CHAPTER 4

Gene Expression in Learning and Memory

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I. INTRODUCTION

Memories can last a long time, even a lifetime. Science has shown that memories reside in the brain. Since the principal cell of the brain is the neuron, it follows that memories exist in neurons. A memory could be in one neuron that recognizes a particular face (Rolls, 1992), or it could be in a distributed network of neurons (Martinez and Derrick, 1996). Regardless, a memory is a physical entity. As we've said before, "memory is a thing in a place in a brain (Martinez and Derrick, 1996, p. 173)," although the place may vary with time (Kim et al., 1995).

Neurons are eukaryotic cells and all share many properties. Each neuron contains the entire DNA library for a person. However, only a portion of the genes coded in the neuron is active at anytime. Hence we have the environment acting on the genome. In the case of learning, the environment acts in a way that is described as "experience-dependent plasticity." For the most part, cells make permanent changes by expressing genes and translating proteins that make the change. One common example of permanent change is the transformation of children to adolescents, directed by hormone-induced gene expression (Rossmanith et al., 1994). A similar process occurs in your brain when you create a memory (Martinez and Derrick, 1996). Neurons change the shape of their synaptic connections to each other and even create new connections. Interestingly, there is no *a priori* reason to expect the brain to engage is this metabolically costly process. Altering the strength of existing connections could form memories, and, indeed, some nonpermanent memories may be formed in this way (Huang and Kandel, 1994). It appears that the brain seldom engages in the simplest processes to achieve its ends in spite of Occam's razor.

The knowledge that the brain engages in gene expression to form memories is recent. "Genes" were hypothetical constructs as late as 1957, when the structure of DNA was deduced (Watson and Crick, 1953). Soon thereafter the genetic code was discovered (Nirenberg, 2004), and the sequencing of the human, mouse, and *c. elegans* genomes propelled us forward at a tremendous rate. Other discoveries — such as the polymerase chain reaction (PCR), which allows the amplification of RNA, gene knock-in and knockout animals, where genes are added or deleted, and recent RNA silencing techniques — allow the manipulation of genes involved in memory. The development of gene array technology allows the assessment of the state of an entire genome on a few chips. All of these technologies have converged to provide us with a new, though far from complete, understanding of how genes function to form memories. What we know is the subject of this chapter.

Historically there has been much interest in RNA and memory. Early researchers thought a memory could be encoded in an RNA sequence and that the resulting peptide would encode the memory. Thus *scotophobin* a peptide that encodes fear of the dark, was isolated by Ungar (1970). The infamous "worm runners" thought memories encoded in RNA sequences could be transferred from animal to animal (McConnell, 1966). All of this early work kept scientists thinking about RNA and DNA and memory. It turns out there is no "memory molecule," just as there are no unique "memory genes." Memories are represented in cells as long-term changes in the function of proteins translated from mRNA transcribed from genes. If there are 30,000 genes in a mouse genome, then how many of these genes could be involved in memory formation and maintenance? The answer is more than one and less that 30,000. The studies reviewed in this chapter suggest that the number is uncomfortably high, suggesting many parallel processes, but not so high that they cannot be studied.

A memory takes time to create, because it involves gene expression. Hence a synapse that is activated and that becomes part of a memory network has to send a message to the nucleus that its state is now changed, and the nucleus has to send a message back, acknowledging and maintaining that memory state. Thus, there is a time-dependent cascade of events inside a cell that represents the memory. As you might imagine, some of the gene changes have to do with excitability, which is a salient property of neurons; some have to do with transcription to recruit new genes products into the process; some have to do with responding to the bodies reaction to the learning situation in the form of hormones; some are involved in altering the structure of neurons, especially at the synapse where neurons communicate. We do not yet have a clear description of a memory in terms of the cascade of cellular function. However, such an understanding is not too far in the distant future.

II. GENE EXPRESSION AND LEARNING AND MEMORY

A. The Hippocampus and Learning and Memory

1. Types of Learning and Memory

Memory is not a unitary construct. Rather, the memories one has are as diverse as the experiences that produce them. According to Squire and Zola-Morgan, memory is comprised of two broad categories, declarative memory and procedural memory. *Declarative memory* encompasses one's explicit recollection for facts and events and working memory processes. *Procedural memory* includes one's implicit knowledge for skills, priming, and simple classical conditioning (Squire and Zola-Morgan, 1991). Research has shown the anatomical substrates that underlie declarative and procedural memory to be mutually exclusive. For example, the hippocampus is critically required for declarative memory but not for procedural memory, and other cortical and subcortical structures mediate procedural memory and not declarative memory (Eichenbaum and Cohen, 2001).

In humans, memory is relatively easy to examine because we are capable of verbalizing what we know. Because nonhuman animals are nonverbal beings, however, scientists developed innovative strategies by which declarative knowledge could be assessed. Spatial tests of learning and memory are excellent ways in which declarative memory can be assessed in nonhuman animals, because they can behaviorally demonstrate to the experimenter their knowledge of previous experiences. In the Morris water maze (Morris, 1984), for example, animals rely on extramaze environmental cues to remember where the hidden platform is located, and performance is based on measures such as the latency to reach the platform and the percent of time spent in the quadrant containing the platform.

Classical conditioning is sensitive to hippocampal disruption, but only when there is a trace in time between the conditioned stimulus (CS) offset and the unconditioned stimulus (US) onset. It is believed that to learn the US-CS association, the hippocampus must temporarily "hold" the US representation in a working memory store (Clark and Squire, 1998). Damage to the hippocampus disrupts contextual fear conditioning in rodents (McEchron et al., 1998; Rogers et al., 2006) and humans (McGlinchy-Berroth et al., 1997). Evidence to demonstrate the hippocampus' role in trace conditioning comes from studies using the neurotoxin methylazoxymethanol-acetate (MAM) to inhibit neurogenesis. The data show that compared to saline-treated controls, MAM-treated animals exhibit impairments in trace eye-blink conditioning, suggesting that basal levels of hippocampal neurogenesis are required for the normal acquisition of this task (Shors et al., 2002).

B. Learning and Memory Genes

1. Using Invertebrate Model Systems to Examine the Neurobiology of Learning and Memory

A great deal of what we know today about the molecular bases of mammalian learning and memory is based on experiments on the sea snail, Aplysia (see Kandel, 2001, for review). Although the Aplysia is an invertebrate organism with a relatively simple neural network, it is an ideal model system to examine the neurobiology of mammalian learning and memory. The small quantity of very large neurons, many of which are visible to the naked eye and morphologically distinguishable, make it relatively easy to identify the precise neurons involved in learning and memory-related processes. More importantly, the Aplysia demonstrates implicit learning and memory that is similar to mammalian implicit learning and memory. In the Aplysia, implicit learning and memory is measured behaviorally in the form of habituation and sensitization of the gill-withdrawal response. In habituation, the repeated administration of an electrical stimulus results in a progressive attenuation in its gill withdrawal, until eventually the animal fails to respond to the stimulus. During sensitization, a weak stimulus is applied to the siphon, and precedes a noxious stimulus to the tail. The temporal pairing of the weak and aversive stimuli results in an augmented gill-withdrawal response when subsequent mild stimulation to the siphon is administered. These manipulations led to the identification of genes involved in both invertebrate and vertebrate memory mechanisms.

2. The Cyclic-AMP Response Element Binding Protein (CREB)

The first protein identified as being associated with the neurobiology of learning and memory was cyclic AMP (cAMP). In the *Aplysia*, cAMP facilitates synaptic transmission between neurons involved in the gill-withdrawal response (Brunelli et al., 1976), and today cAMP-mediated gene expression is known to play a role in a variety of physiological processes (Montminy and Bilezikjian, 1987; Hoeffler et al., 1988; Sassone-Corsi et al., 1988; Montminy et al., 1990),



FIGURE 4-1 CREB activity mediates the expression of genes involved in a variety of physiological processes. [From Samuel Feldman (www.cns.nyu.edu/~sam/old/1030_Lect17.ppt).]

including learning and memory (Dash et al., 1990). cAMP affects memory processes by initiating a signaling cascade that leads to the phosphorylation of cyclic AMP responsive element binding protein (CREB) (Dash et al., 1990). CREB is a nuclear transcription factor that mediates the expression of genes involved in a number of physiological processes (see Fig. 4-1) in organisms such as amphibians (Lutz et al., 1999), fish (Yoshida and Mishina, 2005), and mammals (Montminy and Bilezikjian, 1987). In humans, CREB is estimated to mediate the expression of over 4,000 genes, most of which are involved in transcription processes (Zhang et al., 2005), by binding the cyclic AMP response element (CRE).

CREB-mediated gene transcription occurs via a number of signaling pathways, each of which is initiated at the cell surface (Fig. 4-2). Briefly, neurotransmitter, neuromodulator, and growth factor ligands bind to receptors that, when activated, lead to the expression of protein kinases such as protein kinase C (PKC), protein kinase A (PKA), mitogen-activated protein kinase (MAPK), and calcium/calmodulin-dependent protein kinase II (CamKII) (Kim, Lu, and Quinn, 2000; Josslyn and Nguyen, 2005). Protein kinases phosphorylate CREB at several residue sites (Giebler et al., 2000; Fimia et al., 1998). However, phosphorylation at the serine 133 residue (ser133) site is



FIGURE 4-2 CREB-mediated gene expression occurs via several protein-kinase pathways. GPCR: G-protein coupled receptors; VSCC: voltage-sensitive calcium channels; RTK: receptor tyrosine kinase (binds growth factors). [From: Samuel Feldman (www.cns.nyu.edu/~sam/ old/1030_Lect17.ppt).]

necessary for CREB-activated gene transcription to occur (Gonzales and Montminy, 1989; Shaywitz and Greenberg, 1999).

In the vertebrate, learning and memory enhances CREB expression in a region-specific manner. Animals trained on tasks dependent on the hippocampus, such as passive avoidance and contextual fear conditioning, exhibit significant increases in hippocampal CREB expression (Impey et al., 1998). When treated with the *N*-methyl-D-aspartate (NMDA) antagonist, APV, animals fail to exhibit long-term memory for conditioned fear (Athos et al., 2002) and they show no increase in CREB expression in the hippocampus when compared to vehicle-treated rats (S.M. Rodriguez et al., 2004). In contrast to contextual fear conditioning, auditory cue fear conditioning is not dependent on the hippocampus (Kim and Fanselow, 1992). Rather, learning this memory requires an intact amygdala (Maren et al., 1994), and following auditory cue fear conditioning CREB expression is upregulated in the amygdala and not the hippocampus (Impey et al., 1998).

The importance of CREB in learning and memory processes is strengthened further by the observation that disruption of hippocampal CREB impairs an animal's performance on spatial tasks such as the Morris water maze and contextual fear conditioning (Pittenger et al., 2002; Bourtchuladze et al., 1994). Animals with mutations to the CREB protein exhibit learning and memory deficits. The degree to which CREB mutations affect learning and memory is dependent on how extensive the CREB mutations are. When compared to control animals, animals with mutations to the CREB α and Δ allele (CREB_{$\alpha\Delta$}) perform less well on the Morris water maze. However, when compared to animals in which all CREB alleles are mutated (CREB_{comp}), $CREB_{<math>\alpha\Delta$} perform relatively well on the Morris water maze because they take significantly less time than CREB_{comp} animals to locate the submerged platform (Gass et al., 1998). Further support for this observation includes experiments using antisense oligonucleotides (ODN) that inhibit the expression of most of CREB's known isoforms. Animals that receive CREB ODN have greater escape latencies and longer swim paths than animals treated with scrambled ODN (Guzowski and McGaugh, 1997).</sub>

CREB is also involved in memories that are dependent on other structures besides the hippocampus. For example, in the amygdala-dependent conditioned taste aversion (CTA) task, animals are presented with either a novel taste that produces illness or a novel taste that does not produce illness. In normal animals, a novel taste that produces illness will be avoided when presented on subsequent trials. In contrast, animals with damage to the amygdala continue to sample the aversive taste despite its negative effect on the animal. To investigate whether CTA is mediated by CREB activity, animals with CREB deletions were presented with either flavored water (no malaise) or flavored water with added lithium chloride (LiCl) (malaise). Twenty-four hours later, presenting animals with both flavored waters assessed memory. Results revealed that CREB-mutant mice consumed significantly more of the LiCl-flavored water when compared to wild-type controls, suggesting that their memory for the aversion to the water was significantly impaired (Josselyn et al., 2004). Interestingly, the learning and memory impairments exhibited by CREBmutant animals are only evident when animals are tested at least 24 hours after training, suggesting that CREB is required for long-term, but not short-term, memory (Kaang et al., 1993; Silva and Jasselyn, 2002).

3. Neuronal Growth-Associated Proteins (nGAPs)

Neuronal growth-associated proteins (nGAPs) are a family of gene products involved in the growth and regeneration of the nervous system and include but are not limited to GAP-43 and SCG10. nGAPs are expressed in the brain throughout life; however, during organism development their expression is most robust (Higo et al., 1999). Recent work has shown that in adulthood nGAP expression may be mediated by experience and is involved in synaptic changes that underlie learning and memory processes. GAP-43, which is involved with neurite outgrowth (Karns et al., 1987) and signal transduction (Akers and Routtenberg, 1985), is altered following tasks such as the Morris water maze (Pascale et al., 2004) and contextual fear conditioning (Young et al., 2000) as well as during drug abuse (Park et al., 2002; Vukosavic et al., 2001) and aging (Sugaya et al., 1998). Routtenberg and colleagues recently examined the extent to which GAP-43 is involved in cognitive processes. By using a transgenic mouse line that overexpressed GAP-43, they found that compared to controls, the GAP-43-enhanced animals performed significantly better on delayed-matching-to-sample tasks and delayed-nonmatching-tosample tasks (Routtenberg et al., 2000). The role of GAP-43 in hippocampaldependent memory processes is also illustrated in studies using GAP-43 knockout mice. Rekart and colleagues (2005) trained heterozygous GAP-43 knockout animals (GAP⁺/⁻) on a contextual fear conditioning task in which a tone was paired with a foot shock. Twenty-four hours later animals received a single test to assess memory retention for the context in which they received the foot shock. When compared to wild-type animals, GAP⁺/⁻ animals exhibited significantly less time freezing, thus indicating substantial memory impairment. As a control, both wild-type and GAP+/- animals were trained and tested for cue fear conditioning, which is not dependent on the hippocampus. Using this strategy, no behavioral differences were observed between the two groups, suggesting that GAP-43 may be involved specifically in hippocampaldependent learning and memory.

nGAPs are also involved in learning and memory at the cellular level. GAP-enhanced animals exhibit more robust LTP when compared to controls (Routtenberg et al., 2000); and in both normal and GAP-enhanced animals, LTP induction results in a significant reduction in hippocampal GAP-43 expression (Meberg et al., 1993; Routtenberg et al., 2000). The down-regulation of GAP-43 following LTP induction is presumed to reflect the synaptic stabilization of connections formed during LTP and, in this view, long-term memory maintenance (Meberg et al., 1993).

The nGAP SCG10 (superior cervical ganglia clone 10) is expressed abundantly in the developing brain. The localization of SCG10 to the growth cones and axons of neurons during development suggests that it is involved in synaptogenesis (Stein et al., 1998). Although its expression is significantly lower in the brains of adult animals (Stein et al., 1998), SCG10 is transiently expressed during adulthood in an experience-dependent fashion. Peng et al. (2003) found that LTP induction at the Schaeffer collateral–CA1 pathway in rats resulted in regional changes in SCG10 expression in the hippocampus. Specifically, SCG10 expression was most robust in CA3 when compared to either CA1 or the dentate gyrus (Peng et al., 2003). The LTP-mediated expression of SCG10 in the hippocampus occurs rapidly and is short-lived. Within three hours of LTP induction, SCG10 expression is nearly doubled, and by 24 hours it is back to basal levels (Peng et al., 2004). Like GAP-43, the down-regulation of SCG10 in the hippocampus one day following LTP may reflect its role in synaptic stabilization, and therefore memory.

4. Immediate Early Genes

Immediate early genes (IEGs) are named for their rapid response to cell stimuli. To date, as many as 40 IEGs are known to exist (Lanahan and Worley, 1998), some of which are involved in learning and memory processes. The IEGs Ar, zif/268, and *c-fos* were examined in the dorsal hippocampus either immediately following or two hours after training in a Morris water maze. While each IEG was significantly up-regulated immediately after training, by two hours they had returned to basal levels (Guzowski et al., 2001). In a more recent study, the expression of Arc mRNA in the hippocampus was performed in a region-specific manner. Specifically, Arc expression was examined in both the dorsal and ventral regions of CA1, CA3, and the dentate gyrus as well as in the dorsal and ventral regions of the subiculum. Arc mRNA was enhanced throughout the hippocampus 24 hours following Morris water maze training and that at 1 month following training all regions except CA1 continued to exhibit enhanced Arc mRNA, suggesting that Arc may contribute to both memory formation and memory maintenance (Gusev et al., 2005).

Martinez and colleagues used Affymetrix oligonucleotide microarrays and a subtractive hybridization technique to examine regulatory processes underlying spatial learning and LTP induction in the MF-CA3 pathway (Thompson et al., 2003). The results revealed significant changes in hippocampal IEG expression one hour following Morris water maze training or LTP induction. Pathway analysis (Ingenuity Systems, Inc.) was then performed to examine further the array results for MF-CA3 LTP and water maze-trained animals based on their biological functions. The pathway analysis following MF-CA3 LTP included an attractive array of genes, one of which is the IEG myc (Fig. 4-3A). In addition to its involvement in MF-CA3 LTP induction, myc is known to provoke sustained cell proliferation. Interestingly, stimulation of the granule cell mossy fibers sufficient to induce MF-CA3 LTP increases the number of newly formed granule cells in the dentate gyrus. This indicates that granule cell neurogenesis may be regulated by the induction of LTP at the MF-CA3 pathway (Derrick et al., 2000), a process that may also be associated with certain hippocampal-dependent learning tasks (Shors et al., 2001, 2002).

With regard to water maze training, pathway analysis identified important connections between genes such as *krox24* (EGR1), *Ania-3* (*Homer1* splice variant), and *jun-d*, all of which are linked to memory processes (Fig. 4-3B). *Arc*, an activity-dependent cytoskeleton-associated protein, is also significantly up-regulated following MF-CA3 LTP induction. *Arc* as well as the IEG *Homer* have also been shown to modify dendritic connections in order to strengthen synaptic connectivity (Vazdarjanova et al., 2002). *Homer1* in particular is critically involved in activity-dependent changes of synaptic function (Ammon et al., 2003). As mentioned earlier, a similar expression pattern was observed



FIGURE 4-3 (A) Path analysis (Ingenuity Systems, Inc.) following MF-CA3 LTP indicates important pathways centered around the IEG myc (enclosed in black oval). (B) Path analysis following spatial learning on a Morris water maze indicates important pathways centered around krox24, Homer-1, and jun-d (enclosed in black oval). MTPN and MT1A are also activated following spatial learning. Red and green color represents increased or decreased expression, respectively. Labels include: (B)inding, (E)xpression, (A)ctivation, (T)ranscription, (P)hosphorylation, Tall rectangle (G-protein coupled receptor), long rectangle (nuclear receptor), circle (other), downward triangle (kinase), square (growth factor), oval (transcription factor) and diamond (enzyme).

for MTPN and MT1A between the MF-CA3 LTP animals and those animals trained on the water maze. This indicates that the MTPN and MT1A-related pathways are potentially altered due to overall plasticity (which occurs in the presence of both LTP and learning), whereas the *krox24*, *Homer1*, *jun-d* pathways are directly related to spatial learning.

Pathway analyses also demonstrated a bidirectional pattern of expression between *myc* and genes such as MTPN (myotrophin), metallothionein 1A (MT1A), and BDNF. MTPN attracts integral membrane proteins to cytoskeletal elements, whereas MT1A is protein that is transcriptionally regulated by both heavy metals and glucocorticoids. Both of these genes are also an important component of the network analysis following spatial training in a water maze (Fig. 4-3), and thus the MF-CA3 LTP and spatial learning pathways are connected, as one would expect if LTP were a substrate of memory.

Drugs of abuse also increase several IEGs, including *jun-b*, *zif/268*, and a family of *fos* proteins (for review, see NIDA research monograph #125), suggesting their involvement in the neurobiology of addiction, which, as described at length later, is also a form of learning. The expression of IEGs following drug administration depends on whether drug delivery is acute or chronic. During acute drug administration, IEGs are rapidly and transiently up-regulated (Hope et al., 1992; Burchett et al., 1999; Parelkar and Wang, 2004); whereas following chronic drug administration, IEG expression is comparable to that of control animals. The pattern of IEG expression during prolonged drug administration suggests that IEGs may develop drug tolerance (Hope et al., 1992).

5. Genes Involved in Drug Addiction and Abuse

Drug addiction is a form of learning. Animals and humans display sensitization and tolerance to a drug's effect, both of which are forms of nonassociative learning. In addition, they exhibit place preference to drugs of abuse, which is a form of associative learning. Amphetamine (AMPH) self-administration affects gene expression in the nucleus accumbens (NAc), a brain region associated with reward-mediated behavior. Intracranial self-administration (ICSA) upregulates the expression of the G protein beta 1 subunit (rGB1) as well as genes associated with neuroplasticity, such as C-CAM4, k-cadherin, and vimentin (J.S. Rodriguez et al., 2002). Animals trained to lever press for methamphetamine (METH) exhibit a robust augmentation in NAc oxytocin (OXT) expression; and when pretreated with the OXT antagonist vasotosin, drug-seeking behavior is abolished (J.S. Rodriguez et al., 2002). Drug self-administration also increases CREB in a variety of mesolimbic structures, including the NAc (J.S. Rodriguez et al., 2002), and striatum (Konradi et al., 1994). In these brain regions, CREB activates the cocaine- and amphetamine-related transcript (CART), thus leading to changes in dopamine (DA) release (Kuhar

et al., 2005). People who have abused cocaine exhibit altered CART mRNA levels in the VTA (Albertson et al., 2004). Interestingly, CART gene mutations are also associated with alcohol abuse (Flatscher-Bader et al., 2005). Together these data suggest that CREB-mediated CART activation in mesolimbic structures plays a role in the neurobiology of drug addiction.

III. LTP AND GENE EXPRESSION

Let us assume that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability. (Hebb, 1949)

Gene expression has been extensively examined in the hippocampus, a brain structure that is essential for learning and memory. Long-term potentiation (LTP) refers to a persistent increase in synaptic strength that is produced by brief high-frequency stimulation at excitatory afferents. Since its discovery, LTP has been the most accepted model for studying the neural mechanisms that underlie learning and memory in the hippocampus (Bliss and Lomo, 1973). In fact, LTP and its sister process, long-term depression (LTD), are considered cellular correlates of learning and memory. Although LTP is generally associated with the activation of NMDA receptors, other receptor systems are also implicated (see Fig. 4-4). NMDA-dependent LTP is characteristic of the Schaffer collateral–CA1 pathway, the medial perforant path projection to the dentate gyrus, and the commissural–CA3 pathway (Hebb, 1949;



FIGURE 4-4 Characteristic hippocampal pathways. Small arrows indicate whether LTP at this pathway is dependent on or independent of NMDA receptor (NMDA-R) activation.

Harris et al., 1984; Harris and Cotman, 1986), and most agree that this form of LTP requires a postsynaptic increase in Ca^{2+} (Bliss and Collingridge, 1993; Collingridge and Bliss, 1995). Subsequent changes in protein kinases and other cellular cascades, in turn, modulate the increase in synaptic strength.

The lateral perforant path to dentate gyrus and the mossy fiber projection to area CA3 of the hippocampus display a form of LTP that is not as well known as the typical NMDA receptor-dependent forms (Bliss and Lomo, 1973; M.R. Martin, 1983; Harris and Cotman, 1986; Ishihara et al., 1990; Jaffe and Johnston, 1990; Bramham et al., 1991a, 1991b; Derrick et al., 1991; Breindl et al., 1994; Urban and Barrionuevo, 1996). LTP induction at the mossy fiber CA3 synapse depends on the activation of μ -opioid receptors (Derrick et al., 1992) and metabotropic glutamate receptors (MGluRs) (Thompson et al., 2005) and on repetitive mossy fiber activity (Jaffe and Johnston, 1990; Zalutsky and Nicoll, 1990; Zalutsky and Nicoll, 1992; Derrick and Martinez, 1994a, 1994b). The time course for LTP at the mossy fiber-CA3 pathway also differs from NMDA receptor-dependent LTP. Whereas NMDA-dependent LTP reaches its maximum almost immediately and then begins to decay, mossy fiber-CA3 LTP induced in vivo takes about 1 hr to reach its maximum and does not decay (Derrick et al., 1991; Derrick et al., 1992; Breindl et al., 1994; Derrick and Martinez, 1994a, 1994b). These differences are important in understanding the mechanisms underlying each form of LTP.

Similar to memory storage, LTP at most hippocampal synapses has distinct temporal phases (Nguyen et al., 1994). In the early phase of LTP, which lasts 1–3 hr, preexisting proteins are covalently modified, whereas the late phase of LTP, typically induced by repeated stimulation, is dependent on new RNA transcription and protein translation and lasts for at least several hours. Although these defined time frames vary across hippocampal synapses, altered gene expression is important across synapses and at all stages of hippocampal LTP.

As mentioned in the introduction to this chapter, recent advances in PCR and oligonucleotide microarray technology have allowed us to monitor the expression patterns of thousands of genes simultaneously following LTP (Thompson et al., 2003; Lee et al., 2005). Genes associated with LTP are temporally classified as immediate early genes (IEGs) or late-responder genes (Abraham et al., 1991; Dragunow, 1996; Peng et al., 2003). In the initial hours after LTP induction, cell surface receptors activate second messenger systems that result in IEG transcription (Walton et al., 1999). These, in turn, encode transcription factors, which, once translated, reenter the nucleus and regulate the expression of late-responder genes. Although many important IEGs and late-responder genes have been identified, further understanding of genes involved in LTP induction and maintenance may help unveil the molecular mechanisms that underlie information storage in the brain. In this section, we outline some of the genes that are implicated in hippocampal LTP.

A. Transcription Factors and Hippocampal LTP

As discussed earlier, the cAMP responsive element binding protein (CREB) is possibly one of the best-studied transcription factors implicated in hippocampal learning and memory. CREB activity is regulated by both cAMP and calcium influx (Brindle and Montminy, 1992), and it is critical for long-term memory (Josselyn and Nguyen, 2005). CREB modulates transcription of genes containing cAMP-responsive elements (CRE sites) in their promoters and is commonly activated by calcium/calmodulin-dependent protein kinase IV (CaMKIV) (Marie et al., 2005). LTP induction and maintenance display a delayed onset of CREB phosphorylation (Bito et al., 1996; Bito, 1998), whereas alphaCaMKII and MAPK2 display an enhanced phosphorylation state throughout the induction, early-, and late-LTP. Interestingly, only the late enhancement of pCREB is clearly dependent on protein synthesis (Ahmed and Frey, 2005). Another group (Balschun et al., 2003) found that conditional knockout strains with a marked reduction or complete deletion of all CREB isoforms in the hippocampus showed no deficits in lasting forms of hippocampal LTP and LTD. Thus, in the adult mouse brain, CREB deletions spare LTP and LTD in paradigms that are sensitive enough to detect deficits in other mutants. This suggests a species-specific or regionally restricted role of CREB in the brain.

The IEG *c-fos* is another transcription factor that is implicated in learning and memory. Mice that lack *c-fos* in the brain show a reduced LTP at CA3to-CA1 synapses (Gass et al., 2004). Interestingly, LTP-induced levels of *c-fos* mRNA are significantly higher in aged animals, suggesting that age-dependent hippocampal dysfunction may be associated with a selective change in the dynamic activity of signaling pathways upstream of *c-fos* (Lanahan et al., 1997). Similar rapid increases in the IEGs *c-jun* and *jun-B* RNA are associated with dentate gyrus LTP (Abraham et al., 1991), whereas *jun-D* mRNA and protein display a more delayed and persistent increase (Demmer et al., 1993).

Other studies show that induction of LTP at the perforant path to the dentate gyrus synapse results in increases in the expression of the IEGs zif268 (also termed NGFI-A, Egr-1, or Krox 24), activity-regulated cytoskeletal associated protein (Arc, also termed Arg 3.1), and Homer (Matsuo et al., 2000; French et al., 2001; Jones et al., 2001). LTP induction in the dentate gyrus also results in robust NMDA-R-dependent transcription of zif268 (Cole et al., 1989). Further work using zif286-mutant mice shows that, although the early phase of dentate gyrus LTP is normal in these mice, the later phases are not present, and the ability of the mice to maintain learned information over a 24-hr period is deficient (Bozon et al., 2002). Recent work has focused on the expression of Arc mRNA, which is delivered to dendrites and translated within minutes after tetanic (high-frequency) stimulation. Arc protein binds to actin, possibly to regulate cytoskeletal restructuring after synaptic activation (Lynch, 2004). Arc disruption using antisense oligonucleotides inhibits LTP

maintenance (Guzowski et al., 2000) but not induction. Similar increases in $A\pi$ and Homer were observed following mossy fiber-CA3 LTP induction (Thompson et al., 2005). Others have also shown colocalization of $A\pi$ and Homer 1a, indicating that they may function together to modify dendrites in order to increase synaptic efficacy (Vazdarjanova et al., 2002). However, no changes in $A\pi$, Homer, or zif268 were found following LTP at the commissural projection to CA1 pyramidal cells in vivo (French et al., 2001), indicating that the activation of these IEGs is pathway specific.

Tissue plasminogen activator (tPA) mRNA is significantly increased following mossy fiber LTP (Thompson et al., 2003). Previous work has shown tPA as a serine protease that plays an important role in tissue remodeling and LTP. tPA serves as an immediate early gene and is induced in the hippocampus during seizures, kindling, and LTP (Qian et al., 1993). tPA knockout mice show a decrease in late-phase LTP (Carmeliet et al., 1994) and show deficits in two-way avoidance tasks (Frey et al., 1996). Additionally, overexpression of tPA results in enhanced CA1 LTP and learning (Madani et al., 1999). To date, the role of tPA on hippocampal function is not clear. One possibility is that tPA converts plasminogen, which is the enzyme's main substrate and known to be found in the hippocampus, to the protease plasmin, which in turn can cleave many other extracellular substrates (laminin, for example) to result in alterations of hippocampal structure and function (Chen and Strickland, 1997). Other studies found that binding of tPA to the low-density lipoprotein (LDL) receptor-related protein (LRP) in hippocampal neurons enhances the activity of cAMP-dependent protein kinase, a key molecule in LTP (Zhuo et al., 2000). Overall, the findings suggest an important role for tPA in LTP.

Although CREB, *c-fos, zif268, Arc, Homer*, and tPA expression appear to be important for LTP, their specific role in memory storage is still unclear.

B. Protein Synthesis and LTP

At most NMDA-dependent pathways, the transition from early- to late-phase LTP requires gene expression and protein synthesis. Protein synthesis inhibitors disrupt late-phase LTP at multiple hippocampal synapses (Krug et al., 1984; Otani and Abraham, 1989; Otani et al., 1989; Barea-Rodriguez et al., 2000; Calixto et al., 2003). The expression of early-phase mossy fiber-CA3 LTP is also disrupted by the protein synthesis inhibitor anisomycin in vivo and in vitro (Barea-Rodriguez et al., 2000; Calixto et al., 2003), indicating that early-phase mossy fiber LTP is dependent on protein synthesis. This finding supports the previously described differences between NMDA-dependent and NMDA-independent LTP.

One theory is that LTP-induced protein synthesis induces the morphological changes that occur following LTP and that are essential for synaptic restructuring. For example, LTP increases spine number, spine area, as well as the distribution of synaptic vesicles (Applegate et al., 1987; Desmond and Levy, 1988, 1990; Meshul and Hopkins, 1990). These ultrastructural changes have been observed both pre- and postsynaptically following LTP (Lisman and Harris, 1993; Edwards, 1995; Lynch, 2004), indicating that gene expression (protein) changes are necessary on both sides of the activated synapse.

Many proteins have been linked to hippocampal LTP. For example, proenkephalin was found to be up-regulated following mossy fiber-CA3 LTP (Thompson et al., 2003). This corresponds with previous literature showing that enkephalin peptides released from hippocampal mossy fibers lower the threshold for induction of LTP at mossy fiber synapses (Roberts et al., 1997). Neuropeptide Y (NPY), which was also up-regulated following mossy fiber-CA3 LTP (Thompson et al., 2003), has been previously linked to inhibition of glutamate release and LTP in the dentate gyrus (Whittaker et al., 1999).

The neurotrophin brain-derived neurotrophic factor (BDNF) has been widely implicated in NMDA-R-dependent LTP (Barco et al., 2005) and, more recently, NMDA-R-independent LTP (Thompson et al., 2003). BDNF is thought to trigger long-lasting synaptic strengthening through MEK/ERK pathways (Messaoudi et al., 2002; Ying et al., 2002). Interestingly, BDNF-induced synaptic strengthening in cultured hippocampal neurons increases the expression of the IEGs *c-fos*, early growth response gene 1 (EGR1), and *Arc*, all of which increase following LTP (Alder et al., 2003). Other growth factors, such as vascular growth factor (VGF) and nerve growth factor (NGF), are altered following both NMDA-receptor-dependent and -independent LTP and learning (Sugaya et al., 1998; Alder et al., 2003; Thompson et al., 2003), suggesting a possible role of growth factors in synaptic modification.

C. Synaptic Tagging and LTP

Neural networks allow single synapses or groups of synapses to be activated simultaneously during LTP (and learning). This activation requires both transcription and translation. However, the mechanisms that underlie the movement of gene products to the activated synapse are still not known (K.C. Martin and Kosik, 2002). The synaptic-tagging hypothesis (Frey and Morris, 1997) proposes that a short-lasting LTP tags the activated synapse, allowing it to seize proteins synthesized by the nucleus (Frey and Morris, 1997). On the contrary, weak stimuli do not induce long-lasting changes (L-LTP), because they do not stimulate transcription of mRNAs that encode proteins essential for strengthening the synapse. In this model, learning or LTP-induced protein synthesis targets newly synthesized proteins to the activated synapses in order to make the change permanent (Kandel, 2001). In general, the synaptic-tagging model has been supported, yet there is still some disagreement surrounding the issue of "new" protein synthesis. As part of the controversy, one

group proposed that posttranslational modification (PTM) of proteins already located at the synapse is the crucial mechanism underlying LTP and long-term memory (Routtenberg and Rekart, 2005).

Despite the disagreement, multiple mechanisms seem to be involved in LTP induction and maintenance. As a result, multiple genes (synaptic tags) can be activated, depending on the pathway, the temporal distribution of synaptic activity, and many other factors. It appears that subfields of the hippocampus display different transcriptional responses that may contribute to their regionally specific involvement in learning and memory. Although many LTP-related genes have been identified using microarray analysis and cDNA subtractive hybridization, these technologies are still emerging. One of the biggest pitfalls of microarray technology is the precise statistical analysis required to avoid false positives when dealing with thousands of genes simultaneously. Advances in these areas will contribute greatly toward the identification of the gene expression changes that underlie hippocampal LTP and, ultimately, learning and memory.

Learning and memory has a significant genetic influence, and evidence shows that many genes are critically required for both memory formation and long-term memory storage. Knowing which genes are involved in cognitive processes is an important step in the search for treatments for learning and memory disorders, such as Alzheimer's disease. Given how rapidly technology has advanced over the past several years, it is very possible that future therapeutic treatments for cognitive disorders may include the use of pharmacological agents to manipulate gene expression.

III. SUMMARY

1. Memory is a thing in a place in a brain.

2. There are two types of memory. Explicit memories are declarative in nature, whereas implicit memories are not. Behavioral tests such as the Morris water maze and classical conditioning permit scientists to examine memory in nonhuman animals.

3. Memories are represented in cells as long-term changes in the function of proteins translated from mRNA transcribed from genes.

4. The cyclic-AMP response element binding protein (CREB) is the most widely studied transcription factor and is involved in a variety of physiological processes, including learning and memory.

5. Behavioral tests of learning and memory, such as the Morris water maze, trace fear conditioning, and conditioned taste avoidance, lead to significant changes in CREB expression in the brain structures that mediate the type of learning being assessed, and animals with CREB mutations exhibit learning and memory deficits.

6. Neuronal growth-associated proteins (nGAPs), such as SCG10 and GAP-43, as well as immediate early genes (IEGs), such as *cFOS*, *arc*, and *zif/268*, are also mediated by learning and memory tasks.

7. Experience-dependent increases in synaptic strength underlie memory formation in networks of neurons and are known as Hebb's postulate and long-term potentiation (LTP).

8. There are two fundamental types of LTP, one that is NMDA receptor dependent and one that is NMDA receptor independent, here called opioid-receptor-dependent LTP.

9. Knockout mutant mice revealed the importance of several gene products in LTP induction and learning of a hippocampally dependent learning task, including tissue plasminogen activator (tPA), cFOS, and CREB.

10. The mRNA transcription that encodes proteins essential for strengthening the synapse occurs during late-phase LTP (l-LTP) but not during earlyphase LTP (e-LTP), suggesting that synapse strengthening during l-LTP is protein synthesis dependent.

REFERENCES

- Abraham, W.C., M. Dragunow, et al. (1991). The role of immediate early genes in the stabilization of long-term potentiation. *Mol Neurobiol* 5(2-4):297-314.
- Ahmed, T., and J.U. Frey (2005). Plasticity-specific phosphorylation of CaMKII, MAP-kinases and CREB during late-LTP in rat hippocampal slices in vitro. *Neuropharmacology* 49(4): 477-492.
- Akers, R.F., and A. Routtenberg (1985). Protein kinase C phosphorylates a 47 Mr protein (F1) directly related to synaptic plasticity. *Brain Res* 13:147-151.
- Albertson, D.N., B. Pruetz, C.J. Schmidt, D.M. Kuhn, G. Kapatos, and M.J. Bannon (2004). Gene expression profile of the nucleus accumbens of human cocaine abusers: Evidence for dysregulation of myelin. *Journal of Neurochemistry* 88:1211-1219.
- Alder, J., S. Thakker-Varia, et al. (2003). Brain-derived neurotrophic factor-induced gene expression reveals novel actions of VGF in hippocampal synaptic plasticity. J Neurosci 23(34):10800-10808.
- Ammon, S., P. Mayer, U. Riechert, H. Tischmeyer, and V. Hollt (2003). Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone precipitated withdrawal. *Brain Res Mol Brain Res* 112:113-125.
- Applegate, M.D., D.S. Kerr, et al. (1987). Redistribution of synaptic vesicles during long-term potentiation in the hippocampus. *Brain Res* **401**(2):401-406.
- Athos, J., S. Impey, V.V. Pineda, X. Chen, and D.R. Storm (2002). Hippocampal CREmediated gene expression is required for contextual memory formation. *Nat Neurosci* 5(11):1119–1120.
- Balschun, D., D.P. Wolfer, et al. (2003). Does cAMP response element-binding protein have a pivotal role in hippocampal synaptic plasticity and hippocampus-dependent memory? *J Neurosci* 23(15):6304-6314.
- Barco, A., S. Patterson, et al. (2005). Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. *Neuron* 48(1):123–137.

- Barea-Rodriguez, E.J., D.T. Rivera, et al. (2000). Protein synthesis inhibition blocks the induction of mossy fiber long-term potentiation in vivo. J Neurosci 20(22):8528-8532.
- Bito, H. (1998). [A potential mechanism for long-term memory: CREB signaling between the synapse and the nucleus]. Seikagaku 70(6):466-471.
- Bito, H., K. Deisseroth, et al. (1996). CREB phosphorylation and dephosphorylation: A Ca(²⁺)- and stimulus duration-dependent switch for hippocampal gene expression. Cell 87(7):1203-1214.
- Bliss, T.V., and G.L. Collingridge (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* 361(6407):31–39.
- Bliss, T.V., and T. Lomo (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232(2):331-356.
- Bourtchuladze, R., B. Frenguelli, J. Blendy, D. Cioffi, G. Schutz, and A.J. Silva (1994). Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive elementbinding protein. *Cell* 78:59-68.
- Bozon, B., S. Davis, et al. (2002). Regulated transcription of the immediate-early gene Zif268: Mechanisms and gene dosage-dependent function in synaptic plasticity and memory formation. *Hippocampus* 12(5):570-577.
- Bramham, C.R., N.W. Milgram, et al. (1991a). Activation of AP5-sensitive NMDA receptors is not required to induce LTP of synaptic transmission in the lateral perforant path. *EurJ Neurosci* 3(12):1300–1308.
- Bramham, C.R., N.W. Milgram, et al. (1991b). Delta opioid receptor activation is required to induce LTP of synaptic transmission in the lateral perforant path in vivo. *Brain Res* 567(1):42-50.
- Breindl, A., B.E. Derrick, et al. (1994). Opioid receptor-dependent long-term potentiation at the lateral perforant path-CA3 synapse in rat hippocampus. *Brain Res Bull* **33**(1):17–24.
- Brindle, P.K., and M.R. Montminy (1992). The CREB family of transcription activators. Curr Opin Genet Dev 2(2):199–204.
- Brunelli, M., V. Castellucci, and E.R. Kandel (1976). Synaptic facilitation and behavioral sensitization in *Aplysia*: Possible role of serotonin and cyclic AMP. *Science* 194(4270): 1178–1181.
- Burchett, S.A., M.J. Bannon, and J.G. Granneman (1999). RGS mRNA expression in rat striatum: Modulation by dopamine receptors and effects of repeated amphetamine administration. J Neurochem 72:1529–1533.
- Calixto, E., E. Thiels, et al. (2003). Early maintenance of hippocampal mossy fiber long-term potentiation depends on protein and RNA synthesis and presynaptic granule cell integrity. *J Neurosci* **23**(12):4842–4849.
- Carmeliet, P., L. Schoonjans, et al. (1994). Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* 368(6470):419-424.
- Chen, Z.L., and S. Strickland (1997). Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. *Cell* **91**(7):917-925.
- Clark, R.E., and L.R. Squire (1998). Classical conditioning and brain systems: The role of awareness. *Science* 280:77-81.
- Cole, A.J., D.W. Saffen, et al. (1989). Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* **340**(6233): 474-476.
- Collingridge, G.L., and T.V. Bliss (1995). Memories of NMDA receptors and LTP. *Trends Neurosci* **18**(2):54-56.
- Dash, P.K., B. Hochner, and E.R. Kandel (1990). Injection of the cAMP-responsive element into the nucleus accumbens of *Aplysia* sensory neurons blocks long-term facilitation. *Nature* 345:718-721.

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- Demmer, J., M. Dragunow, et al. (1993). Differential expression of immediate early genes after hippocampal long-term potentiation in awake rats. *Brain Res Mol Brain Res* 17(3-4):279–286.
- Derrick, B.E., and J.L. Martinez, Jr. (1994a). Frequency-dependent associative long-term potentiation at the hippocampal mossy fiber-CA3 synapse. Proc Natl Acad Sci USA 91(22): 10290-10294.
- Derrick, B.E., and J.L. Martinez, Jr. (1994b). Opioid receptor activation is one factor underlying the frequency dependence of mossy fiber LTP induction. J Neurosci 14(7):4359–4367.
- Derrick, B.E., S.B. Rodriguez, et al. (1992). Mu opioid receptors are associated with the induction of hippocampal mossy fiber long-term potentiation. J Pharmacol Exp Ther 263(2): 725-733.
- Derrick, B.E., S.B. Weinberger, et al. (1991). Opioid receptors are involved in an NMDA receptor-independent mechanism of LTP induction at hippocampal mossy fiber-CA3 synapses. *Brain Res Bull* 27(2):219-223.
- Derrick, B.E., A.D. York, and J.L. Martinez (2000). Increased granule cell neurogenesis in the adult dentate gyrus following mossy fiber stimulation sufficient to induce long-term potentiation. Brain Res 857:300-307.
- Desmond, N.L., and W.B. Levy (1988). Synaptic interface surface area increases with long-term potentiation in the hippocampal dentate gyrus. *Brain Res* **453**(1-2):308-314.
- Desmond, N.L., and W.B. Levy (1990). Morphological correlates of long-term potentiation imply the modification of existing synapses, not synaptogenesis, in the hippocampal dentate gyrus. *Synapse* 5(2):139–143.
- Dragunow, M. (1996). A role for immediate-early transcription factors in learning and memory. Behav Genet 26(3):293-299.
- Edwards, F.A. (1995). LTP a structural model to explain the inconsistencies. *Trends Neurosci* 18(6):250-255.
- Eichenbaum, H., and Cohen, N.J. (2001). From Conditioning to Conscious Recollection: Memory Systems of the Brain. New York: Oxford University Press.
- Fimia, G.M., D. De Cesare, and P. Sassone-Corsi (1998). Mechanisms of activation by CREB and CREM: Phosphorylation, CBP, and a novel coactivator, ACT. Cold Spring Harb Symp Quant Biol. 63:631-642.
- Flatscher-Bader, T., M. van der Brug, J.W. Hwang, P.A. Gochee, I. Matsumoto, S. Niwa, and P.A. Wilce (2005). Alcohol-responsive genes in the frontal cortex and nucleus accumbens of human alcoholics. J Neurochem 93:359–370.
- French, P.J., V. O'Connor, et al. (2001). Subfield-specific immediate early gene expression associated with hippocampal long-term potentiation in vivo. Eur J Neurosci 13(5):968– 976.
- Frey, U., and R.G. Morris (1997). Synaptic tagging and long-term potentiation. Nature 385(6616):533-536.
- Frey, U., M. Muller, et al. (1996). A different form of long-lasting potentiation revealed in tissue plasminogen activator mutant mice. J Neurosci 16(6):2057–2063.
- Gass, P., A. Fleischmann, et al. (2004). Mice with a fra-1 knock-in into the c-fos locus show impaired spatial but regular contextual learning and normal LTP. *Brain Res Mol Brain Res* **130**(1-2):16-22.
- Gass, P., D.P. Wolfer, D. Balschun, D. Rudolph, W. Frey, H. Lipp, and G. Schutz (1998). Deficits in memory tasks of mice with CREB mutations depend on gene dosage. *Learning Memory* 5:274-288.
- Giebler, H.A., I. Lemasson, and J.K. Nyborg (2000). p53 recruitment of CREB binding protein mediated through phosphorylated CREB: A novel pathway of tumor suppressor regulation. *Mol Cell Biol* 20:49–58.
- Gonzalez, G.A., and M.R. Montminy (1989). Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59:675-680.

- Gusev, P.A., C. Cui, D.L. Alkon, and A.N. Gubin (2005). Topography of Arc/Arg3.1 mRNA expression in the dorsal and ventral hippocampus induced by recent and remote spatial memory recall: Dissociation of CA3 and CA1 activation. J Neurosci 25:9384-9397.
- Guzowski, J.F., G.L. Lyford, et al. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J Neurosci 20(11):3993-4001.
- Guzowski, J.F., and J.L. McGaugh (1997). Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proc Natl Acad Sci USA* 94:2693-2698.
- Guzowski, J.F., B. Setlow, E.K. Wagner, and J.L. McGaugh (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: A comparison of the immediate-early genes Arc, c-fos, and zif268. J Neurosci 21(14):5089-5098.
- Harris, E.W., and C.W. Cotman (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl-D-aspartate antagonists. *Neurosci Lett* 70(1):132-137.
- Harris, E.W., A.H. Ganong, et al. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Res 323(1):132-137.
- Hebb, D. (1949). Organization of Behavior: A Neuropsychological Theory. New York: Wiley.
- Higo, N., T. Oishi, A. Yamashita, K. Matsuda, and M. Hayashi (1999). Quantitative nonradioactive in situ hybridization study of GAP-43 and SCG10 mRNAs in the cerebral cortex of adult and infant macaque monkeys. *Cerebral Cortex* 9:317-331.
- Hoeffler, J.P., T.E. Meyer, Y. Yun, J.L. Jameson, and J.F. Habener (1988). Cyclic AMP-Responsive DNA-binding protein: Structure based on a cloned placental cDNA. Science 242:1430–1433.
- Hope, B., B. Kosofsky, S.E. Hyman, and E.J. Nestler (1992). Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. *Proc Nat Acad Sci USA* 89:5764-5768.
- Huang, Y.Y., and E.R. Kandel (1994). Recruitment of long-lasting and protein kinase Adependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. *Learning Memory* 1:74-82.
- Impey, S., D.M. Smith, K. Obrietan, R. Donahue, C. Wade, and D.R. Storm (1998). Stimulation of cAMP response element (CRE)-mediated transcription during contexual learning. *Nature Neurosci* 1(7):595-601.
- Ishihara, K., H. Katsuki, et al. (1990). Different drug susceptibilities of long-term potentiation in three input systems to the CA3 region of the guinea pig hippocampus in vitro. Neuropharmacology 29(5):487-492.
- Jaffe, D., and D. Johnston (1990). Induction of long-term potentiation at hippocampal mossyfiber synapses follows a Hebbian rule. J Neurophysiol 64(3):948-960.
- Jones, M.W., M.L. Errington, et al. (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nat Neurosci* 4(3):289-296.
- Josselyn, S.A., S. Kida, and A.J. Silva (2004). Inducible repression of CREB function disrupts amygdala-dependent memory. *Neurobiol Learning Memory* 82:159-163.
- Josselyn, S.A., and P.V. Nguyen (2005). CREB, synapses and memory disorders: Past progress and future challenges. Curr Drug Targets CNS Neurol Disord 4(5):481-497.
- Kaang, B.K., E.R. Kandel, and S.G. Grant (1993). Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in Aplysia sensory neurons. *Neuron* 10:427-435.
- Kandel, E.R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science* **294**(5544):1030-1038.
- Karns, L.R., S.C. Ng, J.A. Freeman, and M.C. Fishman (1987). Cloning of complementary DNA for GAP-43, a neuronal growth-related protein. *Science* 236:597–600.
- Kim, J., J. Lu, and P.G., Quinn (2000). Distinct cAMP response element binding protein (CREB) domains stimulate different steps in a concerted mechanism of transcription

activation. Proceedings of the National Academy of Science USA, Oct 10; 97(12):11292-11296.

- Kim, J.J., R.E. Clark, and R.F. Thompson (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuro*science 109:195-203.
- Kim, J.J., and M.S. Fanselow (1992). Modality-specific retrograde amnesia of fear. Science 256:675-678.
- Konradi, C., R.L. Cole, S. Heckers, and S.E. Hyman (1994). Amphetamine regulates gene expression in rat striatum via transcription factor CREB. Journal of Neuroscience 14(9): 5623-5634.
- Krug, M., B. Lossner, et al. (1984). Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. *Brain Res Bull* 13(1):39-42.
- Kuhar, M.J., J.N. Jaworski, G.W. Hubert, K.B. Philpot, and G. Dominguez (2005). Cocaine- and amphetamine-regulated transcript peptides play a role in drug abuse and are potential therapeutic targets. AAPS J 7:259-265.
- Lanahan, A., G. Lyford, et al. (1997). Selective alteration of long-term potentiation-induced transcriptional response in hippocampus of aged, memory-impaired rats. J Neurosci 17(8): 2876-2885.
- Lanahan, A., and P. Worley (1998). Immediate-early genes and synaptic function. Neurobiol Learn Mem 70:37-43.
- Lee, P.R., J.E. Cohen, et al. (2005). Gene expression in the conversion of early-phase to latephase long-term potentiation. Ann NY Acad Sci 1048:259-271.
- Lisman, J.E., and K.M. Harris (1993). Quantal analysis and synaptic anatomy integrating two views of hippocampal plasticity. *Trends Neurosci* 16(4):141-147.
- Lutz, B., W. Schmid, C. Niehrs, and G. Schutz (1999). Essential role of CREB family proteins during Xenopus embryogenesis. *Mech Dev* 88:55-66.
- Lynch, M.A. (2004). Long-term potentiation and memory. Physiol Rev 84(1):87-136.
- Madani, R., S. Hulo, et al. (1999). Enhanced hippocampal long-term potentiation and learning by increased neuronal expression of tissue-type plasminogen activator in transgenic mice. *Embo J* 18(11):3007-3012.
- Maren, S., G. Aharonov, and M.S. Fanselow (1996). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. *Behav Neursci* 110:718-726.
- Marie, H., W. Morishita, et al. (2005). Generation of silent synapses by acute in vivo expression of CaMKIV and CREB. *Neuron* **45**(5):741-752.
- Martin, K.C., and K.S. Kosik (2002). Synaptic tagging who's it? Nat Rev Neurosci 3(10): 813-820.
- Martin, M.R. (1983). Naloxone and long-term potentiation of hippocampal CA3 field potentials in vitro. Neuropeptides 4(1):45-50.
- Martinez, J.L., Jr., and B.E. Derrick (1996). Long-term potentiation and learning. Annu Rev Psychol 47:173-203.
- Matsuo, R., A. Murayama, et al. (2000). Identification and cataloging of genes induced by longlasting long-term potentiation in awake rats. J Neurochem 74(6):2239-2249.
- McConnell, J.V. (1966). Comparative physiology: Learning in invertebrates. Annu Rev Physiol 28:107-136.
- McEchron, M.D., H. Bouwmeester, W. Tseng, C. Weiss, and J.F. Disterhoft (1998). Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. *Hippocampus* **6**:638-646.
- McGlinchey-Berroth, R., M.C. Carrillo, J.D. Gabrieli, C.M. Brawn, and J.F. Disterhoft (1997). Impaired trace eyeblink conditioning in bilateral, medial-temporal lobe amnesia. *Behav Neurosci* 111:873-882.

- Meberg, P.J., C.A. Barnes, B.L. McNaughton, and A. Routtenberg (1993). Protein kinase C and F1/GAP-43 gene expression in hippocampus inversely related to synaptic enhancement lasting 3 days. *Proc Nat Acad Sci USA* **90**:12050–12054.
- Meshul, C.K., and W.F. Hopkins (1990). Presynaptic ultrastructural correlates of long-term potentiation in the CA1 subfield of the hippocampus. *Brain Res* **514**(2):310–319.
- Messaoudi, E., S.W. Ying, et al. (2002). Brain-derived neurotrophic factor triggers transcriptiondependent, late phase long-term potentiation in vivo. J Neurosci 22(17):7453-7461.
- Montminy, M.R., and Bilezikjian, L.M. (1987). Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature* **328**:175-178.
- Montminy, M.R., G.A. Gonzalea, and K.K. Yamamoto (1990). Regulation of c-AMP inducible genes by CREB. Trends in Neuroscience 13:184–188.
- Morris, R.G.M. (1984). Development of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11:47-60.
- Nirenberg, M. (2004). Historical review: Deciphering the genetic code a personal account. Trends Biochem Sci 29(1):46-54.
- Nguyen, P.V., T. Abel, et al. (1994). Requirement of a critical period of transcription for induction of a late phase of LTP. *Science* 265(5175):1104–1107.
- Otani, S., and W.C. Abraham (1989). Inhibition of protein synthesis in the dentate gyrus, but not the entorhinal cortex, blocks maintenance of long-term potentiation in rats. *Neurosci Lett* **106**(1-2):175-180.
- Otani, S., C.J. Marshall, et al. (1989). Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. *Neuroscience* 28(3):519-526.
- Parelkar, N.K., and J.Q. Wang (2004). mGluR5-dependent increases in immediate early gene expression in the rat striatum following acute administration of amphetamine. *Brain Res Mol Brain Res* 122:151–157.
- Park, Y.H., L. Kantor, K.K. Wang, and M.E. Gnegy (2002). Repeated, intermittent treatment with amphetamine induces neurite outgrowth in rat pheochromocytoma cells (PC12 cells). *Brain Res* 951:43–52.
- Pascale, A., P.A. Gusev, M. Amadio, T. Dottorini, S. Govoni, D.L. Alkon, and A. Quattrone (2004). Increase of the RNA-binding protein HuD and posttranscriptional up-regulation of the GAP-43 gene during spatial memory. *Proc Natl Acad Sci USA* 101:1217–1222.
- Peng, H., B.E. Derrick, et al. (2003). Identification of upregulated SCG10 mRNA expression associated with late-phase long-term potentiation in the rat hippocampal Schaffer-CA1 pathway in vivo. J Neurosci 23(16):6617-6626.
- Peng H., B.E. Derrick, and J.L. Martinez, Jr. (2004). Time-course study of SCG10 mRNA levels associated with LTP induction and maintenance in the rat Schaffer-CA1 pathway in vivo. *Brain Res Mol Brain Res* 120:182–187.
- Pittenger, C., Y.Y. Huang, R.F. Paletzki, R. Bourtchouladze, H. Scanlin, S. Vronskaya, and E.R. Kandel (2002). Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. *Neuron* 34:447-462.
- Qian, Z., M.E. Gilbert, et al. (1993). Tissue-plasminogen activator is induced as an immediateearly gene during seizure, kindling and long-term potentiation. *Nature* 361(6411):453-457.
- Rekart, J.L., K. Meiri, and A. Routtenberg (2005). Hippocampal-dependent memory is impaired in heterozygous GAP-43 knockout mice. *Hippocampus* 15:1–7.
- Roberts, L.A., C.H. Large, et al. (1997). Long-term potentiation in perforant path/granule cell synapses is associated with a post-synaptic induction of proenkephalin gene expression. *Neurosci Lett* 227(3):205-208.
- Rodriguez, J.S., C.F. Phelix, and J.L. Martinez, Jr. (2002). Microarray analysis of altered gene expression associated with D-amphetamine self-administered into the nucleus accumbens via microdialysis. *Soc Neurosci Abstr* **808**:19.

- Rodriguez, S.M., G.E. Schafe, and J.E. LeDoux (2004). Molecular mechanisms underlying emotional learning and memory in the lateral amygdala. *Neuron* 44:75-91.
- Rogers, J.L., M.R. Hunsaker, and R.P. Kesner (2006). Effects of ventral and dorsal CA1 subregional lesions on trace fear conditioning. *Neurobiol Learning Mem*.
- Rolls, E.T. (1992). Neurophysiological mechanisms underlying face processing within and beyond the temporal cortical visual areas. *Philos Trans R Soc Lond B Biol Sci.* 335:11-20.
- Rossmanith, W.G., D.L. Marks, D.K. Clifton, and R.A. Steiner (1994). Induction of galanin gene expression in gonadotrophin-releasing hormone neurons with puberty in the rat. *Endocrinology*, Oct; 135(4):1401-1408.
- Routtenberg, A., I. Cantallops, S. Zaffuto, P. Serrano, and U. Namgung (2000). Enhanced learning after genetic overexpression of a brain growth protein. *Proc Natl Acad Sci USA* 97(13):7657-7662.
- Routtenberg, A., and J.L. Rekart (2005). Posttranslational protein modification as the substrate for long-lasting memory. *Trends Neurosci* 28(1):12–19.
- Sassone-Corsi, P., J. Visvader, L. Ferland, P.L. Mellon, and I.M. Verma (1988). Induction of proto-oncogene fos transcription through the adenylate cyclase pathway: Characterization of a cAMP-responsive element. Genes Dev 2:1529-1538.
- Shaywitz, A.J., and M.E. Greenberg (1999). CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821-861.
- Shors, T.J., G. Miesegaes, A. Beylin, M. Zhao, T. Rydel, and E. Gould (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature* **410**:372–376.
- Shors, T.J., D.A. Townsend, M. Zhao, Y. Kozorovitskiy, and E. Gould (2002). Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* 12:578-584.
- Silva, A., and S.A. Josselyn (2002). The molecules of forgetfulness. Nature 418:929-930.
- Squire, L.R., and S. Zola-Morgan (1991). The medial temporal lobe memory system. *Science* 253:1380-1386.
- Stein, R., N. Mori, K. Matthews, L.C. Lo, and D.J. Anderson (1998). The NGF-inducible SCG10 mRNA encodes a novel membrane-bound protein present in growth cones and abundant in developing neurons. *Neuron* 1:463-476.
- Sugaya, K., R. Greene, et al. (1998). Septo-hippocampal cholinergic and neurotrophin markers in age-induced cognitive decline. *Neurobiol Aging* 19(4):351-361.
- Thompson, K.J., M.L. Mata, et al. (2005). Metabotropic glutamate receptor antagonist AIDA blocks induction of mossy fiber-CA3 LTP in vivo. J Neurophysiol 93(5):2668-2673.
- Thompson, K.J., W.J. Meilandt, P. Lingala, J. Orfila, H. Peng, and J.L. Martinez (2005). Arc and Homer-1 Are Differentially Expressed Following in vivo Induction of Early-Phase LTP at the Hippocampal Mossy Fiber-CA3 Pathway. Washington, DC: Society for Neuroscience.
- Thompson, K.J., J.E. Orfila, et al. (2003). Gene expression associated with in vivo induction of early phase-long-term potentiation (LTP) in the hippocampal mossy fiber-Cornus Ammonis (CA)3 pathway. Cell Mol Biol (Noisy-le-grand) 49(8):1281-1287.
- Ungar, G. (1970). Chemical transfer of learned behavior. Inflammation Research 1(4), 155-163.
- Urban, N.N., and G. Barrionuevo (1996). Induction of Hebbian and non-Hebbian mossy fiber long-term potentiation by distinct patterns of high-frequency stimulation. J Neurosci 16(13):4293-4299.
- Vazdarjanova, A., B.L. McNaughton, et al. (2002). Experience-dependent coincident expression of the effector immediate-early genes arc and Homer 1a in hippocampal and neocortical neuronal networks. J Neurosci 22 (23):10067–10071.
- Vukosavic, S., S. Ruzdijic, R. Veskov, L. Rakic, and S. Kanazir (2001). Differential effects of amphetamine and phencyclidine on the expression of growth-associated protein GAP-43. *Neurosci Res* 40:133-140.

- Walton, M., C. Henderson, et al. (1999). Immediate early gene transcription and synaptic modulation. J Neurosci Res 58(1):96-106.
- Watson, J.D., and F.H. Crick (1953). Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* 171(4356):737-738.
- Whittaker, E., E. Vereker, et al. (1999). Neuropeptide Y inhibits glutamate release and long-term potentiation in rat dentate gyrus. *Brain Res* 827(1-2):229-233.
- Ying, S.W., M. Futter, et al. (2002). Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: Requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. J Neurosci 22(5):1532-1540.
- Yoshida, T., and M. Mishina (2005). Distinct roles of calcineurin-nuclear factor of activated Tcells and protein kinase A-cAMP response element-binding protein signaling in presynaptic differentiation. J Neurosci. 25:3067–3079.
- Young, E.A., E.H. Owen, K.F. Meiri, and J.M. Wehner (2000). Alterations in hippocampal GAP-43 phosphorylation and protein level following contextual fear conditioning. *Brain Res* **860**:95-103.
- Zalutsky, R.A., and R.A. Nicoll (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* 248(4963):1619–1624.
- Zalutsky, R.A., and R.A. Nicoll (1992). Mossy fiber long-term potentiation shows specificity but no apparent cooperativity. *Neurosci Lett* **138**(1):193-197.
- Zhang, X., D.T. Odom, S. Koo, M.D. Conkright, G. Canettieri, J. Best, H. Chen, R. Jenner, E. Herbolsheimer, E. Jocobsen, S. Kadam, J.R. Ecker, B. Emerson, J.B. Hogenesch, T. Unterman, R.A. Young, and M. Montminy (2005). Genome-wide analysis of cAMPresponse element binding protein occupancy, phosphorylation, and target gene activation in human tissues. *Proc Natl Acad Sci USA* 102(12):4459-4464.
- Zhuo, M., D.M. Holtzman, et al. (2000). Role of tissue plasminogen activator receptor LRP in hippocampal long-term potentiation. J Neurosci 20(2):542-549.