

9 TO CONSOLIDATE OR NOT TO CONSOLIDATE: WHAT ARE THE QUESTIONS?

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INTRODUCTION

The concept of memory consolidation was introduced into the scientific discourse over a century ago (Muller and Pilzecker, 1900). However, its consolidation into the collective memory of the neuroscience community remains incomplete. For, although extensively discussed, the term consolidation means different things to different people. In its most general meaning, it refers to the idea that recently formed memories can sometimes be subject to stabilization over time, rendering them less susceptible to disruption by both new information and brain dysfunction. This process of stabilization manifests itself at different levels of brain organization and at multiple time windows. It is both conceptually and methodologically useful to distinguish between cellular consolidation and system consolidation (Dudai, 1996). The first refers to processes that take place locally, in individual nodes of neuronal circuits, i.e. synapses and neurons, during the first hours or days after learning. The second refers to processes that occur at a circuit level, may involve the progressive reorganization of memory traces throughout the brain and seem to last, in some cases, weeks or longer. In the simplest of circuits, cellular and system consolidation may be isomorphic. In more complex circuits, system consolidation probably involves additional mechanisms that reflect the selective activity of different circuits; a consequence of this selectivity is the potential, after consolidation, to retrieve memory using neural circuits different from those encoding memory traces at an earlier stage of their existence (Squire and Zola, 1996; Shadmehr and Holcomb, 1997).

In the present chapter, we restrict our discussion to cellular consolidation. It is this facet of consolidation that has, in recent years, benefited most from exciting developments at the cutting edge of cellular and

molecular biology. Hence, from now on, whenever we say 'consolidation', unless otherwise specified, we are referring to events that take place in synapses and individual neurons within the first hours after training. The field of system consolidation, which is being rapidly pushed into new vistas by sophisticated neuropsychology and neuroimaging, is not discussed.

In spite of the recent developments in understanding mechanisms of neuronal plasticity, which potentially relate to consolidation, many questions remain unsettled. These include basic issues such as *how*, *where* and *when* consolidation takes place in local nodes in the neuronal circuits that encode experience-dependent internal representations. (On learning and memory defined in terms of experience-dependent internal representations, and on the advantages of defining them in these terms, see Dudai, 1989, 1992.) One approach that we will discuss makes reference to the idea that local 'synaptic tags' set at the time of memory formation have the job of capturing diffusely targeted gene products to consolidate otherwise unstable synaptic connections (Frey and Morris, 1997). This concept raises the specter of the potential for consolidation without, necessarily, setting in train at the locus of consolidation all the neural events that would inexorably lead to it. In the present context, it is noteworthy that Gabriel Horn himself proposed a similar idea in the context of his work on filial imprinting (Horn, 1998).

COMMON THEMES

Experimental data from a variety of species, memory systems and preparations are often crystallized to yield a number of common themes concerning consolidation. It is useful to start by listing those that epitomize the *Zeitgeist* in this dynamic field:

- (i) Over time, new memories become resistant to certain kinds of interference. These include behavioral distractors, drugs, seizures and brain lesions. The time window of susceptibility depends on the task and type of interference. It may range from seconds to minutes (e.g. electroconvulsive shocks in associative conditioning, McGaugh, 1966), through hours (distractor tasks in motor skills, Shadmehr and Holcomb, 1997; macromolecular synthesis inhibition in a variety of tasks, Davis and Squire, 1984), up to weeks or more (hippocampal lesions in so-called declarative memory tasks, Squire and Zola-Morgan, 1991; Winocur, 1990). The latter, longer time windows of susceptibility are usually considered to reflect system consolidation, and will not be discussed further here.
- (ii) The application of drugs that are known to inhibit the synthesis of RNA or of proteins during a time window of up to a few hours after training blocks the formation of memories that last for longer than a day (Davis and Squire, 1984; Montarolo *et al.*, 1986; Rosenblum *et al.*, 1993; Freeman *et al.*, 1995). In the behaving animal, drug doses that result in massive reduction in protein synthesis (>90–95%) are required to achieve such an effect. Often, a similar transient reduction in macromolecular synthesis does not significantly affect perception, short-term memory or either the retention or the retrieval of long-term memory once relevant traces have been established. (The claim that retention is unaffected must be qualified since, in the limit, broad-spectrum and long-lasting protein synthesis inhibition is expected to result in neuronal malfunction and ultimately death.)
- (iii) The transformation of short- into long-term memory is not a step-like function, and various drugs or mutations can be used to dissect it further into what appear to be intermediate phases (e.g. Grecksch and Matthies, 1980; Rosenzweig *et al.*, 1993; DeZazzo and Tully, 1995; Ghirardi *et al.*, 1995; Winder *et al.*, 1998). However, it recently became evident that the time course of such phases is not a given, and might be altered (Frey and Morris, 1997). Moreover, it is not clear whether intermediate phases of memory consolidation must take place in a prescribed order, or even that the creation of long-term memory traces requires a short-term one; the processes may occur in parallel (e.g. Emptage and Carew, 1993).
- (iv) Signal transduction cascades, and specifically the cAMP-response element (CRE)-mediated modulation of gene expression by such cascades, are thought to be important players in the consolidation of short- into long-term memory. This has been established in behaving animals as well as in neuronal models of plasticity (e.g. Kaang *et al.*, 1993; Frank and Greenberg, 1994; Yin *et al.*, 1994; Deisseroth *et al.*, 1996; Lamprecht *et al.*, 1997). An example is the cAMP cascade (Kaang *et al.*, 1993; Frank and Greenberg, 1994; Deisseroth *et al.*, 1996). It involves activation of a cAMP-dependent kinase, which phosphorylates and activates isoforms of cAMP-response element-binding proteins (CREBs); the balance between activator and repressor forms of CREB may be critical in triggering long-term information storage (Bourtchuladze *et al.*, 1994; Yin *et al.*, 1994). CREB modulates the expression of CRE-regulated genes, including a number of immediate-early genes (IEGs such as transcription factors that, in turn, regulate the expression of late response genes). Other IEGs identified so far include ubiquitin C-terminal hydrolase (Hegde *et al.*, 1997), extracellular protease plasminogen activator (Frey *et al.*, 1996) and neural cell adhesion molecule (NCAM; Fields and Itoh, 1996). Gabriel Horn and his colleagues have also reported evidence implicating NCAMs in long-term memory (Solomon *et al.*, 1998). Compared with immediate and early response genes, much less currently is known about late response genes, though candidates have been suggested (Kennedy *et al.*, 1992; Kuhl *et al.*, 1992; Cavallaro *et al.*, 1997).
- (v) There is evidence suggesting that long-term synaptic plasticity and long-term memory are correlated with morphological changes in synapses (e.g. Horn *et al.*, 1985; Bailey and Kandel, 1993; Weiler *et al.*, 1995).

THE ANALOGY WITH DEVELOPMENT

The aforementioned themes are usually fitted into a conceptual paradigm that depicts the cellular manifestations of memory consolidation as growth or developmental processes. This paradigm exerts a marked influence on current research in molecular and cellular neurobiology, and is behind much of the striking merger of the concepts of developmental

neurobiology and the molecular biology of memory (e.g. Corfas and Dudai, 1991; Davis *et al.*, 1996; Martin and Kandel, 1996). As such, it is probably the most influential in current research on consolidation.

The notion that neural remodeling subserves behavioral plasticity (James, 1890) and that experience-dependent cellular growth processes take place in the mature brain (Cajal, 1911; Hebb, 1949) are tenets of neurobiology at large. The response of neural tissue to stimulation was shown long ago to be accompanied by alteration in proteins (Hamberger and Hyden, 1945). Soon after, the suggestive data and the aforesaid dogma combined to yield a proposal that new memory traces depend on proteins (Monne, 1949), and paved the way for the reports that synthesis of brain RNA (Dingman and Sporn, 1961) and protein (Flexner *et al.*, 1963; Agranoff and Klinger, 1964) are obligatory for the formation of long-term memory.

At its outset, the role of macromolecular synthesis in memory consolidation was embedded in two radically different conceptual frameworks. The first was the 'macromolecular code' hypothesis. It stated that neuronal memory, similarly to genetic memory, is encoded in the primary structure of nucleic acids and proteins, and hence that learning is an experience-dependent modification in the structure of these macromolecules (Dingman and Sporn, 1961; Hyden and Egyhazi, 1962). The macromolecular code hypothesis stimulated work which contributed valuable data on the metabolism of brain nucleic acids and proteins (Hyden and Egyhazi, 1962; Hyden and Lange, 1965), but it also resulted in some dead ends. These included attempts to transfer specific memories from one individual to another—attempts that followed directly from the supposition that memories were encoded in the primary structure of the molecules (e.g. Ungar, 1970). The appeal of the macromolecular code dwindled as these experiments failed or, in some cases, where apparently positive results were shown to be artifactual (e.g. Byrne *et al.*, 1966). Consequently, the second conceptual framework—the 'macromolecular synthesis' hypothesis—came to dominate the field. In brief, it states that newly synthesized gene products do not themselves encode memory, but rather promote and sustain modifications in neuronal circuits that encode internal representations (Dudai, 1989). Over the years, two types of methods have been used to reveal the possible roles that RNA and protein synthesis might play in memory. The first is interventional, based on inference of function from dysfunction. The second is cor-

relative, involving direct measurement of macromolecular synthesis in brain during or after learning and/or artificial models of neural plasticity.

Initially, antibiotics that specifically inhibit RNA or protein synthesis were used to determine the necessary role of macromolecular synthesis in behavior (reviewed in Davis and Squire, 1984), this approach being complemented by the gene targeting techniques of today (Winder and Kandel, Chapter 10). Despite occasional worries about the exact site of action of the drugs used (e.g. Kyriakis *et al.*, 1994), the overwhelming conclusion was that protein synthesis during or shortly after training is required for consolidation of long-term memory but not for acquisition, short-term memory or retrieval once long-term memory had been formed (Davis and Squire, 1984). However, injection of inhibitors into the brain could not provide definitive evidence that their critical effect was on neurons. This problem was resolved only in the mid-1980s, when preparations were developed which allowed both the isolation of individual memory-subserving neurons in relatively simple nervous systems and, separately, the analysis of cellular experience-dependent modifications in discrete pathways in the mammalian brain. Application of antibiotics to these preparations confirmed the general approach, showing macromolecular synthesis to be required for long-term heterosynaptic facilitation of the sensory-to-motor synapse in the *Aplysia* defensive reflexes circuits (Montarolo *et al.*, 1986), conditioning in *Hermisenda* photoreceptors (Crow and Forrester, 1990), long-term potentiation (LTP) in various fields of the hippocampal formation (dentate gyrus, Krug *et al.*, 1984; Otani *et al.*, 1989; hippocampal area CA1, Stanton and Sarvey, 1984; Nguyen *et al.*, 1994) and long-term depression (LTD) in the cerebellum (Linden, 1996). In parallel, local microinjection of antibiotics into circumscribed brain areas known to subservise specific experience-dependent behaviors also confirmed that protein synthesis is required for various kinds of memory in the brain of the behaving organism (Grecksch and Matthies, 1980; Horn *et al.*, 1985, Chapter 19; Mizumori *et al.*, 1985; Rosenblum *et al.*, 1993; Brennan and Keverne, Chapter 6). More recently, novel variants of perturbational methodology have been deployed to investigate the role of gene expression in memory consolidation; these include local microinjection of oligodeoxynucleotides antisense to transcription factors into the brain (Guzowski and McGaugh, 1997; Lamprecht *et al.*, 1997), local application of antibodies to transcription

factors (Bartsch *et al.*, 1995) and the use of transgenes and knockouts of genes that encode such factors (Bourtchuladze *et al.*, 1994; Yin *et al.*, 1994; Blendy *et al.*, 1996).

The second and complementary approach, based on looking for correlations between macromolecular synthesis in the brain and parameters of learning or memory, was also applied early but with only limited success (Hyden and Egyhazi, 1962; Zemp *et al.*, 1966; Shashua, 1979). The development of cellular preparations and the improved sophistication of cellular and molecular neurobiology [including, for example, the single-cell polymerase chain reaction (PCR) techniques] have opened new vistas for the correlative approach. This has led to the identification of experience-dependent modulation of protein synthesis in both invertebrate and vertebrate nervous systems (Mayford *et al.*, 1992; Kaang *et al.*, 1993; Meberg *et al.*, 1995; Impey *et al.*, 1996; Hegde *et al.*, 1997; Martin *et al.*, 1997a; Casadio *et al.*, 1999).

On the basis of the above and similar findings, a picture thus is commonly portrayed of cellular consolidation being analogous to a developmental process. In both cases, gene expression is regulated by extracellular signals via intracellular signal transduction cascades and, in both cases, similar ubiquitous signaling cascades, such as the cAMP (see above) and mitogen-activated protein kinase cascades (MAPKs; Hill and Treisman, 1995; Martin *et al.*, 1997b; Berman *et al.*, 1998; Crow *et al.*, 1998), are recruited, culminating in cellular remodeling and growth. However, at this stage, the scenes in this picture justify a closer look. We will consider the questions of *how*, *where* and *when* does cellular consolidation takes place in turn, turning toward the end of this chapter to the more speculative issue of *why* it takes place at all.

HOW?

A birds-eye view of the current data on cellular consolidation may lead one to conclude that the process is triggered in a step-function-like manner depending upon the availability of an input signal, and that it later unfolds as an orderly, multiple phase cascade of 'developmental decisions' culminating in the modulation of gene expression. Is this an adequate account?

Let us start with the trigger. Experimental protocols used to elicit consolidation in both *Aplysia* and LTP are usually based on increasing the intensity of input.

For example, one pulse of serotonin is used to induce short-term facilitation in the sensory-to-motor synapse in *Aplysia*, whereas five spaced pulses are required to elicit the long-term facilitation (Montarolo *et al.*, 1986). However, the notion that consolidation is a function of the intensity of a single input might well be too simplistic. It also is not in accord with the natural situation, in which much of the information that we consolidate frequently is distinguished from the non-consolidated by virtue of context and association rather than intensity. We therefore favor the notion that a time-dependent convergence of two or more events usually is required.

To illustrate this latter possibility, let us take a closer look at mechanisms of LTP in hippocampal area CA1. It has long been thought that *N*-methyl-D-aspartate (NMDA) receptor-dependent LTP is a homosynaptic event, i.e. glutamatergic synapses are capable of inducing and maintaining all processes required to enhance the efficacy at the particular synapse. However, activation of ionotropic glutamatergic receptors alone leads only to a short- (<1 h) but not a long-term potentiation, provided that the extracellular ion concentrations are not manipulated (Kullmann *et al.*, 1992). Further, it has been shown that late-LTP in CA1 and dentate gyrus can be prevented or stimulated by inhibitors and activators, respectively, of aminergic, opioid or metabotropic glutamate receptors coupled to the cAMP cascade (for a review, see Frey and Morris, 1998). We therefore suggest that late-LTP requires concomitant activation of multiple extracellular signaling systems. The typical LTP experiment, involving a brief period of high-frequency stimulation (or, with intracellular recording, the pairing of pre- and postsynaptic activation), overlooks the more likely situation in the behaving organism where the synaptic population of an individual CA1 cell is likely to have a unique history and change dynamically with time. Potentiation will occur at some sites matched by heterosynaptic and homosynaptic decreases in synaptic efficacy elsewhere. The artificial massive stimulation, involving simultaneous activation of hundreds of fibers, probably activates more than one kind of neurotransmitter input, and it is that cooperative action of inputs that, experimentally, enables late-LTP. The main 'take-home' message is that consolidation is not a function merely of more-of-the-same (i.e. a single transmitter), but also of coincidence (i.e. external stimuli, internal states or both).

Having set the process in motion, two main classes of scenarios can be considered for the conversion of

this short- into a more long-term synaptic change (though others can also be envisaged—see Lisman and Fallon, 1999):

- (i) the same molecular species and cellular processes that subserve the short-term changes, also subserve the long-term change;
- (ii) some of the molecular species and cellular processes subserving the short- might also subserve the long-term changes, but additional molecular species and processes must be recruited with time.

The first scenario is theoretically possible, and can be subserved, for example, by a durable shift in the kinetics of autocatalytic protein kinase–protein phosphatase cascades in the synapse (Crick, 1984; Lisman, 1985; Buxbaum and Dudai, 1989). In this scenario, macromolecular synthesis is not expected to have a causal role in establishing the persistence of traces over time, but rather to have a role in supplying the synapse with resources for enhanced molecular turnover, in expanding synaptic space for future plasticity, and in homeostatic functions. Using a simplistic metaphor of the synapse as a motor vehicle, it has the engine required for a very long ride, but will evidently stop running in the absence of an appropriate supply of fuel and spare parts.

The second scenario regards modulation of macromolecular synthesis as causally required for retention of memory over time. This may be done locally at or near the synapse, or cell wide. Adhering to the above metaphor, the non-consolidated synapse does not have an engine, merely a starter. The combustion engine, powerful enough to travel for years, must be assembled during consolidation from new parts that are either manufactured on the spot, or shipped from the main factory, i.e. the nucleus and cytoplasm.

What macromolecules might be involved in each of the above scenarios? If we focus on the synapse as the critical (and most thoroughly investigated) locus of change, both pre- and postsynaptic changes could be entertained. Presynaptic modifications are expected to consist of alterations in the efficacy of transmitter release. These might be induced either by changes in the availability of Ca^{2+} (from external as well as from internal stores, Reyes and Stanton, 1996) or directly in the release machinery. There are various ways of realizing such processes. Experience-dependent phosphorylation of ion channels, for which there is experimental evidence, is one of them (Kandel and Schwartz, 1982). So is, potentially, a direct modi-

fication in the release machinery. An appealing memory-keeping step is persistent activation of the appropriate protein kinase(s), resulting in continuous phosphorylation and rephosphorylation of the relevant substrate proteins in the synapse (Schwartz, 1993; Chain *et al.*, 1995). Postsynaptic mechanisms are expected to involve alterations in receptors for neurotransmitters and their coupled intracellular signal transduction cascades. Examples are α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in LTP, modulated by phosphorylation (Barria *et al.*, 1997); here again, persistent activation of protein kinases is one of the candidates for the memory-keeping devices. Calcium/calmodulin-dependent protein kinase II is a prime suspect, but definitely not the only one (Otmakhov *et al.*, 1997; Ouyang *et al.*, 1997). Modulation of kinases and receptors need not be expressed only in altered receptor responsiveness to an incoming stimulus; it can also involve modification in the interfacing of a membrane-associated receptor with the cytoskeleton and intracellular signal transduction cascades (e.g. Niethammer *et al.*, 1996; Otmakhov *et al.*, 1997), and in the availability of receptor molecules (Hayashi *et al.*, 2000).

So what might consolidation be in each of the above scenarios? In the first scenario, the initiation of long-term memory actually coincides with the short-term process, and consolidating consists of recruiting supportive mechanisms. In the second scenario, macromolecular synthesis might be required to generate more of the same or new variants or types of protein that augment and extend the function of the those modified in the short term—such as proteases that degrade the inhibitory, regulatory subunit of cAMP-dependent protein kinase (Hegde *et al.*, 1993). In addition, mechanisms not required in the short term might be called into service, e.g. intra- and extracellular proteolysis for synaptic remodeling, and alterations in intra- and extracellular architecture brought about by members of the large family of cell adhesion molecules (Fields and Itoh, 1996; Martin and Kandel, 1996; Solomon *et al.*, 1998). This scenario indeed depicts consolidation as including mechanisms similar if not identical to those recruited in growth and differentiation (Davis *et al.*, 1996). However, since most cellular preparations used to investigate experience-dependent modifications in synaptic efficacy use rather strong, non-physiological stimulation protocols, it is not unlikely that they reveal molecular events that *in vivo* are recruited only in response to stress and injury. The specificity of the transcriptional

modulation in such preparations to trace formation in the behaving animal must therefore be regarded with some caution. Whatever is the case, in the long term, the synapse, whether strengthened by proliferation or not, is still expected to harbor modified channels, enzymes or receptors of the kinds mentioned above (Martin and Kandel, 1996). Again, this hints at the notion that too much emphasis on the nucleus, an understandable outcome of the impressive success of developmental cell biology, might be luring us away from mechanisms specific to representational changes in the circuit which operate over a much faster time scale (Dudai, 1997b; Singer, Chapter 3).

WHERE?

The discussion so far leads to the inevitable conclusion that consolidation proceeds at multiple sites within neurons. Consider in this respect the engineering problem that neurons face in determining where consolidated changes in neuronal function are to occur. Cortical pyramidal cells have many thousands of excitatory glutamatergic synapses receiving afferent input from a large number of other cells. An individual cell functions, therefore, in numerous distinct but overlapping circuits, and the spatial distribution of synaptic weights on such a cell will reflect this at any one time. It follows that any decision to consolidate or not to consolidate the synaptic strength of an individual synapse is not likely to be taken in the cell body exclusively if the desired change is to be specific to a subset of inputs onto that cell. What the cell body can do, however, is to effect a change that creates the potential for consolidation, but allow the final decision as to whether and where this is to occur to be determined locally. This solution is attractive, but it requires a potentially complex interaction between local and central mechanisms within a neuron.

One engineering solution for avoiding this complexity would be to allow all decision making to be done locally, once the relevant permissive or instructive information is available. Individual synapses could not only have the machinery for changing synaptic efficacy, but also all the machinery for ensuring that this change is persistent in the face of protein turnover (e.g. Davis *et al.*, 1987; Feig and Lipton, 1993; Ouyang *et al.*, 1999). In this view, the cell body would have only the housekeeping role of synthesizing the mRNAs and proteins required at the periphery, but play no part in determining whether or where local

consolidation occurred. Such an arrangement is, however, wasteful, for at least two reasons. First, the neuron is an integrative computing element that, in addition to summing excitatory postsynaptic potentials (EPSPs) and enabling action potential propagation (over a fast time scale), could also maintain a record of its own recent history of activation. This history could play a part in making decisions about consolidation. To take advantage of this opportunity, aspects of the decision making cannot be only at the local elements of connectivity, but must also be at either the cell body or, allowing one further level of complexity, within individual dendritic domains. The key concept here is to remove part (but not all) of the decision making from sites where only limited information is available to sites where afferent information can accumulate. Secondly, an exclusively local mechanism of consolidation may be biochemically wasteful. If macromolecules are necessary for consolidation, and similar if not identical molecules are required at all synapses, it may make no sense to endow each of the thousands of synapses on a cell with the intricate machinery for achieving persistent change. Thus, irrespective of the specific mechanisms involved, a distributed process has the merits of intraneuronal integration and biochemical economy—subject to the eventual necessity for achieving input specificity.

The aforementioned reference to dendritic domains deserves further comment. In the cerebral cortex, inputs are received in particular cortical layers that correspond to specific domains of a pyramidal cell's architecture (Creutzfeld, 1995). This state of affairs allows the possibility that synapse formation and/or synaptic change and consolidation may be required in these domains of the cell but not others. Under these circumstances, the question arises of the efficiency of having only the options of local or central decision making; it may be advantageous to traffic cellular machinery to intermediate locales, i.e. those cellular domains where it will be used most intensively. By way of illustration, it may be advantageous to have specific types of RNA or protein synthesis in layer IV of the neocortex where thalamic inputs are received; or in specific segments of the medial perforant path where entorhinal input to the dentate gyrus terminates. It should be recognized, however, that this gain in efficiency cannot be at the expense of the necessity for shared decision making by the synapse and the cell body, and the advantages it confers upon the neuron.

For a process involving decision making at distributed loci in a neuron to be effective and to evade

catastrophes, it is essential that there is appropriate overall cellular coordination. Furthermore, this coordination may enrich and optimize the metabolic and computational repertoire of the neuron. This is one of the ideas behind the concept of 'synaptic tagging' (Frey and Morris, 1997), whereby local sites at which consolidation is presumed to occur (i.e. individual synapses) can sequester the products of consolidation processing elsewhere. The first experiment to illustrate this point established that it is possible to induce protein synthesis-dependent late-LTP during the inhibition of protein synthesis provided that the macromolecules required for this local consolidation have been synthesized earlier. Moreover, weak stimulation, that ordinarily gives rise only to transient changes in synaptic efficacy, can also result in a lasting change if this stimulation follows much stronger prior activation of the neuron by stimulus patterns that are sufficient for triggering macromolecular synthesis. Consolidation at local sites in a neuronal domain (such as a small population of synapses) is therefore determined in a dual fashion—by the cell-wide availability of macromolecules that may (and often will) have been synthesized in response to other events, and by local postsynaptic 'tags' that sequester or 'hijack' these proteins and so render permissive the 'final common path' of synaptic consolidation. Frey and Morris (1998) have also reported that there is an appealing symmetry about these dual-location arrangements—persistent synaptic change can also occur if the weak stimulation on a pathway (that sets a local synaptic tag) precedes the stronger stimulation of a population of neurons on another pathway, provided that the interval between the two patterns of stimulation is quite short (<1–2 h). The success of both the original 'strong-before-weak' and the newer 'weak-before-strong' protocols in inducing persistent change on the weakly stimulated pathway offers strong evidence that consolidation is a consequence of two or more neuronal processes operating at different loci within cells. Each creates the potential for persistent change; neither can induce it alone.

These experiments were conducted using extracellular recording techniques and stimulation of large numbers of afferent fibers and neurons. Striking evidence for synaptic tagging occurring within individual cells has been obtained in *Aplysia* cell cultures. In a preparation in which one sensory cell was afferent onto two motor neurons, weak facilitatory activation of one synaptic terminal following prior strong activation of the other revealed a lasting presynaptic facilitation at

both (Martin *et al.*, 1997a). Furthermore, whereas repeated application of serotonin to an individual synapse produced a CREB-mediated, synapse-specific long-term facilitation, repeated pulses of serotonin to the cell soma produced a CREB-dependent cell-wide facilitation that, alas, was only short lived. However, if a single pulse of serotonin, which by itself produced only transient synaptic facilitation, was then applied to an individual synapse, that synapse acquired persistent facilitation and synapse-specific growth. The effect of the single serotonin pulse on the synapse and its priming for capturing and locally stabilizing the cell-wide facilitation involves cAMP-induced post-translational modifications as well as local protein synthesis (Casadio *et al.*, 1999). All in all, the results from *Aplysia* extend our understanding of synaptic tagging in several important respects: it establishes that tag-macromolecule interactions occur within single cells, it indicates that this can happen presynaptically (as well as postsynaptically), it shows that the local synaptic processes involve both protein synthesis-dependent and protein synthesis-independent mechanisms, and it firmly establishes that dual control of cellular consolidation by the synapse and the nucleus occurs in both invertebrate and vertebrate nervous systems.

WHEN?

A frequent claim is that consolidation involves a series of discrete phases. This way of thinking invites the notion of critical periods at which it is feasible to interfere with specific players in this cascade of events. In this view, consolidation may be said to have both a start- and an end-point, with each of its component processes having characteristic durations after some unique triggering event. Several factors will determine the time course of these constituent elements of the machinery, including the typical half-life of synaptic proteins, the speed with which molecules are transported to and from the nucleus, rates of protein synthesis, and so forth (e.g. Huh and Wentfold, 1999; Xu and Sapleter, 1999). Knowledge of these time courses, coupled with the assumption that they occur in a prescribed sequence, would enable one to work out the important time points of consolidation including the duration of critical periods after learning has taken place.

However, if, as discussed previously, decision making in a neuronal domain is distributed, it seems

to us more likely that different triggers will be responsible for different elements of the consolidation machinery. Temporal intersection of the products of these different mechanisms at local sites presumably will be both necessary and sufficient for consolidation to occur, but there may then be no rigorously prescribed time course or critical periods at which interference with consolidation will always be successful. To spell out this quite radical suggestion more precisely, suppose local consolidation involved the utilization of somatically synthesized plasticity-related proteins at sites at which specific receptor proteins recently had been phosphorylated (e.g. AMPA receptors, Wyllie and Nicoll, 1994; Barria *et al.*, 1997). If these plasticity proteins were to be synthesized in advance, perhaps in response to neuromodulatory input elsewhere on the cell, there is no *a priori* reason why consolidation could not occur very rapidly after the local phosphorylation events had taken place. The time-consuming cycle of translocation to and from the nucleus would be finessed by virtue of the prior history of activation of the neuron. Thus, a phase of consolidation usually described as being a 'late' phase could often occur quite early. In the case of 'late-LTP', for example, the prior induction of early-LTP may still be a necessary condition, but the local changes at synapses that characterize a consolidated alteration to receptor proteins may nonetheless occur very soon thereafter. This suggestion does, incidentally, have the immediate practical importance that it could enable techniques such as intracellular or patch-clamp electrophysiology to be deployed in the analysis of 'phases' of memory formation that have hitherto seemed beyond its reach.

A final aspect of the 'when' question has to do with whether consolidation ever ends. We suspect not. A typical pedagogical scenario is the supposition that a labile memory trace is established and it is then subject to consolidation beginning at time t_1 and ending at time t_2 . Consolidation complete, it can now survive the winds and waves of any brainstorm that the nervous system may throw at it. Some memories may be like this—being dredged up years after they supposedly were forgotten. However, everyday reality is likely to be a more dynamic process of storage, consolidation, retrieval, integration with other new information, re-storage, a new cycle of consolidation, and so on (e.g. Roulet and Sara, 1998). This more dynamic perspective sees memory traces assuming a status where they acquire both persistence and resistance to interference, yet somehow maintain

the capacity to be resculpted and so transformed. There is a mystery embedded in such a state of affairs, but one which our present understanding of how internal representations are retained, reactivated or reconstructed in retrieval is still poorly equipped to address.

WHY?

We do not intend to ask the metaphysical question of why is there long-term memory to start with, but rather why it does not stabilize instantaneously. Though well aware of the teleological nature of the question and the speculative nature of any answers, we deem it useful to delve into the game, because, apart from its mere theoretical interest, it could pinpoint potential experimental avenues.

One potential explanation to be kept in the back of one's mind is that the gradual nature of the maturation of synaptic changes that subserve memory traces arises, in part at least, by virtue of the mechanistic constraints imposed on the nervous system by phylogenesis. These could be of two types. First, synapses and neurons, having to react quickly to incoming stimuli, must rely in their detection and immediate registration machinery on fast biophysical and post-translational modifications. However, alas, the latter are also short lived and, additional mechanisms, more immune to molecular turnover, must therefore be engaged to keep the trace going (e.g. Crick, 1984; Goelet *et al.*, 1986; Lisman and Fallon, 1999). This transition establishes consolidation by definition. Secondly, the possibility should be considered that it was phylogenetically parsimonious for evolving phyla to utilize in memory systems the same cellular building blocks used in development, growth and response to injury. The resemblance of cellular processes of growth and learning and the overlap of the molecular machinery between the two have already been mentioned above (see also Carew *et al.*, 1998). It has also been suggested specifically, for example, that behavioral plasticity stems from a more primitive compensatory plasticity (e.g. Walters *et al.*, 1991). The reliance on primitive mechanisms may have imposed some basic constraints on neurons, and shaped them to function in certain ways which might not necessarily be optimal.

Mechanistic and phylogenetic constraints notwithstanding, one could envisage theoretically a situation in which newly formed memories do stabilize instantaneously, evading the risk of erasure by confounding

input and metabolic interference. This indeed may happen occasionally, as in certain instances of flash-bulb memory (Brown and Kulik, 1977), but, routinely, there are good reasons not to have such a step-function change. Contemplating such hypothetical rationales must take into account not merely cellular consolidation but also system consolidation, which the former is postulated to subservise. One of the reasons to assume that instantaneous stabilization of memory is a counterproductive routine is that immediate conversion of information into a long-term store might waste brain space on events that have to be retained for intermediate periods but can then be safely forgotten. Presumably, most of the sensory information that impinges on our senses does not culminate in lasting traces, for, if it were to do so, it would impede our ability to construe the world efficiently and react to it (Dudai, 1997a). Another potential drive may have to do with the way in which the brain constructs narratives to construe the world and react to it. The construction of such internal narratives may take the form of pruning and rearrangement of mental items, a process that takes time and has to be performed against the background of ongoing brain activity. Consolidation, including cellular consolidation immediately after learning, may allow such processes to take place. Furthermore, while reconstructing internal narratives, consolidation may promote generalization and categorization (McClelland *et al.*, 1995), without which the perceived world may become confusing and the reaction to it ineffective (Luria, 1968). Finally, the reason for the gradual stabilization of memories in brain systems may be an algorithmic one; for example, gradual interweaving of new information can, with certain learning algorithms, avoid catastrophic interference (McClelland *et al.*, 1995).

Turning again from the circuit to the cellular processes, and refocusing on cellular consolidation and its mechanisms, what might be the functional advantage of having cell-wide in addition to synaptic changes in cellular consolidation? The following possibilities could be entertained.

Cell-wide changes might fulfill a storehouse function, needed because synapses do not have sufficient metabolic assets. More than 20 years ago, Squire and Barondes (1976) concluded, based on kinetic argumentation and on data from systemic inhibition of protein synthesis, that the effect of macromolecular synthesis inhibition on consolidation is not due to the depletion of constitutively expressed proteins. This might

indeed hold for the critical initial period, but does not reflect the synaptic need for protein supplies over longer time periods. Despite the theoretical ability of neurites and synapses to sustain long-term change autonomously (Crick, 1984; Lisman, 1985; Buxbaum and Dudai, 1989; Friedrich, 1990), and their possibility of sustaining some degree of protein synthesis locally (e.g. Davis *et al.*, 1987; Feig and Lipton, 1993; Ouyang *et al.*, 1997; Casadio *et al.*, 1999), it is plausible to assume that nuclear transcriptional changes must be invoked to supplement and replenish the synapse with housekeeping and building material. Furthermore, the fact that a synapse is active might signal to the nucleus that it should be strengthened properly and supplied to withstand the demands of extensive use. It reminds one of the instantaneous recruitment of blood to activated brain areas, so successfully exploited in functional neuroimaging (Cohen and Bookheimer, 1994). The capillary blood flow is not part of the computational machinery, but rather fuels the machinery to keep the computations going. This raises issues of causality and specificity. Though obligatory for sustaining the process over time, modulation of transcription might be neither causal nor sufficient for determining the specificity of changes in a neuronal circuit; specificity could be determined locally and autonomously. If this is true, one should not expect to elucidate mechanisms of specificity, so critical to learning and memory, by analyzing cell-wide transcriptional alterations alone.

A quite separate issue has to do with some facets of integration, context and generalization of information. Additional dissociations emerge from recent studies on hippocampus and *Aplysia*. One is spatial—between the hijacker and the hijacked—namely the dissociation between the local tag (enabling spatial specificity and some measure of temporal specificity) and the proteins captured from other components of the neuron (reinforcing the embodiment of the long-term change at the specific tagged location). Another is definitively temporal, between the event marking specificity (and leading to the creation of the local tag) and the time window surrounding it (during which activity-dependent proteins can be captured by the tagged synapse). At the cellular level, these spatial and the temporal linkages might subservise binding and encoding of context over time and space (Frey and Morris, 1997). Hence it might be advantageous to 'inform' other synapses on a neuron that a salient event has occurred, and to have a cellular mechanism of 'local attention' that—provided the context signifies

saliency—permits mild stimuli to be construed as important ones (and ultimately resulting in a more permanent record of their occurrence). Such a mechanism might also contribute to generalization and categorization, mirroring at the cellular level functional drives that operate at the system level (e.g. McClelland *et al.*, 1995). What is unveiled here could be regarded as an additional manifestation of an elementary property of perceptual, mnemonic and cognitive systems, namely the ability to bind representational elements into a coherent whole over time and space. This is realized in the brain at different levels of resolution. For example, perceptual binding of instantaneous events in the sub-second range by some sort of fast coincidence detection in neuronal assemblies (which refers to the current popular usage of 'binding' in the neurosciences, see also Singer, Chapter 3); binding of context and events on a time scale of minutes to hours which might be subserved among others at the cellular level by local consolidation; and binding of events into narratives and categories over weeks to years in system consolidation (Dudai, 1996, 1997b). It should also be noted that local, cellular processes of integration and generalization, embodied in the interdependency of remote synapses on the same neuron in cellular consolidation, may pose as yet unknown constraints on computations made over elements of internal representations.

A third factor is metaplasticity. In both vertebrate (Kirkwood *et al.*, 1996) and invertebrate (Fischer *et al.*, 1997) nervous systems, the induction of synaptic plasticity also results in a modulation of the ability of synapses to induce or maintain plasticity subsequently. This form of higher order plasticity is dubbed metaplasticity (Abraham and Bear, 1996). The induction by activated synapses of cell-wide waves of protein synthesis, and the resulting effects of these waves on synapses in which local protein synthesis had not been triggered, could be another example of metaplasticity.

CONCLUSIONS

We have focused on a number of open questions concerning local, cellular consolidation in neuronal circuits. Recent evidence suggests that consolidation need not be triggered in an abrupt, step-like function manner by intensive input, and does not necessarily unfold in a predetermined, fixed cascade of developmental decisions. Rather, it appears to involve decision making at multiple sites in the neuronal domain,

and an intricate interaction between local and central mechanisms within the neuron. It may, *in vivo*, need coincident inputs in order to start rolling. Its time course depends on the history of the neuron and the circuit, and is not expected to follow rigid time windows and phases. Whereas macromolecular synthesis appears obligatory for the process to proceed, the possibility that it does not play a causal role in altering representational properties and does not contribute to the specificity of the change, but rather fulfills post-factum supportive, homeostatic and possibly preparatory functions, should not be ignored. In spite of its appeal, the analogy to developmental processes, which emerges from the cellular analysis of consolidation, should not blur the search for the unique representational properties of synapses and neurons in the brain and their stabilization over time. A developing neuromuscular junction may use molecular cascades similar to those that are detected in a consolidating synapse but, for all their capacity to move minds, muscles did not think up *Hamlet*.

Consolidation is not only indispensable for some types of memory; it is also a potential window into the functions of memory at large, the processes that subserve these functions, the mechanisms that embody these processes and the interaction between levels of organization and function in the brain. Recent investigations of the cellular biology of consolidation begin to expose fine distinctions between the specific and the general, the local and the global, the tokens and the types of the cellular processes and mechanisms that subserve the conversion of precepts into long-lasting internal representations. These processes and mechanisms now become definable in a molecular language. This makes it attractive to consider the formulation of rudimentary correspondence rules (Nagel, 1979) for the translation of certain aspects of behavioral and physiological phenomena related to consolidation, such as attention, association and generalization, into cellular and molecular events, and vice versa.

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