such as motor control and coordination simultaneously encode and store environmental information. Normal physiology and "higher functions," such as memory, are inseparable. The mechanisms underlying this unity are the subject of this article.

Initially, we focus on studies performed in the relatively simple and well-defined peripheral nervous system of the rat in vivo and in vitro. We then extend our discussion to the central nervous system to determine whether similar mechanisms regulate brain function. Throughout our inquiry we focus on the mechanisms that translate brief stimuli into relatively long-lasting molecular changes of high specificity and precision.

## Transmitter Plasticity in the Sympathetic System

The peripheral sympathetic nervous system is a relatively well-defined, accessible model, long used for the study of neural function. In particular, the transmitter norepinephrine (NE), which mediates many of the physiologic consequences of sympathetic discharge and is involved in the "fight-or-flight" reaction, has been the focus of intense interest. Classical work characterized the actions of NE and fostered a rather static view of sympathetic function in which impulse activity simply led to well-delineated physiologic effects. Current studies indicate, however, that impulse activity has far-reaching effects of direct relevance to information storage.

Trans-synaptic impulse activity elicits long-term changes in the metabolism of NE in postsynaptic sympathetic neurons. Specifically, stressful stimuli that reflexively increase sympathetic discharge, a category that includes drugs such as reserpine and environmental stress, biochemically induce tyrosine hydroxylase (TH), the rate-limiting enzyme in NE biosynthesis (Fig. 1) (*8*). The number of TH molecules increases in response to these environmental stimuli (*9*). Moreover, the increase in TH protein is relatively long-lasting with respect to the inciting stimuli.

In fact, the kinetics of TH induction have been defined in detail and illustrate one molecular basis for information storage. Environmental stress and activation of the sympathoadrenal axis result in a two- to threefold increase in TH in sympathetic neurons within 2 days, and enzyme activity remains elevated for at least 3 days after increased impulse activity has ceased. In related experiments, direct

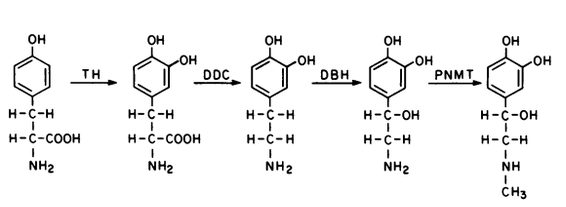
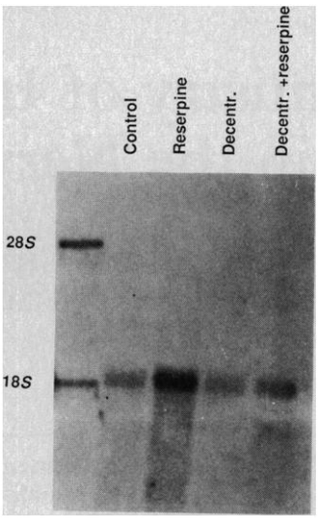



**Fig. 1.** Catecholamine biosynthetic pathway. The relevant biosynthetic enzymes are indicated for each reaction. Abbreviations: TH, tyrosine hydroxylase; DDC, aromatic amino acid decarboxylase (L-Dopa decarboxylase); DBH, dopamine-β-hydroxylase; PNMT, phenylethanolamine-*N*-methyltransferase.



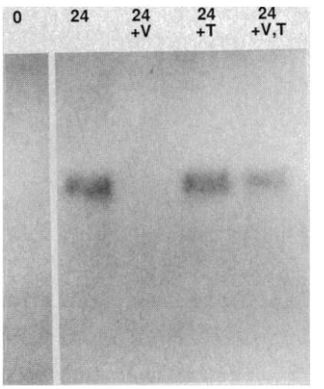




**Fig. 2 (left).** Northern blot analysis of TH mRNA in superior cervical ganglia. Five micrograms of total RNA was denatured and separated by electrophoresis in 1.1% agarose gels containing formaldehyde. RNA was transferred to nitrocellulose, probed with pTH4, and exposed to x-ray film for 2 days. Marker lane, 18*S* and 28*S* ribosomal RNA; control, vehicle treated and intact; reserpine, reserpine treatment and intact; decentr., denervated and vehicle treated; decentr. + reserpine, denervated and reserpine treated. [Data derived from (*12*)]. **Fig. 3 (right).** Northern blot analysis of PPT mRNA. RNA was extracted from uncultured ganglia immediately after removal from the animal (0) or from control ganglia cultured for 24 hours with no drug (24), $5 \times 10^{-5}M$ veratridine (24 + V), $10^{-7}M$ tetrodotoxin (24 + T) or with both veratridine and tetrodotoxin (24 + V,T). Five micrograms of total RNA was used in each sample.

electrical nerve stimulation for 30 to 90 minutes increases TH activity for at least 3 days (*10*). Thus, direct nerve electrical stimulation reproduced the effects of environmental stress and reserpine treatment, supporting the contention that these latter manipulations result in increased impulse flow. In summary, a brief stimulus evokes a long-term neural molecular change, constituting the temporal amplification that must transpire for memory to occur. Moreover, this biochemical alteration is physiologically significant, because the rise in TH catalytic activity results in an increase in NE synthesis. We now have the opportunity to relate environmental stress, altered TH activity, and the sympathetic fight-or-flight behavioral repertoire.

What molecular mechanisms are responsible for this information-storing process? The trans-synaptic induction of TH is inhibited by treatment with cycloheximide or actinomycin D, suggesting that ongoing protein and RNA synthesis are necessary (*9*). Indeed, immunotitration with specific antisera directed against TH indicates that an increase in enzyme molecule number accompanies the trans-synaptic increase in catalytic activity (*11*). Consequently, indirect evidence suggested that increased impulse activity elicited a rise in RNA synthesis and increased TH synthesis with a resultant elevation of NE biosynthesis that persists long after the exciting stimulus has been removed.

The availability of a complementary DNA (cDNA) probe complementary to TH messenger RNA (mRNA) allowed us to test these conjectures experimentally (*12*). Our goal was to determine whether trans-synaptic impulse activity increased the steady-state levels of mRNA encoding TH. Rats were subjected to unilateral denervation (decentralization) of the sympathetic superior cervical ganglion, and the contralateral ganglion served as a control. The rats were then divided into control and experimental groups. The former was treated with vehicle, and the latter received reserpine, an agent

that causes a reflex increase in sympathetic impulse activity (see above). We examined TH mRNA and enzyme activity in the four groups of ganglia: (i) control (intact, vehicle), (ii) intact, reserpine, (iii) denervated, vehicle, and (iv) denervated, reserpine. As expected, reserpine elicited a marked increase in TH enzyme activity and denervation blocked this rise (*12*). In addition, Northern blot analysis indicated that TH mRNA increased markedly in response to reserpine (Fig. 2). Moreover, decentralization prevented this increase, indicating that trans-synaptic impulse activity does elicit a specific rise in TH mRNA (Fig. 2). Finally, inspection of the gel indicated that the cDNA probe hybridized to a single band of approximately 1900 nt in all groups, in agreement with results obtained in adrenal medulla (*13*).

A number of conclusions are warranted. The long-term induction of TH by relatively brief stimuli appears to be mediated by a rise in TH mRNA and increased biosynthesis of TH molecules. In turn, the rise in TH mRNA does not appear to be attributable to altered mRNA processing or to readout of alternative genes because TH mRNA size is identical in all ganglia. However, because only steady-state levels of mRNA were examined, we cannot yet determine whether the increase was attributable to increased mRNA synthesis (increased gene readout) or mRNA stabilization. (The issue of stabilization versus synthesis is examined below.) Nevertheless, it is apparent that one form of information storage in sympathetic neurons is dependent on altered expression of specific mRNAs coding for transmitter-regulatory molecules. It is now important to determine whether environmental stress and direct nerve stimulation reproduce the effects of pharmacologically induced increases in impulse activity.

## Multiple Transmitters and Information Storage

The foregoing illustration indicates that transmitter mutability confers one form of information storing capacity on the individual neuron. Recent studies, however, indicate that individual neurons are capable of expressing and releasing multiple transmitters simultaneously [for review see (*14*)], vastly augmenting the potential for information storage in the single neuron. Do other transmitter species vary with alteration in impulse activity? If so, do different colocalized transmitters vary independently with environmental stimuli, leading to a variety of potential states representing external reality? These rather complex questions may be approached in the sympathetic system as well.

In addition to the classical transmitters, NE and acetylcholine (Ach), sympathetic neurons also contain neuropeptide transmitters such as substance P (SP) and somatostatin (*15*). Moreover, the peptides also respond to trans-synaptic activity. Denervation of sympathetic ganglia in vivo or treatment of rats with pharmacologic agents that block ganglionic transmission results in a marked increase in SP (*16*). Conversely, activation of sympathetic impulse flow depresses SP (*16*). In this instance, then, it appears that trans-synaptic stimulation decreases the putative postsynaptic transmitter, SP.

To analyze underlying molecular mechanisms, ganglia were explanted to the well-controlled culture environment. Explanation and consequent denervation resulted in a 50-fold rise in SP within 4 days, and the peptide was localized exclusively to ganglionic neurons (*15*). SP remained elevated for at least 3 weeks, the longest time examined. Further, somatostatin, an entirely different peptide, derived from a different precursor molecule, also increased in the denervated, explanted ganglia (*15*). Finally, veratridine, which elicits depolarization by inducing sodium influx, completely prevented the rise in SP; tetrodotoxin, which prevents transmembrane sodium influx, blocked the veratridine effect (*15*). Thus, depolarizing stimuli suppress SP in culture as in the live animal.

In sum, denervated, cultured ganglia exhibit an increase in SP neuropeptide, mimicking the effects of surgical or pharmacological denervation in vivo. Moreover, depolarizing influences prevent the rise in SP. Notably, then, trans-synaptic impulse activity apparently affects sympathetic catecholamines and peptides differently. As discussed above, we have already begun to analyze mechanisms governing the regulation of catecholaminergic traits. What molecular mechanisms translate environmental events and membrane depolarization into altered neuropeptide metabolism?

To begin approaching this issue, we used a cDNA probe complementary to mRNA coding for the SP precursor protein, preprotachykinin (PPT). Initial experiments revealed that PPT mRNA was detectable in the ganglion in vivo, indicating that the low basal levels of SP peptide present in normal ganglia are synthesized endogenously and not transported from an external source (*17*). We turned our attention to the mechanisms mediating the rise of SP in culture. In fact, PPT mRNA increased before the rise in SP peptide. The amount of mRNA increased by 6 hours in culture, reached a plateau by 24 hours, and remained high for at least 1 week, the longest time examined (*17, 18*). Moreover, an identical single band on Northern blot analysis was observed in all groups in which the PPT mRNA was detectable, suggesting that the same species of mRNA was synthesized in basal and explanted, denervated ganglia. Consequently, the increase in SP peptide after denervation and explanation may well have been mediated by a rise in specific precursor mRNA.

Does membrane depolarization regulate the steady-state levels of PPT mRNA? In fact, veratridine depolarization prevented the rise in PPT mRNA in a tetrodotoxin-sensitive fashion (Fig. 3). Viewed in conjunction with the in vivo studies, these results suggest that impulse activity with attendant membrane depolarization depresses PPT mRNA, leading to a decrease in PPT precursor peptide synthesis, and a decrease in SP peptide itself. Conversely, decreased impulse activity results in an increase in specific mRNA, precursor peptide, and SP. Note, however, that these sequelae are opposite to effects on TH mRNA, TH enzyme, and NE synthesis. The individual sympathetic neuron encodes and stores the same, or similar, sequences of environmental events differently in different colocalized molecular species.

Consequently, multiple molecular species are available in the neuron to code for environmental events over time. Further, at least some of these different transmitter molecules are differentially regulated by external stimuli. In brief, the neuron may employ a combinatorial strategy, in which a limited set of elements is used in multiple combinations, to generate specificity and diversity in the representation of external influences.

Moreover, these molecules are not simply indifferent repositories of environmental information. Rather, the molecules are central to neural function, regulating communication among neurons in synaptic systems. Impulse activity and neuronal depolarization, the very processes that mediate millisecond-to-millisecond function in the nervous system, apparently alter gene expression and the storage of neural information. Although the foregoing experiments suggest that impulse activity and depolarization may directly alter gene expression, indirect mechanisms remain to be examined. For example, even in the relatively simple sympathetic ganglion, impulse activity may affect multiple populations leading to indirect effects on specific neurons.

Although it appears that impulse activity alters the expression of transmitter-specific genes, the precise intracellular mechanisms that mediate this effect are unknown. It is not yet clear, for example, if impulse activity primarily affects gene readout itself or regulates

message stability in sympathetic neurons. We have been able to approach this issue in the closely related adrenomedullary cell.

## Gene Transcription in the Adrenal Medulla

Parallel studies were undertaken in the adrenal medulla, because this tissue is available in quantities adequate to examine gene transcription in response to depolarizing influences. Previous experiments had indicated that the opiate peptide enkephalins and catecholamines were colocalized to adrenomedullary cells in a number of species (*19–21*). A considerable body of evidence already indicated that impulse activity decreased enkephalin synthesis in the rat medulla in vivo (*19, 22*). We found that surgical denervation of the rat medulla or pharmacologic blockade of impulse activity, that is, depriving medullary cells of depolarizing influences in vivo, increased the amount of leucine-enkephalin (leu-enk) (*23*). Following protocols outlined above, we explanted medulla (now denervated) to culture, and observed a 50-fold rise in leu-enk with a concomitant increase in the mRNA encoding the enkephalin precursor [preproenkephalin mRNA (PPE mRNA)] (*23, 24*). As expected, depolarizing influences blocked the increases in leu-enk peptide and in PPE mRNA (*23, 24*). None of these regimens altered the activity of colocalized TH.

Thus, depolarizing influences prevented the rises in mRNA coding for precursor and in the enkephalin peptide, mimicking mechanisms regulating sympathetic neuropeptides. In both instances steady-state levels of transmitter mRNA were decreased by depolarizing influences. However, in the case of the medulla, we now had enough tissue to examine gene transcription itself.

To determine whether depolarizing influences altered transcription of the PPE gene, nuclear run-off assays were performed (*25*) and nucleotides incorporated into PPE mRNA were measured (Fig. 4). In fact, depolarization profoundly inhibited transcription of the PPE gene: incorporation was virtually undetectable, compared to that in baseline, denervated controls (Fig. 4). This effect was highly specific, since transcription of the actin gene was unchanged by exposure to depolarizing stimuli. Of even greater interest, transcription of the TH gene was also unaffected, indicating that expression of different neurohumoral genes is differentially regulated by depolarization at the level of transcription.

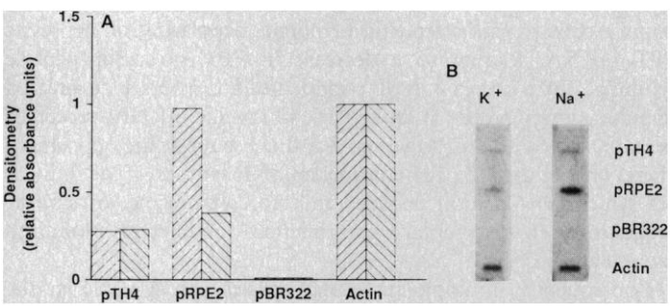



**Fig. 4.** Transcription "run-off" assay in adrenal medullae. The rate of transmitter gene transcription was compared in medullae grown for 48 hours in standard culture medium (RPMI 1640 supplemented with fetal horse serum and calf serum) plus 50 m*M* NaCl ($Na^+$) or 50 m*M* KCl ($K^+$), with minor modifications of published run-off protocols. Briefly, nuclei from ten explanted rat adrenal medullae dissected from the adrenal cortex were obtained by Dounce homogenization (pestle A) in 0.3*M* sucrose plus 0.1% Triton X-100 in 5 m*M* Hepes (*p*H 7.4, 1 m*M* $MgCl_2$), and 1 m*M* dithiothreitol. Nuclei were pelleted by gentle centrifugation (2500*g*) and washed twice with reaction buffer: 20 m*M* tris (*p*H 7.9), 20% glycerol, 140 m*M* KCl, 10 m*M* $MgCl_2$, 1 m*M* $MnCl_2$, and 14.3 m*M* 2-mercaptoethanol. Nascent RNA chains were labeled in situ with [$^{32}$P]uridine triphosphate (3000 mCi/mmol) in reaction buffer plus creatine phosphokinase (20 μg/ml) and 10 m*M* phosphocreatine, 0.5 m*M* each of adenosine triphosphate, cytidine triphosphate, and guanosine triphosphate for 15 minutes at 30°C. The reaction was terminated by lysis of nuclei, treatment with ribonuclease-free deoxyribonuclease, proteinase K, and separation from unincorporated label with a Sephadex G50 column. Labeled RNA chains were randomly cleaved with alkali (1*M* NaOH) before hybridization to cloned DNA (pTH4, tyrosine hydroxylase; pRPE2, preproenkephalin; pBR322, vector; or actin) immobilized on a nitrocellulose filter. Autoradiographs were analyzed by densitometry and recorded in relative absorbance units normalized to actin. (**A**) Left hatched bars represent results from the sodium-treated explants and right hatched bars from potassium-treated explants. (**B**) Autoradiograph of the mRNA from sodium- ($Na^+$) and potassium- ($K^+$) treated explants.

Clearly, we are just beginning to understand the neural genomic mechanisms that participate in the storage of information in the nervous system, and a multitude of questions remain. It is not even known, for example, whether the phenomenon of depolarization-regulated gene transcription occurs throughout the nervous system. Emerging data, however, suggest that this process is generalized. For example, recent studies indicate that cholinergic stimulation of neuronally differentiated pheochromocytoma PC12 cells rapidly induces transcription of the c-*fos* proto-oncogene (*26*). This observation is of particular interest, because c-*fos* protein appears to regulate nuclear events, occurring in response to environmental stimuli (*27*) that could result in long-term neuronal change.

To summarize, study of the peripheral neurohumoral system is beginning to define molecular mechanisms that mediate the long-term storage of functionally important neural information in response to external stimuli. Depolarization induced by presynaptic signals regulates the steady-state levels of postsynaptic transmitter mRNA, probably by altering gene transcription. Brief external events elicit long-term changes in neuronal function. Information about the external world is thereby stored through the very mechanism that serves neuronal intercommunication. Do related processes occur in brain neurons?

## Molecular Plasticity and Information Storage in the Brain

In fact, extensive emerging evidence indicates that brain neurons transduce environmental stimuli into relatively long-lasting transmitter changes. It may be helpful to focus on central catecholaminergic systems, thereby building on our knowledge of peripheral neurons. The locus coeruleus (LC) has been a particularly intensively studied system. These remarkable, bilateral brainstem nuclei innervate structures throughout the neuraxis, projecting rostrally to the cerebral cortex, superiorly to the cerebellar cortex, and inferiorly to multiple segments of the spinal cord [for review see (*28*)]. These neuroanatomic relations allow LC neurons to influence widespread centers in the central nervous system and also are integral to information storage, as described below.

The LC has been implicated in setting levels of arousal, attention, and vigilance throughout the nervous system (*29*). According to this view, the tonic release of NE, which occurs during such activities as grooming, sleeping, and eating, conditions the animal to internal vegetative demands. In contrast, phasic release of NE evoked by environmental stimulation prepares the nervous system for attention to external demands. The LC thus globally biases the nervous system toward internal or environmental demands and activities, and thereby participates in transitions among behavioral states.

TH occupies a position in LC function that is entirely analogous to that already described in the sympathetic system. As the rate-limiting enzyme, TH determines flux through the NE biosynthetic pathway and, consequently, the amount of transmitter available for release.

Treatment of rats with reserpine, which induces peripheral sym-

pathetic TH (see above), also induces the enzyme in the LC (*30*) (Fig. 5). After a single dose of reserpine, which acts immediately, TH increases 3.5-fold, reaching a peak in the nucleus at approximately 4 days, but remaining significantly elevated for at least 2 weeks (*31*). Although these results were provocative, interpretation of the in vivo experiments was difficult; for example, there is no simple, direct way to selectively denervate the LC in the brainstem to determine whether the effect is trans-synaptic. In fact, any approach to the brainstem is hazardous because this area is the crossroads of multiple life-support faculties.

We decided to approach mechanisms in culture of the LC. The LC nucleus was grown in explant culture and the effects of depolarizing agents were examined. Exposure of the explant to veratridine resulted in a significant increase in TH activity, and this rise was blocked by tetrodotoxin (*32*), suggesting that depolarization with attendant sodium influx mediated the enzyme increase. Moreover, exposure to elevated potassium also increased TH activity, suggesting that the effect was attributable to depolarization per se and not to nonspecific effects of a single pharmacologic agent (*32*). Was the increase in catalytic activity associated with true enzyme induction, an increase in molecule number, or with activation of pre-existing enzyme? To approach this question, immunoblot analysis was performed with a specific antiserum against TH to quantitate enzyme protein after depolarization. TH molecule number was increased by depolarization (Fig. 6), suggesting that impulse activity may increase enzyme molecules in brain neurons, as in the periphery (*32*).

Is the induction of brain TH by depolarizing stimuli dependent on altered gene expression as in the periphery? Indirect evidence suggests that this is the case. Treatment of rats with reserpine elicited over a fourfold increase in the steady-state levels of TH mRNA in the brain nucleus (*33*). Viewed in conjunction with work discussed above, these observations suggest that depolarization of LC neurons increases TH mRNA with a resultant rise in TH protein synthesis, leading to elevated NE synthesis. Similar, or even common, molecular events in brain and periphery may transduce environmental stimuli into neural information. The neural molecular information persists over time, forming the rudiments of a storage system.

## Molecular Information in Other Brain Systems

Are the neurons of the LC peculiar, or do other functionally dissimilar neuronal populations alter transmitter metabolism or expression in response to external stimuli? One additional system that we have been studying is the dopaminergic substantia nigra (SN). The SN, which is located in the midbrain, plays a critical role in coordinated movement, and its degeneration leads to Parkinson's disease. Consequently, although catecholaminergic (dopaminergic), the SN serves a neurophysiologic role that is entirely different from that of the LC. Yet the SN too, exhibits transmitter plasticity in response to external events.

Increased impulse activity increases TH catalytic activity in the SN in vivo. We examined the SN in culture and found that depolarizing stimuli increased TH activity, and that this rise was attributable to an increase in TH enzyme protein, that is, was true enzyme induction (*34*). Moreover, in the case of the SN we were able to examine the effects of the physiologic presynaptic transmitters, because these had been identified; the SN appears to receive an excitatory SP-containing pathway from the striatum (*35*). In fact, exposure to a stable analogue of SP, or to substance K (NKA), another active peptide derived from the SP precursor, elevated TH activity in TH molecule number (*34*).

We are presently attempting to determine whether the induction of TH in the SN is also dependent on increased TH mRNA, and whether increased gene transcription is involved. Regardless of underlying mechanisms, however, it is apparent that diverse central systems exhibit plastic responses to external stimuli, mimicking responses in the periphery. Both central and peripheral systems are equipped with the molecular machinery to receive, transduce, encode, store, and transmit environmental information.

## Spatial Organization and Information Processing

Thus far, we have considered molecular mechanisms while largely ignoring geometry of neurons and their connections. Yet the spatial, neuroanatomic dimension confers special characteristics on the processes under discussion. The peptide transmitters and transmitter enzymes are synthesized within the perikaryon (cell body) and transported through the axon to terminals in different regions. Because axonal transport requires a finite period of time, and because different terminals are located at varying distances from the cell body, the temporal profile of transmitter change differs at different synapses even in the same neuron. Synaptic specificity of altered metabolism derives in part, from unique neuronal geometry.

The LC serves as a good example because the nucleus innervates widespread populations of cells (see above). After exposure to an external stimulus, TH increases in different LC terminals with very different temporal profiles (Fig. 5). In the brainstem perikarya, TH peaks at 4 days, while peaking at 8 days in the proximate cerebellum and at 12 days in the distant frontal cortex. Consequently, from a single stimulus leading to a common series of molecular, and presumably genomic, events in the nucleus, information is related to different terminal fields in distinct fashions. In the present example, the cerebellum experiences peak elevation of TH 4 days before frontal cortex, potentially leading to entirely different behavioral consequences and profiles in these functionally diverse regions.

It follows that the decay of information will vary with distance from the cell body as well. This contention is supported by comparing the decay of TH in cerebellum and frontal cortex (Fig.

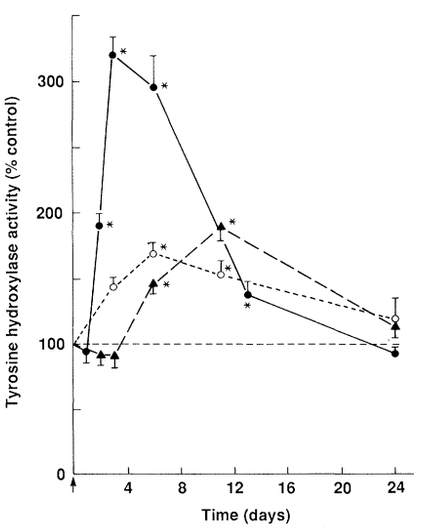



**Fig. 5.** Time course of the increases in TH activity in locus coeruleus (closed circles), cerebellum (open circles), and frontal cortex (closed triangles) after reserpine treatment. Rats were treated with reserpine (10 mg per kilogram of body weight, subcutaneously) at time zero (arrow) or with saline. At varying times after injection groups of six control and six reserpine-treated animals were killed, and enzyme activity was assayed in the indicated areas. Results are expressed as percentage of respective controls ± SEM (vertical bars). Thus 100% represents enzyme activity in controls, which was 692.5 ± 52.56 pmol of L-Dopa per pair per hour for locus coeruleus, 80 ± 6.7 fmol per microgram of protein per hour for frontal cortex, and 25 ± 1.2 fmol per microgram of protein per hour for cerebellum. *Differs from respective control at $P < 0.001$. [Reprinted with permission from (*31*)]

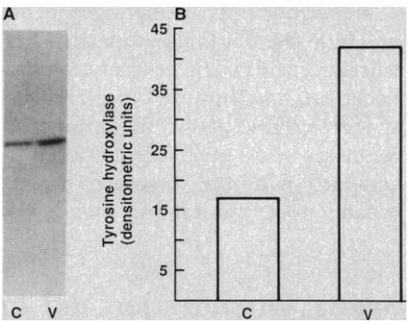



**Fig. 6.** (**A**) Visualization of immunoreactive TH protein extracted from locus coeruleus cultures grown for 1 week and then exposed to control nutrient medium (C) or veratridine-supplemented nutrient medium (V) for an additional week. The TH was visualized with specific antisera and $^{125}$I-labeled protein A. (**B**) Densitometric analysis. The density of the bands is expressed in arbitrary densitometric units. [Reprinted with permission from (*32*)]

5), although a finer-grained analysis will be necessary to elucidate this point. Although sequelae must be documented in detail, it is clear that the temporal characteristics of functional neuronal change elicited by the environment are determined by geometry of the nervous system as well as the rules governing molecular interactions.

## Some Implications for Memory Mechanisms

The single neuron is apparently endowed with enough complexity to exhibit the rudiments of mnemonic mechanisms. The very cellular processes that sense environmental change, trans-synaptic communication, and membrane depolarization set in motion a series of cellular and nuclear mechanisms that constitute information storage. Different patterns of impulse activity, presumably, will be found to activate and repress different families of genes, leading to different patterns of long-term information storage. Different patterns of activity have already been shown to alter the ratio of colocalized transmitters that are released by neurons (*36*).

Although differential kinetics of molecular turnover may lead to differences in information storage by molecules in the same or different neurons, an entirely novel specificity is conferred by neuronal geometry. The topological characteristics of neurons as highly branched structures projecting for vast distances in three-dimensional space transform a neuron-wide event, such as altered gene expression, into multiple events with differing synaptic specificities. Because axonal transport of any molecular species is constant, while terminal fields lie at varying distances from the perikaryon, the temporal pattern of molecular changes will differ in different synapses of the same neuron. That is, synaptically selective alteration of function is generated both by the kinetics of molecular processing and by the structure of the neuron and nervous system itself. Thus, a neuronal change may be translated into changes that are specific for individual synapses and reverberating synaptic arrays, as suggested by Hebb [see above and (*1*)].

Our observations indicate that diverse peripheral and central neuronal populations exhibit a biochemistry of information storage. Indeed, persistence of biochemical change may be a general property of neurons and their aggregates. Consequently, the potential for mnemonic function may be widely distributed in the nervous system and not restricted to a few populations of neurons. The particular physiologic, behavioral, or cognitive manifestations of information storage may simply reflect the specific neural populations involved.

It should be stressed that the narrow focus on genome, mRNA, and transmitter does not necessarily explain how multimodal memories become associated or how some memories persist for decades. However, molecular approaches in conjunction with the elegant molar approaches that are defining information processing at the neural systems level (*37*) may begin defining higher order problems in molecular terms. A clearer understanding of the molecular plasticity of synapses, which connect neurons and their arrays, may help integrate mnemonic function in the molecular, cellular, and systems domains.

### REFERENCES AND NOTES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