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Time to Remember

Minireview

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A sigh for every so many breath / And for so many sigh a death. / That's what I always tell my wife / Is the multiplication table of life. Neuroscientists searching for the multiplication table of the brain cannot, unfortunately, use the poetic calculus of Robert Frost (1927); as a matter of fact, to date, they are not even sure what the computations are and over which terms they are performed. Recent advances in neurophysiology only fueled the debate over the syntax and semantics of neural codes (Softky, 1995; Konig et al., 1996). It is therefore of interest to note that molecular and cellular neurobiologists attempt to contribute their own novel insight into mechanisms that embody, retain, and modify information in neural circuits. And even though, as noted below, molecular neurobiology illuminates only certain facets of brain function but not others, the findings are intriguing indeed and specifically point to a crucial component of biological codes, recurrently unveiled on different scales: time.

Three new reports that demonstrate the importance of doing the right molecular thing at the right cellular time in the nervous system deal either directly or indirectly with the cAMP cascade. Since the early discoveries that adenylyl cyclase, cAMP, cAMP-dependent protein kinases, and their substrates play a role in neuronal plasticity (Kandel and Schwartz, 1983), several notions became evident: the cAMP cascade subserves plasticity in neuronal systems and functions far apart on the phylogenetic scale; triggering the cAMP cascade can culminate in short, intermediate, and long-term neuronal and behavioral change; and the cAMP cascade does not function in isolation but rather is involved in an extensive cross talk with other signal-transduction cascades, thus forming an intricate intracellular network whose spatiotemporal activity contributes to the determination of the structural and functional status of the cell at any given moment.

Cellular Integration

The activity of the cAMP cascade in cellular subcompartments is a function of the ambient level of cAMP. Hempel et al. (1996) have determined, for the first time, the spatiotemporal dynamics of cAMP in response to neuromodulation in an intact neural circuit. They used fluorescence ratio imaging of cAMP, a clever method in which the catalytic (C) and the regulatory (R) subunits of cAMP-dependent protein kinase are labeled with fluorescein and rhodamine, respectively, and injected into the cell. In the holoenzyme, the dyes are close enough to permit resonance energy transfer; this does not happen when cAMP binds to R and dissociates it from C. The change in the profile of the fluorescence emission spectrum thus allows cAMP concentration to be determined. Combined with confocal microscopy, the method allows relatively high resolution imaging of cAMP in individual cells and in cell systems. It was previously used to image cAMP in single neurons (Bacskai et al., 1993) and now recruited to analyze the spatiotemporal dynamics of cAMP in an intact circuit.

The circuit chosen was the well-studied stomatogastric ganglion of the spiny lobster Panulirus interruptus, which comprises about 24 interconnected motoneurons and 6 interneurons. Two modes of stimulation of the cAMP cascade were employed. In the first, neuromodulators such as octopamine, dopamine, and serotonin, known to be present in the ganglion, or drugs affecting neuromodulation, were added to the bath solution. In the second, afferent fibers in the somatogastric nerve were stimulated electrically, resulting in synaptic release of endogenous modulators. Bath application produced transient increases in cAMP ($\tau_{1/2} \sim$ minutes) in the soma of specific sets of neurons. Electrical stimulation of neuromodulatory afferent fibers unveiled in addition to the spatial resolution a temporal one, dependent on the duration of the stimulus. As little as 1 s of stimulation was sufficient to produce cAMP increases in fine neurites of identified neurons within a few seconds. Longer stimulation periods revealed a centripetal pattern of cAMP increases, with the finer neurites responding most rapidly, then primary neurites, and finally somata. Hempel et al. (1996) concluded that cAMP is produced primarily in fine neurites and diffuses retrogradely through the extensive neuritic arbor back to the soma. From this study and previous studies (Bacskai et al., 1993) and taking into account the kinetics of decay of the cAMP signal, one can infer that cAMP is a temporal and spatial cellular integrator: short duration or low frequency modulatory input is likely to produce only local changes in cAMP levels, whereas prolonged or repeated synaptic activation is likely to culminate in elevation of cAMP throughout the entire neuron.

In their analysis of the effect of neuromodulators on the activity of identified somatogastric neurons, Hempel et al. (1996) also noted that the pattern of cAMP gradients was accompanied, as expected, by characteristic changes in the electrophysiological output of the circuit. However, comparison of the patterns of cAMP changes with the cell-specific electrophysiological effect of each modulator did not show any simple correspondence between the two. They concluded that the electrophysiological outcome is complex and that other second messenger pathways are probably involved.

The role of cAMP as a cellular spatiotemporal integrator and its interaction with other second messenger cascades further emerge from additional recent investigations performed in the mammalian brain in lieu of an invertebrate ganglion.

From Alliance to Disengagement

The current prevailing hypothesis of long-term cellular alterations that subserve neuronal circuit remodeling in development and memory is that extracellular signals trigger specific patterns of gene expression and hence endow the neurons with immunity to molecular turnover that otherwise would erase experience-dependent post-translational modifications (reviewed by DeZazzo and

Tully, 1995). Studies in both invertebrates and vertebrates have identified some of the players in this external world-to-gene game, among them members of the transcription factor family CREB (cAMP-response element binding protein; reviewed by Sassone-Corsi, 1995). CREB phosphorylation promotes the activation of genes with an upstream CRE (cAMP response element). The state of phosphorylation of CREB is regulated by protein kinases and phosphatases under the control of cAMP, Ca^{2+}, or both. Liu and Graybiel (1996) have set out to investigate the convergence of the cAMP and Ca^{2+} signals and its effect on CREB phosphorylation in the developing rat striatum. They used a slice preparation in which the cAMP cascade can be activated via the dopamine D1/D5 receptors and the Ca^{2+}/calmodulin cascade via L-type voltage-sensitive channels.

The mammalian striatum is characterized by distinct macroscopic compartments, striosomes, and matrix, which have different developmental histories, patterns of neurotransmitter systems, and input–output connections. Activation of the dopamine receptor by a specific agonist induced CREB phosphorylation. However, the distribution of phosphorylated CREB (pCREB) in the tissue was time dependent. At first (7 min), it was spread throughout the striatum, but later (30 min), it was confined to striosomes. Activation of the L-type Ca^{2+} channels by a channel agonist also induced at first widespread pCREB immunoreactivity throughout the striatum, but at 30 min, most pCREB-positive cells were in the matrix and at the edges of the striosomes, i.e., the pattern of pCREB in this case was essentially complementary to that observed after dopaminergic stimulation. Thus, the cAMP and the Ca^{2+} signals first converged and then diverged.

When Liu and Graybiel (1996) proceeded to analyze the expression of a CRE-regulated gene, *c-fos*, they found that this expression matched the divergent (sustained) rather than the convergent (transient) pattern of CREB phosphorylation. In other words, pCREB needed time (and probably the presence of other molecular factors generated with time; see Impey et al., 1996) to activate CRE-regulated genes. Inhibition of protein phosphatases in the presence of either the dopaminergic or the Ca^{2+} channel agonists switched the spatial pattern of pCREB into the convergent, diffused mode. The conclusions of Liu and Graybiel (1996) were: a. Phosphorylation of CREB and subsequent Fos expression are subject to temporal control (the duration of CREB phosphorylation) and spatial control (where sustained phosphorylation takes place); b. The developing striatum processes the cAMP and Ca^{2+} signals either conjunctively (for relatively fast signaling and short-term modifications) or disjunctively (for long-term modifications); c. The switch from conjunction to dysjunction is under protein phosphatase control; and d. The striatum contains compartment-specific dephosphorylation systems that account for the differential spatiotemporal expression of the cAMP and Ca^{2+} signals.

Phosphatases as Neuronal Erasers

CREB reversible phosphorylation was found to act as a stimulus duration-dependent switch in the hippocampus as well. However, in this case, Ca^{2+} rather than cAMP signaling was implicated. Bito et al. (1996) focused on the cellular processes that regulate the phosphorylation of CREB and the subsequent induction of the CRE-regulated genes *c-fos* and somatostatin in cultured hippocampal neurons. They found that relatively brief electrical stimulation (18 s, 5 Hz) was as effective as a longer stimulus (3 min) in triggering CREB phosphorylation, and that this was probably due to fast activation of Ca^{2+}/calmodulin kinases including CaMKIV. However, longer stimulation produced greater stability in the peak level of pCREB and its cellular distribution and was paralleled by increased expression of Fos and somatostatin. The greater stability of pCREB over time was explained by experience-dependent net inhibition of a CREB-protein phosphatase cascade; i.e., once CREB is phosphorylated by a transient stimulus, the maintenance of the active state is regulated by Ca^{2+} dependent protein phosphatase rather than kinase, and the activity of the phosphatase is an inverse function of stimulus duration. Whereas the function of persistent phosphorylation of CREB may be understandable (sustained presence of pCREB in the nucleus could foster efficient CRE-regulated gene expression), the role of the transient rise in the level of pCREB following a brief stimulus is not evident. Bito et al. (1996) suggest several possibilities, among them that the brief increase acts as a priming signal that affects nuclear activity only in conjunction with (an)other signal(s) (i.e., an AND gate; see Impey et al., 1996).

It thus appears that in the lobster somatogastric ganglion, the developing rat striatum, and the rat hippocampus, signal transduction mechanisms function as nets of spatiotemporal integrators, expected to switch on gene expression and hence induce long-lasting circuit modifications only above a certain threshold level of activated transcription factor(s). The principle that cellular decisions on patterns of gene expression are based on sustained activation of signal transduction cascades may be a general one and was also adduced in the context of differentiation and proliferation pathways activated by receptor tyrosine kinases (Marshall, 1995). This principle is in consonance with the current heuristic consensus on the type of molecular switches that transform short- into long-term modifications in neuronal circuits, memories, and behaviors (DeZazzo and Tully, 1995). But the congruity should not blur some open issues and especially the need to clearly recapitulate which functions of the brain are addressed in studies of the kinetics of signal transduction cascades and gene expression and which functions are not.

The Inescapable Uniqueness of Brains

The timescale of the processes described by Hempel et al. (1996), Liu and Graybiel (1996), and Bito et al. (1996) is characteristic of metabolic reactions involved in cellular housekeeping, initiation of differentiation and growth in many tissues, and long-term memory consolidation in nervous systems. It is orders of magnitude above that in which neuronal circuits and assemblies perform computations over internal representations (Table 1). Alas, the most critical and salient distinction between neural and nonneural tissue is the ability of the former to perform typical complex computations in biophysical and electrical dialects within 10^0–10^2 ms, not

Table 1. Time Domains in the Nervous System

Event/Process	Time	Method	Reference
Single Na^+ channel rise time	10^{-1} ms	Electrophysiology	Hille (1992)
Na^+ spike	10^0 ms	Electrophysiology	Johnston and Wu (1995)
Fast synaptic transmission	10^0–10^1 ms	Electrophysiology	Johnston and Wu (1995)
Coincidence detection in a single neuron	10^0–10^1 ms	Electrophysiology	Abeles (1991); Markram et al. (1997)
Electrical integration of input in a single neuron	10^1–10^2 ms	Electrophysiology	Shadlen and Newsome (1994)
Dendritic Ca^{2+} transients	10^1–10^2 ms	Electrophysiology	Markram et al. (1995)
Binding of neuronal assemblies	10^1–10^2 ms	Neuroimaging	Arieli et al. (1995)
Focal visual attention	10^2 ms	Psychophysics	Treisman (1977)
Processing a visual scene	10^2 ms	Psychophysics, ERP	Thorpe et al. (1996)
Recognition of a visual scene followed by a selected motor response	$<10^3$ ms	Psychophysics	Thorpe et al. (1996)
Integrated neuromodulator-induced local rise of cAMP in neurites	10^3 ms	Mol. cell biology	Hempel et al. (1996)
Integrated rise of cAMP over entire neuron	10^5 ms	Mol. cell biology	Hempel et al. (1996)
Time to peak transient CREB phosphorylation by Ca^{2+}	10^4 ms	Mol. cell biology	Bito et al. (1996)
Integration time for sustained CREB phosphorylation by Ca^{2+}	10^5 ms	Mol. cell biology	Bito et al. (1996)
Integration time of cAMP or Ca^{2+} signals for activating CRE-regulated genes	10^6 ms	Mol. cell biology	Liu and Graybiel (1996)
Consolidation of long-term memory	10^6–10^7 ms	Behavior	DeZazzo and Tully (1995)
Long-term memory	10^{10}–10^{12} ms	Behavior	Dudai (1989)

Values are order of magnitude in milliseconds. Only selected references are given. In part of the studies of the time to peak and integration time of signal transduction cascades, the values may represent upper limits, due to insufficient resolution of the detection methods (see text). Consolidation of long-term memory refers to an interference-prone period during which long-lasting changes are initiated at local nodes of circuits subserving memory. Binding of neuronal assemblies is assumed to generate internal representations (for definitions and discussion, see Dudai, 1989). ERP, event-related potential measurement; Mol. cell biology, molecular and cellular biology methods.

the ability to respond by differentiation and growth to chemical signals within minutes. This fact must not become beclouded by the understandable enthusiasm evoked by the ability of sophisticated molecular methods to dissect the dynamics of signal transduction cascades and gene expression in neurons. What is computed by cAMP, integrated by CREB and its kinase–phosphatase systems, and switched on and off by CRE and other gene regulation elements are components of algorithms that construct, maintain, and accommodate the machinery that encodes internal representations and performs computations over them; these are not functions that embody and express those representations and computations in real time. The latter are encoded in a different biophysical language and occur in a different temporal domain; deciphering them requires powerful analytical tools in addition to those of molecular biology.

Two caveats are especially pertinent here. They relate to the notions of generalization and universality. First, the impressive success of state of the art developmental neurobiology carries the risk of superfluous generalization of findings and concepts from this field to models of ongoing brain function. For example, the relevance of physiologically meaningful cortical activity, capable of inducing enduring modifications in internal representations in the adult brain, to modulation of gene expression, must yet be scrutinized. Second, from time to time, expressions of elation find their way into the scientific literature because molecular mechanisms similar to those identified in neural tissues are identified in other tissues or vice versa. But it is exactly this cross-tissue universality that attests to the inability of these mechanisms per se to account for the unique properties of brain. All of the above, of course, does not belittle whatsoever the marvelous achievements of molecular analysis of brain development and function; it only points to the pitfalls inherent in extensive generalizations and search for universals.

Memory Filters

Some time constants reported by Hempel et al. (1996), Liu and Graybiel (1996), and Bito et al. (1996) (Table 1) may actually represent upper limits, due to insufficient resolution of the methodologies used. For example, a physiologically significant increase in the level of cAMP in local neurites of somatogastric neurons may well take place before the first rise in signal is detected, 3 s after a 1 s stimulation (Hempel et al., 1996). However, in other cases, the data impose a lower limit on the inferred duration of the process; e.g., 7 min of cAMP or Ca^{2+} activation are insufficient to induce divergent pattern of sustained CREB phosphorylation and CRE-regulated gene expression, whereas 30 min are sufficient (Liu and Graybiel, 1996). Whatever the exact time windows of transient activation of signal transduction cascades, clearly, even the shortest ones harbor processes that must yet be unraveled. In the example cited above, the convergent activity of the cAMP and Ca^{2+} cascades are expected to subserve short-term functions in striosomes and matrix, still unknown.

Of special interest in this context are the filtering mechanisms that prevent some stimuli from inducing novel patterns of gene expression. Three major principles determine the ability of stimuli to activate long-term changes: intensity, persistence, and convergence. Intensity and persistence determine the magnitude of the integrated signal. Their role is clearly evident in the aforementioned reports by Hempel et al. (1996), Liu and Graybiel (1996), and Bito et al. (1996). Convergence, i.e., activation of a cellular process by conjoint signals, determines the context (and hence significance) of

events. It may play a role in the early occurrences following coactivation of the cAMP and Ca^{2+} cascades in the striatum (Liu and Graybiel, 1996) or when the cAMP cascade interacts with other signal transduction cascades to yield characteristic cellular response in the somatogastric ganglion (Hempel et al., 1996).

Assuming that transient stimuli of the kind that reach our brain every fraction of a second can indeed ever and anon modulate gene expression in the adult brain, the aforementioned filtering mechanisms may have a particular role in a faculty used extensively in our daily lives, including while reading this article. This faculty is working memory. In working memory, the brain transiently combines on-line (sensory) and off-line (memory) information to guide behavior that is relevant for a short while only. Neuronal circuits in the prefrontal cortex have been implicated in this type of memory (Goldman-Rakic, 1995). The activated prefrontal circuits are not expected to convert the ad hoc representational combinations into long-term ones, because they are not usually relevant after a few seconds. If this is the case, then how do the appropriate brain circuits ensure that long-term storage does not happen? (Or does it?) Keeping in mind the above filtering mechanisms, one may suggest a number of possibilities. One is that the intensity of stimuli arriving at these circuits does not suffice to generate an appropriate integrated level of activated transcription factors, such as pCREB; another is that in these neurons, high activity of protein phosphatases or inhibitory forms of transcription factors rapidly suppress the stimulus-induced activation; and yet another possibility is that convergence of inhibitory input from other circuits quenches the short-term activation before it triggers gene expression. It would be of interest to perform in prefrontal cortex analysis similar to that performed in the lobster ganglion and mammalian hippocampus and striatum, in an attempt to identify unique characteristics of the spatiotemporal dynamics of signal transduction cascades.

Can Levels Meet?

The metabolic computations performed by the spatiotemporal waves of intracellular signal transduction cascades in neurons are just beginning to unravel. Given the rapid pace of molecular and cellular biology, there is little doubt that in the coming years, exciting facts will be learned about these intracellular networks. However, a quick glance at Table 1 pinpoints a most critical and difficult problem facing brain research: the gap between the current success of neuroscience in disclosing types and tokens of molecular devices and processes used for maintenance and long-term plasticity on the one hand, and the still enigmatic codes and mechanisms of ongoing representations and computations unique to the brain, on the other. Closing the gap requires not only novel concepts, methodologies, and technologies but also an intimate dialogue between molecular and system neuroscientists, which may eventually yield some interlevel bridge rules (Nagel, 1979). For example, dendritic Ca^{2+} transients may end up as terms in such rules (Table 1). How successful the bridging attempts will be, only time will tell.

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