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Learning-induced activation of transcription factors among multiple memory systems

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Abstract

Experimental evidence for multiple memory systems grew initially from reports that integrity of the medial temporal lobes is necessary for some, but not all, types of memory formation. A primary inference from many studies of multiple memory systems is that they operate independently during encoding, storage, and retrieval of information. An accumulation of recent evidence, however, suggests that multiple memory systems may interact under some conditions. At the cellular level of analysis, it is accepted widely that protein synthesis is necessary for the formation of long-term memory and recent efforts have focused on the mechanisms by which learning-induced gene transcription and translation are regulated. The present review examines learning-induced activation of transcription factors among multiple memory systems. The results indicate that studies of transcriptional regulation, in conjunction with other experimental approaches, can provide complementary lines of evidence to further understanding of the extent to which multiple memory systems are independent or interactive.

1. Introduction

A primary inference from many studies of multiple memory systems is that they operate independently during encoding, storage, and retrieval of information. An accumulation of recent evidence, however, suggests that multiple memory systems may interact under some conditions. Reports of interactions among memory systems have raised many questions regarding the kinds of interactions that may occur and the significance of these interactions. Two fundamental questions that have come under empirical scrutiny only recently are these: (1) Are memory systems independent or interactive primarily and, if interactive, do they function cooperatively or competitively? (2) To the extent that memory systems are interactive, do interactions occur during memory formation, during retrieval, and guidance of behavioral output, or both? The goal of the present review is to provide evidence that investigation of learning-induced changes in the localized activity of transcription factors, and expression of immediate-early genes, can be of substantial benefit to test and extend the multiple memory systems hypothesis.

Experimental evidence for multiple memory systems grew initially from reports that integrity of the medial temporal lobe is necessary for some, but not all, types of memory formation (Scoville & Milner, 1957; see Squire, 2004 in this issue). The early findings stimulated half a century of research in which a primary aim was to identify the forms of memory and the brain systems on which they depend for storage and retrieval. In research using animal subjects, a primary research strategy has been to manipulate, either by lesions or administration of pharmacological agents, the function of one or more brain systems and then test the effects on one or more measures of learning and memory. Perhaps the strongest evidence for multiple memory systems is the triple dissociation (Kesner, Bolland, & Dakis, 1993; McDonald & White, 1993) in which a lesion to one of three brain regions produces

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memory impairment for one of three tasks and damage to each region causes impairment of a different task. The most evident conclusion that can be drawn from dissociation studies is that multiple memory systems process and store information independently.

2. Dissociation of memory systems mediated by the hippocampal formation, the neostriatum, and the amygdala

A growing number of researchers have attributed distinct memory functions to the hippocampal formation, the neostriatum, and the amygdala among humans (Bohbot et al., 1998; Heckers, Zalesak, Weiss, Ditman, & Titone, 2004; Kilpatrick & Cahill, 2003; Knowlton, Mangels, & Squire, 1996; Maguire et al., 1998; Morris, Pickering, Abrahams, & Feigenbaum, 1996; Phelps, 2004; Preston, Shrager, Dudukovic, & Gabrieli, 2004; Poldrack, Prabhakaran, Seger, & Gabrieli, 1999) primates (Amaral, 2003; Fernandez-Ruiz, Wang, Aigner, & Mishkin, 2001; Teng, Stefanacci, Squire, & Zola, 2000), and rats (Eichenbaum, Otto, & Cohen, 1992; Gold, Macri, & McGaugh, 1973; Kesner, 1980; McDonald & White, 1993; Morris, Garrud, Rawlins, & O'Keefe, 1982; Olton, Becker, & Handelman, 1979; Packard, Cahill, & McGaugh, 1994). Lesions or pharmacological manipulations that alter the function of the hippocampal formation, the neostriatum, or the amygdala have provided the primary evidence that these brain systems are specialized for different types of memory.

Interpreted generally, the results of experiments with rats indicate that lesions to the hippocampal formation or fornix selectively impair acquisition of trial-dependent associations among stimuli (Eichenbaum, 1998; White & McDonald, 2002), whereas damage to the neostriatum impairs acquisition of trial-independent associations between stimuli and responses (Colombo, Davis, & Volpe, 1989; McDonald & White, 1994; Packard, Hirsh, & White, 1989; Packard & White, 1990; Packard & McGaugh, 1992, 1996). Lesions to the amygdala tend to impair stimulus-affect associations (Davis, 1992; Kilpatrick & Cahill, 2003; Salinas & White, 1998). It has been proposed, however, that the amygdala may modulate memory formation in the hippocampus and striatum based on states of emotional or affective arousal (Packard & Teather, 1998; Packard & Cahill, 2001). Specialized mnemonic functions tested in rats and attributed to the hippocampus and neostriatum, respectively, include memory for spatial and non-spatial information (Packard & McGaugh, 1992; Packard et al., 1994), memory for allocentric (place), and egocentric (response) information (Kesner, Farnsworth, & DiMattia, 1989; Packard, 1999), and declarative and procedural memory (DeCoteau & Kesner, 2000).

3. Multiple memory systems may operate independently, interactively, or in temporal sequence

The findings cited above show that the hippocampus, neostriatum, and amygdala mediate memory systems that can be measured independently, but they do not address whether or not these systems interact during either memory formation or recall in the intact brain under normal conditions. Among other reports that memory systems may not act independently, McDonald and White (1995) reported a double dissociation of the hippocampus and amygdala based on findings that amygdala, but not fornix, lesions impaired conditioned cue preference (CCP), whereas fornix, but not amygdala, lesions impaired spatial memory. It is important to note that rats with fornix lesions showed a significant enhancement of the CCP in comparison with control rats, suggesting that if one memory system is not intact, then behaviors mediated by another system may be facilitated. Other evidence that memory systems may act competitively is derived from examples of correlations between markers of regional neuronal integrity or activity and performance of individual animals on different components of memory tasks. As an example, Colombo and Gallagher (1998) reported a negative correlation between choline acetyltransferase (ChAT) activity in the dorsolateral neostriatum and working memory performance in a water version of the radial arm maze task. In that example, rats with the highest ChAT activity in the neostriatum, a system necessary for reference memory (Colombo et al., 1989) made the most working memory errors, which are often associated with hippocampal function. McIntyre, Pal, Marriott, and Gold (2002) used in vivo microdialysis to measure release of acetylcholine (ACh) in the hippocampus as rats were trained and tested on an amygdala-dependent conditioned place preference task. In that study, testing-related increases in ACh were correlated negatively with performance, suggesting a competitive interaction between the hippocampus and amygdala during memory recall. In a subsequent study (McIntyre, Marriott, & Gold, 2003), release of ACh in the amygdala was correlated positively with performance during testing on a hippocampus-dependent spontaneous alternation task. Thus, activation of the amygdala may cooperate with the hippocampus under some recall conditions.

Taken together, these results indicate that either damage or activation in one memory system may alter performance mediated by another system, suggesting that memory systems can interact both competitively and cooperatively under some conditions. Packard (1999) reported that rats tend to solve the cross maze task using a place strategy on day 8 of training, but shift to a response strategy by day 16. Administration of glutamate in the hippocampus retarded the shift from place to response learning whereas administration of glutamate in

the caudate facilitated the shift from place to response learning. These results suggest that, in addition to mediating place and response learning, the hippocampus, and caudate may operate in temporal sequence. Further evidence for sequential participation of the hippocampus and striatum in memory was reported by Chang and Gold (2003). In that study, cross maze training-related ACh release in the hippocampus and striatum reflected the shift from a hippocampus-dependent place strategy to a striatum-dependent response strategy. The studies by Gold and colleagues (Mc Intyre et al., 2002; 2003) illustrate the utility of measurements that reveal regional learning-induced activity in the intact brain. While experimental lesions and pharmacological manipulations have been particularly useful to identify multiple memory systems, those techniques also alter structural or functional components of circuitry. In contrast, examination of learning-induced plasticity offers the advantage that measurements reflect the activity of the intact brain during normal memory formation and retrieval (Gall, Hess, & Lynch, 1998).

4. Learning-induced activation of transcription factors as markers of memory formation

When taken together, the multiple memory systems hypothesis and the synaptic plasticity and memory (SPM) hypothesis (Martin, Grimwood, & Morris, 2000) suggest that neuronal modifications occur within brain regions specialized for various forms of memory during acquisition, retrieval, or both. It follows also that the behavioral expression of interactions among neural systems important for memory formation and retrieval are related to time- and region-specific neuronal plasticity. Indeed, measurements of regional learning-induced activation or changes in levels of transcription factors reflect plasticity of the intact brain as it functions normally, thus they are well suited for testing hypotheses regarding independence and interactions among memory systems.

It is accepted widely that protein synthesis is necessary for the formation of long-term memory (Davis & Squire, 1984). Most recently, significant research efforts have focused on the mechanisms by which learning-induced gene transcription and translation are regulated. In general, long-term neuronal plasticity requires stimulus-induced activation of kinases that phosphorylate constitutively expressed transcription factors (e.g., cyclic AMP response element binding protein, CREB). These in turn regulate both inducible transcription factors (e.g., c-Fos, zif268) and late response genes (e.g., *Arc*) that can alter synaptic efficacy (Lonze & Ginty, 2002). Classes of genes with CRE sequences include growth factors, structural proteins, channels/transporters, cellular metabolism, transcription, and signal transduction (Lonze & Ginty, 2002). Inducible transcription factors regulate expression of late response genes which code for receptors, synapsins, and structural proteins (Walton et al., 1999).

The present review compares studies of learning-induced changes in transcription among several dimensions listed in Table 1. The studies included in Table 1 were selected to illustrate factors that are important for purposes of comparison and the table was not intended to provide a comprehensive record of learning-induced changes in transcriptional regulation. Factors illustrated in Table 1 include different forms of learning, the duration of training, the interval between training and measurements, and the brain regions and transcription factors measured. The hippocampus is the brain region in which learning-induced activation of transcription factors and expression of immediate-early genes has been characterized most thoroughly. The paragraphs that follow compare reports of learning-induced activity of transcription factors and late-response genes in the hippocampus, striatum, and amygdala.

5. Learning-induced changes in transcription factors among multiple memory systems

Among the behavioral tasks for which hippocampal or fornix lesions produce memory impairment are inhibitory avoidance (Taubenfeld, Wiig, Bear, & Alberini, 1999), spatial maze learning (Morris et al., 1982; Olton et al., 1979) contextual fear conditioning (Maren & Fanselow, 1997; Phillips & LeDoux, 1995), and socially transmitted food preferences (STFP) (Alvarez, Lipton, Melrose, & Eichenbaum, 2001; Bunsey & Eichenbaum, 1995; Winocur, 1990; Winocur, McDonald, & Moscovitch, 2001). A first step in establishing that learning-induced transcriptional regulation is important for memory formation among multiple neural systems is to determine whether training on each of the tasks indicated above is associated with localized increases in the activity of constitutive transcription factors (e.g., CREB) or increased expression of immediate-early genes (e.g., c-*fos*, *arc*, and *zif268*). In the hippocampus, inhibitory avoidance is associated with increased phosphorylation of CREB (Bernabeu et al., 1997; Cammarota et al., 2000; Taubenfeld et al., 1999, 2001), CRE-mediated transcription (Impey et al., 1998), induction of C/EBP β (Taubenfeld et al., 2001), and expression of c-Fos (Cammarota et al., 2000). Contextual fear conditioning is associated with increased pCREB in the hippocampus (Stanciu, Radulovic, & Spiess, 2001) and *zif268* expression (Hall, Thomas, & Everitt, 2001), and acquisition of socially transmitted food preferences is associated with increased pCREB and c-Fos in the hippocampus (Countryman, Orlowski, Brightwell, Oskowitz, & Colombo, in press). In addition, aversively motivated spatial maze learning is associated with

Table 1
Examples of studies in which learning-induced activation of transcription factors was measured in one or more brain regions implicated in memory formation

Task	Train-test interval (measure)	Dependent variable	Train-test interval (effect)	Reference
Hippocampus				
Inhibitory avoidance	0, 0.5, 1, 3, 6, 9 h	pCREB	0, 3, 6 h	Bernabeu et al. (1997)
Inhibitory avoidance	0, 3, 6, 9 h	pCREB	0, 3, 6 h	Taubenfeld et al. (1999)
Inhibitory avoidance	2 h	pCREB	2 h	Cammarota et al. (2000)
		c-Fos	2 h	
Operant conditioning	1, 2, 3 h after day 1, 2, 5	c-Fos	day 1/60 min (CA3) day 2/1 h (CA1, CA3, DG)	Bertaina-Anglade et al. (2000)
Contextual fear	15, 30, 60 min	EGR-1 (*zif 268*)	Compared to sham trained	Malkani and Rosen (2000)
Inhibitory avoidance	3, 6, 9, 20, 72 h, 1 week	C/EBPβ	9, 20 h	Taubenfeld et al. (2001)
		C/EBPδ	20 h	
		pCREB	12, 20 h	
Water maze; spatial/cued	0, 30, 120, 360 min	*Arc, zif268, c-fos*	30 min	Guzowski et al. (2001)
Contextual fear	0, 7, 30, 60, 90, 180, 360 min	pCREB	180, 360 min (CA1 only)	Stanciu et al. (2001)
		c-Fos	Compared to sham trained	
Radial arm maze	2 h after day 1, 3, 5	c-Fos	day 3	He et al. (2002)
Plus maze	0, 60 min	pCREB, c-Fos	0, 60 min/place; 0/resp	Colombo et al. (2003)
STFP	60 min	pCREB, c-Fos	60 min	Countryman et al. (in press)
Striatum				
Operant conditioning	1, 2, 3 h after day 1, 2, 5	c-Fos	Day 2; 1 h	Bertaina-Anglade et al. (2000)
Radial arm maze	2 h after day 1, 3, 5	c-Fos	Day 3	He et al. (2002)
Plus maze	0, 60 min	pCREB	0, 60 min/resp; 0 min/place	Colombo et al. (2003)
		c-Fos	Compared to sham trained	
Amygdala				
Operant conditioning	1, 2, 3 h after day 1, 2, 5	Fos	Compared to sham trained	Bertaina-Anglade et al. (2000)
Contextual fear	15, 30, 60 min	EGR-1 (*zif 268*)	15, 30, 60 min in LaDL	Malkani and Rosen (2000)
Contextual fear	0, 7, 30, 60, 90, 180, 360 min	pCREB	180 min	Stanciu et al. (2001)
		Fos	Compared to sham trained	
Neocortex				
Water maze (spatial)	30 min	*Arc, zif268, c-fos*	30 min	Guzowski et al. (2001)
Contextual fear	0, 7, 30, 60, 90, 180, 360 min	pCREB	180 min	Stanciu et al. (2001)
		Fos	Compared to sham trained	
Radial arm maze	2 h after day 1, 3, 5	Fos	day 1, 3	He et al. (2002)

increased *arc* and *zif268* RNA in the hippocampus (Guzowski, Setlow, Wagner, & McGaugh, 2001) and appetitively motivated spatial learning increases pCREB and c-Fos in the hippocampus (Colombo, Brightwell, & Countryman, 2003; He, Yamada, & Nabeshima, 2002).

Lesions to the striatum or amygdala produce memory impairment for response learning or contextual fear conditioning, respectively (Colombo et al., 1989; Hitchcock & Davis, 1996; Kapp, Frysinger, Gallagher, & Appelgate, 1979; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Packard et al., 1989). Learning-induced changes in transcription factors and IEGs have been reported in the striatum and amygdala although these regions have not been tested as extensively as has the hippocampus. In the dorsal striatum, sustained increases in CREB phosphorylation and c-Fos were reported among rats that chose a response strategy in comparisons with controls and rats that chose a place strategy to solve an ambiguous plus-maze task (Colombo et al., 2003). In addition, c-Fos was increased in the dorsal striatum of rats trained on a variable position reward task in the radial arm maze in which 4 of 8 arms were baited, but the positions of the baited arms varied in every trial (He et al., 2002). In the amygdala, increased *zif268* expression (Hall, Thomas, & Everitt, 2000, 2001) and pCREB (Stanciu et al., 2001) were reported following contextual fear conditioning.

As noted previously, experiments using localized lesions or pharmacological manipulations have provided the bulk of results supporting the multiple memory systems hypothesis. The studies described above indicate that behavioral training is associated with activation of transcription factors in the same brain regions in which damage produces memory impairment. Taken together, these findings forge an initial link between two different experimental approaches. As the double dissociation is a primary source of support for the multiple memory systems hypothesis, however, it is important to examine next the extent to which learning-induced changes are localized solely to regions associated with memory for a given task. Strong support for the independence of systems during memory formation would result if training on a task that is associated with a specific brain region causes increased activation of transcription factors in

that region, but not in another region. To date, few studies of learning-induced transcriptional regulation have employed tasks that were selected to test hypotheses regarding multiple memory systems. As indicated in Table 1, many studies of learning-induced changes in transcription employ tasks that are sensitive to lesions of more than one brain region implicated in memory. These include inhibitory avoidance, contextual fear conditioning, and operant conditioning which may each be disrupted by lesions to either the hippocampus or the amygdala. He et al. (2002) examined both working and reference memory simultaneously in the radial arm maze. As the working and reference memory components are associated with integrity of the hippocampus and striatum, respectively, it is not possible to conclude from that study if c-*fos* was expressed generally in both regions or selectively in each region by the separate working and reference task demands. One study designed by Colombo et al. (2003) to address the regional selectivity of learning-induced transcriptional regulation among multiple memory systems showed that place and response learning were associated with specific patterns of CREB phosphorylation in the hippocampus and dorsal striatum, respectively. A second study that used training paradigms sensitive to either hippocampus or striatum lesions was conducted by Guzowski et al. (2001). In that study, rats were trained on spatial (hippocampus) or cued (striatum) versions of a water maze task. Among rats trained on the spatial task, a correlation was reported between escape latency and *Arc* RNA in the hippocampus. In contrast, no correlation was found between escape latency and IEG expression in the hippocampus for rats trained on the cued version. The results of those two studies are consistent with evidence for independence among neural systems during memory formation. While it is clear that studies of learning-induced transcriptional regulation can provide convergent evidence in tests of the multiple memory systems hypothesis, it is clear also that those efforts are in early stages of development.

6. Brain region-specific patterns of learning-induced gene expression may reflect brain region-specific mnemonic processes

Differences in information processing among neural systems have been proposed as a factor that may distinguish multiple memory systems (e.g., Kesner, 1980, 1991; White & McDonald, 2002). This suggests that as information is processed and stored in the hippocampus, dorsal striatum, and amygdala, each system will produce a distinct pattern of neuronal plasticity. Consistent with this idea, Guzowski et al. (2001) provided important evidence that experience produces a more generic immediate early gene (IEG) response within than between brain regions implicated in memory formation. In comparisons with naïve controls, significant increases in expression of *Arc*, *c-fos*, and *zif268* were measured in the hippocampus, the entorhinal cortex, and the primary visual cortex 30 min after spatial training. Of particular importance, the relative increase in IEG expression was not uniform among the brain regions. For example, the training-related increase in *zif268* RNA in the hippocampus was not correlated with the increase in either the entorhinal or the visual cortex. In contrast, the training-related increase in *Arc* RNA in the hippocampus was correlated with the increase in the entorhinal but not the visual cortex. In addition, there was no correlation between *Arc* expression in the entorhinal and visual cortices, but *zif268* expression in the same regions was correlated. One conclusion drawn from this study is that, although the overall patterns of expression of IEGs were similar among the regions tested, training-induced expression of IEGs occurs differentially among brain regions involved in memory formation and is related to the type and phase of learning. Studies of synaptic plasticity have also revealed differences in the roles of transcription factors among brain regions during induction and maintenance of long-term potentiation (Chapman, Ramsay, Krezel, & Knevett, 2003; Yaniv, Vouimba, Diamond, & Richter-Levin, 2003). Although many of the cellular mechanisms of learning-induced plasticity are consistent across memory systems of the brain, there is ample evidence for regional differences. Thus proposed differences in processing styles among memory systems (White & McDonald, 2002) appear to have a neurobiological substrate in regional differences in the patterns of expression of IEGs.

7. Time-courses of learning-induced activation or expression of transcription factors among multiple memory systems

There are relatively few reports of systematic investigations of the time-courses of learning-induced activation or expression of transcription factors in the mammalian brain; the number of reports of effects in more than one brain region involved in memory is fewer still. Bernabeu et al. (1997) measured hippocampal CREB phosphorylation 0, 0.5, 1, 3, 6, and 9 h after inhibitory avoidance training. In comparisons with naïve and shocked controls, trained rats showed an immediate increase in hippocampal pCREB-ir that returned to baseline at the 0.5 and 1 h intervals. The authors reported a second learning-induced peak in pCREB 3 and 6 h after training. Stanciu et al. (2001) also reported two phases of hippocampal CREB phosphorylation following contextual fear conditioning in mice. In that case, pCREB was measured 0, 7, 30, 60, 90, 180, and 360 min after training and was elevated among trained mice 7, 180,

and 360 min after training. The authors concluded, however, that the first peak of pCREB was due to exposure to the shock and not associative processes. A similar, but not identical, time-course of CREB phosphorylation was reported by Taubenfeld et al. (1999) in which inhibitory avoidance training caused an increase in pCREB 0, 3, and 6, but not 9 h after training. A subsequent report by the same group indicated that pCREB was increased 12 and 20 h after training (Taubenfeld et al., 2001). It is not clear whether the decrease in CREB phosphorylation at the 9 h time point reported by Taubenfeld et al., reflects two periods of CREB phosphorylation as reported by others (Bernabeu et al., 1997; Stanciu et al., 2001), or more sustained learning-induced CREB phosphorylation. Studies in which learning-induced effects were measured in more than one brain region implicated in memory formation are of primary importance to the present review. For example, if multiple memory systems interact competitively, then competition may occur either at the time of memory formation or during recall. If competition occurs during memory formation, then one hypothesis is that increased activity or expression of transcription factors in one brain region is accompanied by suppression in a competitive system. To date, there is no support for this hypothesis. For example, Malkani and Rosen (2000) measured expression of the early growth response gene (EGR-1, also called *zif268*) in the hippocampus and amygdala after contextual fear conditioning. In that study, delayed shock increased EGR-1 mRNA expression in the dorsolateral portion of the lateral nucleus of the amygdala 15, 30, and 60 min after training in comparisons with context-no shock and immediate-shock controls. In contrast, EGR-1 expression specific to fear conditioning was not altered in the hippocampus. It may be argued that evidence for competitive interactions among systems at the neuronal level of analysis is best studied by examination of constitutively expressed transcription factors as basal activity may be either suppressed or potentiated. Colombo et al. (2003) measured CREB phosphorylation in the hippocampus and dorsal neostriatum of rats trained during one session on an appetitively motivated cross maze task that could be solved using either a hippocampus-dependent place strategy or a

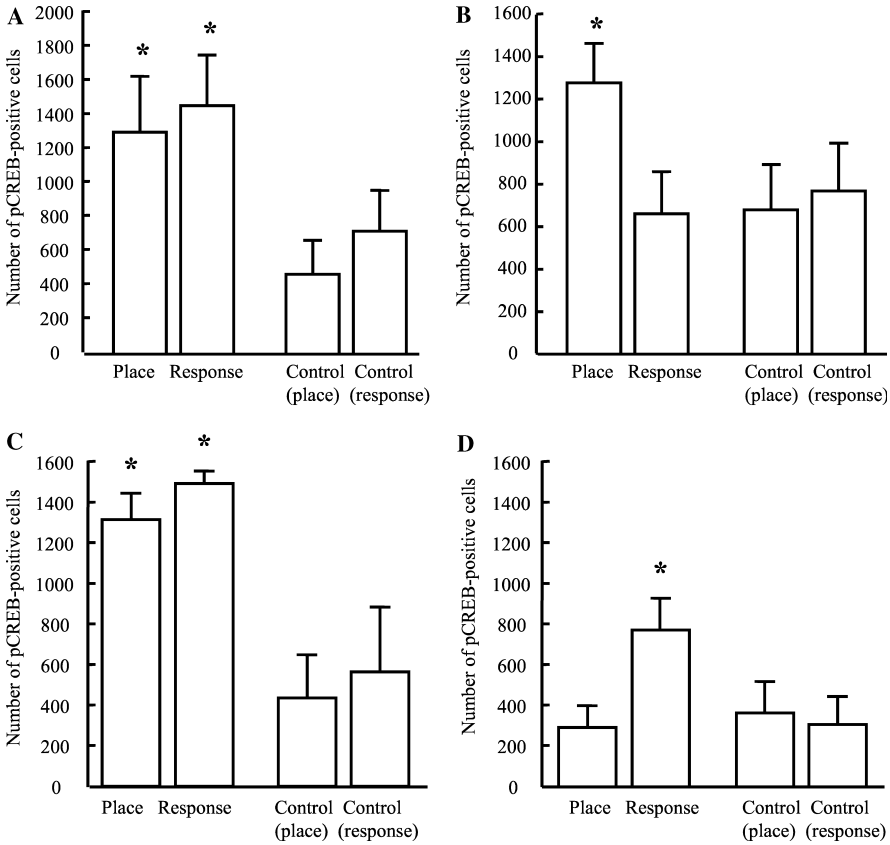

Fig. 1. Numbers of pCREB-positive cells in the hippocampus (A and B) and dorsal striatum (C and D) of place learners, response learners, and controls matched to the numbers and durations of trials of place and response learners either immediately (A and C) or 1 h (B and D) after completion of training. The initial increase in pCREB in the hippocampus (A) and dorsal neostriatum (B) in comparisons with controls was independent of the strategy used. In contrast, sustained pCREB was detected in the hippocampus among the place learners only (B) and in the neostriatum among the response learners only (D).

neostriatum-dependent response strategy. The strategy used by rats to solve the task was determined during a single probe trial. In pilot experiments, lighting and cues were manipulated until approximately equal numbers of rats used place and response strategies after reaching a criterion of 9 out of 10 correct. This was important because reports indicate that the predominant strategy used to solve the task shifts from a place strategy to a response strategy with increasing numbers of trials (Chang & Gold, 2003; Packard, 1999).

As indicated in Fig. 1, an increase in phosphorylated CREB was measured in both the hippocampus and neostriatum immediately after training among place- and response-learners in comparisons with activity-matched controls. That finding indicates that the initial phosphorylation of CREB was learning-induced but not specific to the brain region associated with memory for either strategy. Of particular importance was the finding that when measured an hour after training, place learners, but not response learners, had sustained elevation of pCREB in the hippocampus. In contrast, response learners, but not place learners, had sustained elevation of pCREB in the neostriatum. Thus there was a specific pattern of pCREB in the hippocampus of place learners and in the neostriatum of response learners. Taken together, however, the results of the study by Colombo et al. show that regional learning-induced specificity of transcription factors may be reflected best by sustained, rather than initial, activation. One implication is that memory systems may be activated somewhat indiscriminately during learning and thus evidence for induction of the cellular processes associated with plasticity may be widespread. Mechanisms that terminate learning-induced plasticity, however, such as the activity of phosphatases, may prevent sustained activity in regions that do not contribute to memory formation. In this case, the cellular 'off' switch may prove to be a more important determinant of the contribution of a system to memory than the 'on' switch. A recent report that extinction training causes increased levels of calcineurin within 20 min is consistent with that proposition (Lin, Yeh, Lu, & Gean, 2003). A second implication of the results by Colombo et al. is that the lack of either positive or negative correlations between the learning strategy employed and regional levels of pCREB or c-Fos are consistent with the hypothesis that the hippocampus and neostriatum operate in parallel, but do not interact, during memory formation.

The studies cited above were conducted with tasks that were learned during a single session. Some forms of learning, such as neostriatum-dependent skill or habit learning, may require many repetitions for acquisition. Thus the study of learning-induced changes in transcription using tasks that are learned over many repetitions offers the advantage that they generalize to those forms of learning. One disadvantage, however, is the broad span of time over which learning-induced plasticity must be examined. In one study, Bertaina-Anglade, Tramu, and Destrade (2000) measured c-Fos 60, 120, or 180 min after the first, second, or fifth daily training session on an appetitive operant conditioning task using BALB/c mice. In comparisons with sham-trained mice, the trained mice had increased c-Fos in CA3, anterior cingulate, occipital cortex, and parietal cortex 60 min, but not 120 or 180 min, after training on day 1. On day 2, c-Fos was increased in CA1, CA3, dentate gyrus, entorhinal cortex, posterior cingulate, and striatum 60 min after training; no differences were detected among groups 120 or 180 min after training. On day 5, no differences were detected between trained- and sham-trained mice. One important conclusion that can be drawn from that study is that repetitive training induced c-*fos* expression at about the same time in subcortical and cortical brain regions implicated in memory formation. It has been suggested that multiple memory systems may have a sequential role in formation of memory and that dependence on subcortical systems including the hippocampus and amygdala may precede that of neocortex (see, for example Izquierdo & Medina, 1997). The results of studies reviewed presently, however, indicate that learning-induced activation or expression of transcription factors is generally initiated at about the same time in several regions implicated in memory, including subcortical and cortical regions (see Table 1). For example, when studied in a range from 0 to 6 h after contextual fear training, the earliest increase in pCREB was 3 h after training, and that was observed in the hippocampus, amygdala, and neocortex (Stanciu et al., 2001). In addition, c-Fos was increased in the hippocampus, striatum and neocortex after the third day of training in the radial arm maze, but only in neocortex after day 1 (He et al., 2002). Taken together, these data are consistent with the hypothesis that memory formation occurs in parallel among multiple systems. Thus, the sequential dependence of memory systems inferred from disruption of one or more systems may reflect region-specific differences in the duration of memory consolidation rather than regional differences in the times during which learning-induced neuronal plasticity is initiated (see Attalah, Frank, & O'Reilly, 2004 in this issue).

8. Learning-induced changes in transcriptional regulation?

The studies of learning-induced changes in transcription factors reviewed presently fall within a general experimental design in which the independent variable is a manipulation of behavior and the dependent variable is a somatic measure. This is in contrast to studies in which the independent variable is a lesion or other somatic manipulation and the dependent variable is

behavior. In cases in which the independent variable is behavior, proper controls are critical for isolating learning-induced changes from those produced by unconditioned stimuli or factors such as activity that may be unrelated to learning. As an example of careful behavioral control, Stanciu et al. (2001) used context-dependent fear conditioning to test the time-courses of learning-induced CREB phosphorylation in multiple brain regions. In that study, trained mice received contextual exposure followed by shock whereas control mice were either naïve, exposed to the context alone, or exposed to an immediate shock in the context. In the hippocampus, training caused an early peak at 7 min and later peaks at 3 and 6 h. The early peak was observed also among mice that received immediate shock in the context—a condition that does not produce contextually conditioned fear—whereas the later peak was higher in CA1 of trained mice than that of mice in any of the control conditions. In this case, the two peaks of CREB phosphorylation were each related to a different component of training; the first peak was related to exposure to the shock, but not the context, whereas the later peak was greatest among mice that formed an association between the shock and the context. A similar relationship between early and late pCREB and the training and control conditions was observed in the parietal cortex and in the amygdala. Taken together, these results illustrate that careful evaluation of the precise experimental conditions that activate transcription factors, as well as the time courses of these effects, will be necessary to progress from reports of activity- and experience-dependent changes in transcriptional regulation to strong tests of the multiple memory systems hypothesis.

9. Conclusions

Theories of multiple memory systems have contributed significantly to our understanding of how information is processed and stored in the brain and convergent lines of evidence from different measures of memory formation will be necessary for further progress. Studies of learning-induced activation of transcription factors provide several lines of evidence that can be compared to results using other techniques to extend our understanding of multiple memory systems. Three conclusions can be drawn from the studies reviewed presently. First, training rats on tasks that are sensitive to lesions of a particular brain region is associated with learning-induced activation of transcription factors in the same regions. This general conclusion, however, leads to the following question: does learning-induced activation show brain region-selectivity that corresponds to that shown by dissociation studies? The studies reviewed presently indicate that in some cases there is regional specificity and in some cases there is not. Resolution of this question will require further study in which careful consideration is given to the cognitive task demands and the degree to which those demands are consistent with the processing styles of brain regions in question. Double and triple dissociations have been demonstrated because the tasks used tend to be solved best by processing in one neural system or another, but not by combinations of systems. The win-shift, win-stay, and conditioned cue preference tasks are relatively ''pure'' tasks for assessment of the hippocampus, dorsal striatum, and amygdala, respectively (McDonald & White, 1993). Thus, an experiment employing either these tasks or others with similar information processing requirements is a good candidate for investigating region-specific learning-induced changes among transcription factors.

While there is evidence that interactions occur among memory systems, the conditions under which systems may cooperate or compete are mostly unknown. A second contribution of the studies reviewed presently is that there appears to be no evidence for competition among systems during memory formation. In specific, there is no evidence that learning-induced activation or expression of transcription factors in one brain region is associated with suppression in another region. Evidence of learning-induced activation of transcription factors is consistent with the proposal by White and McDonald (2002) that competition among systems arises during guidance of behavioral output.

Researchers have proposed that multiple memory systems may operate in temporal sequence (Chang & Gold, 2003; Izquierdo & Medina, 1997; Packard, 1999). Studies of learning-induced activation and expression of transcription factors, however, tend to show that these events occur at about the same time among different brain regions and include subcortical and cortical areas, both. Thus, a third conclusion that can be drawn from the present review is that sequential involvement of brain systems in memory may be due to regional differences in consolidation, rather than initiation, of memory formation. Further study of both the time-courses of learning-induced activation and the mechanisms that regulate the duration of activity will be important in resolution of questions of regional specificity. In summary, studies of learning-induced activation of transcription factors, in conjunction with other experimental approaches, can provide complementary lines of evidence to further our understanding of the extent to which multiple memory systems are independent or interactive.

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