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Weaving the Molecular and Cognitive Strands of Memory

Minireview

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Summary

Several recent studies seamlessly blend cognitive, systems, and molecular neuroscience to unravel the temporal organization of memory.

The study of memory has become truly multidisciplinary in recent years, integrating a considerable array of technologies and approaches into a science aimed at understanding how the brain comes to acquire, transform, and store information. Although molecular, systems, and cognitive neuroscience will continue to prosper on their own, there is a growing sense that integration of these three fields will be particularly prolific in the study of memory. Recently, powerful tools emerging from molecular genetics, electrophysiology, and brain imaging have offered novel perspectives to the study of memory formation, and their use has led to an unprecedented ability to both manipulate and observe brain phenomena across levels of biological complexity. In this review, we will discuss several recent examples that highlight this exciting convergence between molecular, systems, and cognitive neuroscience.

Time, Molecules, Synapses, and Memory

Time is a key component of the framework used to organize the dizzying array of molecular, cellular, and systems processes involved in forming the different phases of memory. A great deal of work has indicated that early memory stages involve synaptic processes, but that the stability and permanence of these early changes require the specific transcription and translation of certain genes, whose products are thought to stabilize the synaptic changes triggered during learning. For example, early pharmacological work demonstrated that inhibition of protein synthesis (or RNA transcription) up to 2 hr after training disrupts long-term memory ("days") without affecting short-term memory ("seconds to hours") (Davis and Squire, 1984). Similarly, inhibitors of protein synthesis or RNA transcription are also known to block a late-phase, but not an early phase, of long-term potentiation (LTP), an experimental model of synaptic changes thought to underlie learning and memory.

A likely mediator of transcription processes required for memory is CREB (cAMP response element binding protein). A variety of manipulations of this transcription factor show that it is required for long-term, but not short-term, plasticity and memory (reviewed in Silva et al., 1998). More recent experiments have also demonstrated the role of the transcription factors Zif 268 (Jones et al., 2001) and C/EBPβ (Taubenfeld et al., 2001) in long-term memory. Both of these transcription factors are in part regulated by CREB, and they may be components of a transcriptional cascade that controls the formation and stability of long-term memory. C/EBP, for example, was shown to be required for the initial consolidation of new memories in mammals (Taubenfeld et al., 2001), and for the late phase of a protein-synthesis dependent synaptic facilitation in *Aplysia*.

The simpler nervous system and larger neurons of *Aplysia* have facilitated cellular studies of mechanisms underlying learning and memory. Pioneering electrophysiological studies in neurons mediating the siphon withdrawal reflex, have identified multiple synaptic facilitation phases that track closely similar behavioral phases of this defensive reflex. In addition to short-term and long-term phases of memory, there has also been considerable evidence for intermediate phases of memory in many species studied. Recent studies in *Aplysia* have identified an intermediate memory phase with properties similar to an intermediate phase of synaptic facilitation: both are translation-, but not transcription-, dependent and require persistent activation of protein kinase A (PKA) (Sutton et al., 2001). Similarly in mice, long-term memory may be sensitive to disruption by inhibitors of either protein synthesis or the PKA pathway at two distinct phases: immediately after or 4 hr after weak training, suggesting an intermediate phase of memory (Bourtchouladze et al., 1998).

The compelling parallels between the molecular biology of synaptic plasticity and memory also include the earliest stages of these two processes. Many molecules required for the initial stages of memory formation, such as neurotransmitter receptors, kinases, and phosphatases, are also essential for the early phases of both synaptic facilitation in *Aplysia* and potentiation in mammals.

The findings summarized above demonstrate that time is a useful criterion by which to organize the daunting array of molecular processes underlying both synaptic plasticity and memory. More importantly, the shared molecular requirements between synaptic plasticity and memory substantiate the nearly axiomatic idea that changes in synaptic strength underlie learning and memory.

Time, Brain Systems, and Memory

Cognitive studies have also used time in their efforts to classify and organize memory phenomena. A large number of studies have demonstrated that memory involves multiple temporally distinct processes, including acquisition, consolidation, retention, and retrieval, whereby memories are formed, stored, maintained and recalled, respectively. Many of these processes appear to be mediated by the interplay of several brain systems. For example, patients with damage to the hippocampal formation suffer a severe amnesia for declarative (i.e., semantic and episodic—memory for facts and events, respectively) memories that are a few years old at the time of damage (recent memory), but not for memories that are many years old (remote memory), a phenomenon known as temporally graded retrograde amnesia

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(reviewed in Squire et al., 2001). Similar findings have also been made in rodents. Contextual fear conditioning, a form of learning in which animals learn to fear the environmental context in which they receive footshocks, is severely disrupted by hippocampal lesions made 1 day, but not several weeks, after training (reviewed in Anagnostaras et al., 2001). Collectively, these results suggest that initially, contextual memories are acquired and temporarily stored in the hippocampus, but that gradually these memories become independent of the hippocampus, as other systems come to store the memory permanently. Although difficult to distinguish experimentally, another theory that also explains these data suggests that memory may always be stored in the cortex, but requires the participation of the hippocampus for memory consolidation or retrieval while the memory is still new. Yet another theory suggests that memories are stored in both locations, but that the quality of the memories is different (reviewed in Nadel and Bohbot, 2001). According to this theory, hippocampus-dependent memories form the basis of recent and some remote memories, whereas memories in the cortex, frequently somewhat degraded, form the basis of remote and permanent memories. Although these theories posit different locations for memory storage, they all share the common indisputable timeline that recent memories are dependent on hippocampal function, whereas certain remote memories are independent of hippocampal function.

In agreement with this timeline, a study examining patients with cortical brain damage found that, unlike hippocampal lesions, cortical lesions impair remote autobiographical (Graham and Hodges, 1997) and semantic memories (Hodges and Graham, 1998) more severely than recent memory. This *reverse* temporal memory gradient is consistent with the theory that memory is permanently stored in the cortex and that the hippocampus is only required for its initial processing.

One study examining maze learning in mice also found evidence suggesting that memory is initially stored in the hippocampus and later stored in the cortex (Bontempi et al., 1999). Mice learned to navigate a maze and, following a recall test 1 day or several weeks after training, metabolic activity was measured in the hippocampus and several cortical areas. This study showed that recent memory triggered primarily hippocampal activation, while remote memory activated mostly cortical sites. Just as different molecular mechanisms are required for memory at specific times after acquisition, different brain structures seem to be essential for memory at different times after learning. But how are these two sets of mechanisms integrated in the brain?

Bridging Molecular and Cognitive Neuroscience

Until recently, molecular and cognitive studies of memory progressed in parallel with only occasional interaction and cross-reference. However, new advances have eroded traditional barriers between molecular and cognitive neuroscience and have triggered a wave of studies that tapped into ideas and approaches from both fields. For example, a recent study using molecular genetic techniques provided further evidence for the idea that the hippocampus is only temporarily involved in memory and that remote, permanent memory is dependent on neocortical sites (Frankland et al., 2001). In this study, memory and synaptic plasticity were examined in mice *heterozygous* for the null mutation of the α-calcium/calmodulin kinase II (α-CaMKII), a kinase critical for the induction of LTP and for memory acquisition (reviewed in Lisman and Morris, 2001). Mice homozygous for a null mutation of this kinase show severe deficits in hippocampal and cortical LTP and in the acquisition of several forms of learning and memory (Silva et al., 1992). In contrast, the heterozygous α-CaMKII mutants also show deficient cortical LTP, but have intact hippocampal LTP. The heterozygous α-CaMKII mutants showed spared spatial and contextual memory for up to 3 days after training, but exhibited dramatic forgetting over longer retention intervals (10–50 days). Remarkably, this time course corresponds to that predicted by the neuroimaging (Bontempi et al., 1999) and lesions studies described above (Anagnostaras et al., 1999, 2001). Thus, these findings suggest that during training α-CaMKII is activated in multiple brain systems including the hippocampus for initial memory and the neocortex for remote, permanent memories. Losing half of the levels of this kinase appears to specially affect cortical networks. This cortical deficit does not affect memory for the first few days, since the mutants have intact hippocampal plasticity. Once the hippocampus' ability to support memory fades and memory becomes more dependent on cortical networks, the amnesia of these mutants becomes pronounced.

A complementary pattern of findings was observed with mice carrying hippocampal (CA1) inducible and reversible genetic lesion of the NMDA receptor, a glutamate-gated, and depolarization-dependent calcium channel critical for the induction of LTP (Shimizu et al., 2000). This study showed that disrupting the NMDA receptor gene in CA1 anytime within a week of training disrupted both spatial and contextual memory. In contrast, later disruptions of the NMDA receptor gene in CA1 did not affect these two forms of memory. This result is consistent with the idea that recent, but not remote, memory requires hippocampal function. The striking convergence of data between human and animal studies, cognitive and molecular approaches indicates that temporary storage of memory is primarily dependent on the hippocampus and that remote, permanent memory storage is predominantly dependent on cortical networks (Squire et al., 2001).

Bridging Molecular and Systems Neuroscience: Time Again

Initial efforts to bridge molecular and cognitive neuroscience quickly revealed the need for information concerning the network processes mediated by molecular and cellular mechanisms. Many of the studies to date suggested that changes in synaptic efficacy have a key role in memory formation but revealed very little about how synaptic plasticity is used by neural networks to process and store information. Recent genetic and pharmacological studies have indicated that synaptic plasticity has a key role in the stability of network representations of information. For example, hippocampal neurons fire preferentially in specific areas of an animal's environment called place fields. These fields reflect the ability of hippocampal networks to represent spatial information. A growing number of pharmacological and genetic studies have shown that molecular components engaged in either the induction or maintenance of hippo-

campal LTP are also critical for the stability of these place fields. For example, αCaMKII, PKA, and CREB mutations were previously shown to disrupt LTP and spatial learning in mice. These same mutations have also been shown to disrupt the stability of place fields (Cho et al., 1998; Rotenberg et al., 2000). Similar results were also obtained with NMDA receptors antagonists in rats: blocking NMDA receptors resulted in disruption of hippocampal LTP, deficits in place cell stability and spatial learning impairments (Kentros et al., 1998). Interestingly, none of the genetic or pharmacological manipulations that blocked LTP disrupted the formation of place fields. They only disrupted their stability. Altogether, these findings lay down the foundation for the study of the molecular and cellular basis of place fields, and they strengthen the link between hippocampal molecular mechanisms of plasticity and spatial learning. It is very possible that the mechanisms underlying the formation and stability of place fields will also be involved in other kinds of network representations in the hippocampus and elsewhere in the brain.

Novel Approaches to Memory

This last decade has seen an explosion of molecular technological advances that have opened the doors to innovative and sophisticated research in the field of learning and memory. Previously, most molecular studies were focused on defining the processes involved in acquisition and initial memory consolidation (up to 3 hr). Taking heed of systems-level studies, molecular neuroscientists are now venturing into defining the circuit events and memory systems required for the consolidation and retrieval of different types of memory. Similarly, systems and cognitive neuroscientists are taking advantage of newly developed molecular tools to test complex ideas. For example, the ability to delete genes involved in synaptic plasticity in each of the hippocampal subregions is allowing systems and cognitive neuroscientists to test for the first time key computational ideas about information processing in these subregions. Moreover, the emerging application of functional genomics in neuroscience, for example utilizing DNA microarrays and large-scale phenotypic screening studies of mutants, furthers the prospects of shining light on the plethora of molecular mechanisms subserving memory. In this respect, the study of model organisms is especially enlightening. In *Drosophila*, for example, molecular and genetic tools are being used to dissect the function of synaptic transmission in different regions of mushroom bodies, the neuroanatomical sites of associative olfactory learning. These capabilities place studies of *Drosophila* in an excellent position to fuse genetics and memory. Most importantly, we are educating a new generation of neuroscientists that effortlessly use ideas and tools from these three previously separated disciplines. Thus, a growing number of studies, which seamlessly blend cognitive, systems, and molecular neuroscience, are laying down the outlines of a rich and exciting tapestry that will eventually depict how memories are formed.

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