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MOLECULAR PSYCHOLOGY: Roles for the ERK MAP Kinase Cascade in Memory

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■ **Abstract** In this review we describe an emerging understanding of the roles of the Extracellular-signal regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade in learning and memory. We begin by describing several behavioral memory paradigms and review data implicating ERK as an essential component of the signal transduction mechanisms subserving these processes. We then describe evidence implicating ERK as a critical player in synaptic and neuronal plasticity—a cellular role likely to underlie ERK's behavioral role in the animal. We then proceed to parsing the complexities of biochemical regulation of ERK in neurons and to a description of a few likely cellular targets of ERK. This is in order to begin discussing the possible molecular basis of ERK-mediated behavioral change. We close our review with speculations concerning how the complexities and idiosyncrasies of ERK regulation may allow for sophisticated information processing at the biochemical level in neurons—attributes that may make the ERK cascade well-suited for triggering complex and long-lasting behavioral change. Our goal in this review is not so much to portray ERK as unique regarding its role as a signal transducter in memory, but rather to use ERK as one specific example of recent studies beginning to address the molecules and signal transduction pathways subserving cognition.

INTRODUCTION—HEBB'S POSTULATE

Of the higher-order cognitive processes, learning and memory are likely to be the first to be understood at the cellular and molecular level. Learning and memory stand apart from most areas of cognitive psychology in that there is a central unifying hypothesis concerning their fundamental underlying mechanisms. The hypothesis is captured nicely in its commonly expressed formulation, "Hebb's Postulate": "When an axon of cell A . . . excites cell B and repeatedly or persistently takes part in firing it, some . . . metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased" (1). Thus, information storage in the nervous system is widely held to be based upon enduring, activity-dependent changes in the number, strengths, or downstream consequences of synaptic connections between neurons in the central nervous system.

The availability of a unified hypothesis for information storage in the nervous system allows effective utilization of a reductionist approach to the problem of memory. In the limit such a reductionist approach can begin to address the basis of memory at its most finite level—the molecules and biochemical cascades operating to form and store memory. In this review, we focus on the ERK MAP kinase cascade as an example of a specific signal transduction cascade that has been implicated in behavioral memory, long-lasting synaptic plasticity, and biochemical information processing at the molecular level. We focus on changes in the activity of the ERK cascade as one of the "metabolic changes" implied by Hebb's Postulate.

We feel that before proceeding, a few well-intentioned editorial comments are appropriate concerning Professor Hebb's postulate and the behavioral change it explains. For the adventuresome psychologist who might undertake to read this review, be forewarned that Hebb's "metabolic change" contains deceptively simple phrasing—it covers a world of complexity at the molecular level. For the adventuresome signal transduction biochemist who undertakes to read this review, be forewarned that "memory," while seemingly easily understood intuitively, covers a world of complexity at the behavioral and anatomical level. Finally, for the synaptic physiologist undertaking to read the review, we note that "Hebbian plasticity" is not synonymous with "synaptic plasticity"—many molecular sites outside the synapse are likely targets of metabolic change in long-term memory formation. While we deliver these comments in a light-hearted fashion, we note that in many ways they capture the essential take-home message of the review for each category of reader.

ERK IN MEMORY

The ability to store and recall information is one of the most amazing capacities of higher organisms. As human adults, we can remember events that happened in our earliest childhood. We can recall skills learned far in the past. We can even be influenced by memories that we cannot explicitly remember. These phenomena are termed "memory" in the research at the intersection of neuroscience, computational science, and psychology. Memory has a long history of study in the arena of psychology, where such notables as William James and Hermann Ebbinghaus used the tools of their day to try to understand some of the basic properties of memory. More recently, computational scientists and neuroscientists have worked both independently and together to try to understand the biological basis of memory.

In today's formulations, memory has been divided into different types or categories. The most popular division puts explicit or declarative memories in one category and implicit or nondeclarative memories in another (reviewed in 2). In this division, the declarative category includes memories about facts (sometimes referred to as semantic memories) and events (sometimes referred to as episodic memories) that can be consciously recalled. The nondeclarative category includes memories not explicitly or consciously remembered, including memories introduced by priming, skills, habits, the emotional coloring of memories, and simple

classical conditioning. Decades of research have led to the idea that these different types of memory require different areas of the brain for encoding and consolidation. For example, it is widely accepted that the hippocampus is required for the encoding and consolidation of declarative and spatial memories, while the amygdala is required for implicit emotional memories, such as conditioned fear.

Memories can also be divided into two additional categories: associative and nonassociative. While both these categories cover many different subtypes, the essential difference is that in associative learning an animal learns the predictive nature of one environmental stimulus for another. The classic example of associative learning is Pavlovian associative conditioning, where Pavlov's dogs learned that a sound reliably predicted the delivery of food.

Finally, long-term memory (LTM) is distinguished from short-term memory (STM) by its time phase and its dependence on protein synthesis. STM lasts only a few hours at the most and is not dependent on protein synthesis, while LTM can last a lifetime and is dependent on protein synthesis.

The first direct evidence that the ERK cascade is involved in LTM was reported by Atkins et al. (3) in 1998, who showed that ERK activation is required for the expression of LTM induced by a fear-conditioning paradigm in the rat. In fear conditioning, rats are taught to associate a foot shock with either a cue, a context, or both. Later (up to 24 hours), the extent of the animals' freezing in response to re-presentation of the cue or the context is quantified: rats typically show robust fear-associated "freezing" upon testing with either the cue or the context. Contextual fear conditioning is dependent on both the amygdala and the hippocampus, while cued fear conditioning is dependent only on the amygdala. Atkins et al. found that ERK2 phosphorylation was increased in rat hippocampus one hour after training using either a cued- or cued-plus-contextual conditioning paradigm. It is important to note that this increase was not due to the handling of the rats or to the foot shock itself. In additional studies the authors showed that the MAPK cascade is required for fear conditioning—an inhibitor of ERK activation, SL327, led to a blockade of memory formation upon testing with either the cued or the contextual conditioning paradigms. These results showed that the MAPK cascade is required for long-term fear memory, and in subsequent studies these initial findings have been confirmed and greatly extended using intraventricular injection of an inhibitor of ERK activation in the rat (4), intra-amygdalar infusion of an inhibitor of ERK activation in the rat (5), and using systemic administration of SL327 in the mouse (6).

Furthermore, two laboratories have found that the MAPK cascade is involved in another form of hippocampus-dependent LTM: spatial memory (6, 7). To examine this involvement, both groups made use of the Morris water maze, an experimental paradigm that involves the use of a spatial search strategy to locate a platform hidden in a pool of opaque liquid. Selcher et al. (6) found using mice that the inhibitor of ERK activation SL327 led to impairments in two tests of the animals' spatial memory. First, the SL327-treated animals exhibited longer escape latencies; that is, their ability to find the hidden platform over time (six days) did not improve

relative to vehicle-injected animals. Second, the SL327-injected animals were significantly worse than vehicle-treated controls in a probe trial on the last day of training; the probe trial is designed to test the animals' use of a spatial search strategy. In earlier studies Blum et al. (7) found that intrahippocampal infusion of an inhibitor of ERK activation also blocked acquisition of spatial memory in rats, and furthermore, these investigators showed hippocampal ERK activation occurred during learning. Thus, compelling evidence now exists that the ERK cascade is involved in two forms of hippocampus-dependent LTM—contextual fear conditioning and maze learning.

The ERK cascade also is involved in the formation in the insular cortex of long-term memories for taste. The insular cortex is thought to be the location of the consolidation of long-term taste memory; this memory is usually tested using the conditioned taste aversion paradigm (8, 9). Berman et al. (10) have shown that presentation of an unfamiliar taste leads to activation of ERK in the insular cortex within 30 min. Furthermore, they showed that inhibition of ERK activation in the insular cortex impairs conditioned taste aversion when tested up to 120 h after training. These data suggest that ERK is involved in insular cortex–dependent LTM.

Inhibitory avoidance training is a paradigm that involves learning an association between a footshock and an electric grid on the floor. After several trials in which an animal naturally steps down off a platform to explore the floor of the cage and is subsequently shocked, the animal learns to avoid stepping down off the platform. This type of learning produces hippocampus-dependent LTM. In a series of papers by Walz et al. (11, 12), the MAPK cascade was shown to be involved in this type of learning and memory. The authors found that inhibition of ERK activation in the entorhinal cortex or in hippocampal area CA1 impaired retention of the avoidance when tested up to six hours after training. Thus, not only does ERK appear to be involved in hippocampus-dependent LTM, but evidence now also implicates ERK in insular cortex–(taste) and entorhinal cortex–dependent LTM.

Finally, ERK is activated via a Ras-dependent pathway in many cell types (reviewed in 13), and research using Ras knockout mouse lines from several labs implicates ERK in some types of LTM. These types of ERK-dependent LTM include cued fear conditioning in mice and consolidation of spatial memories in mice (14–16). Overall, a wide variety of evidence using many different behavioral paradigms indicates a broad role for ERK activation in mammalian learning and memory.

ERK IN LONG-TERM POTENTIATION

The involvement of the hippocampus in human long-term memory was first definitively suggested by William Scoville and Brenda Milner in 1957 with the introduction into the scientific literature of their patient H. M. (reviewed in 2). H. M. underwent a bilateral removal of the hippocampus and related structures to relieve epilepsy that was not controllable with the medications available at the time. After his surgery, H. M. showed a complete inability to form new long-term memories,

a condition referred to as anterograde amnesia. In the decades since his surgery, H. M. has been the subject of many neuropsychological tests. Based on the data from studies of H. M., as well as studies of other patients with hippocampal lesions and associated amnesia, it is now widely accepted that the hippocampus is required for the consolidation of many types of LTM in humans.

Fortuitously for research scientists interested in studying the biological basis of the involvement of the hippocampus in LTM, the hippocampus is constructed in such a way as to facilitate electrophysiological, biochemical, and molecular studies of its properties (Figure 1). The neurons in the hippocampus are laid out in a laminar fashion, which facilitates the extracellular electrophysiological study of the synaptic connections in the hippocampus. In addition, the hippocampus is easily removed from the brains of many species of mammals, which makes it possible to study the biochemistry of the hippocampus proper.

The main synaptic circuit in the hippocampus consists of information entering the dentate gyrus of the hippocampus from cortical and subcortical structures

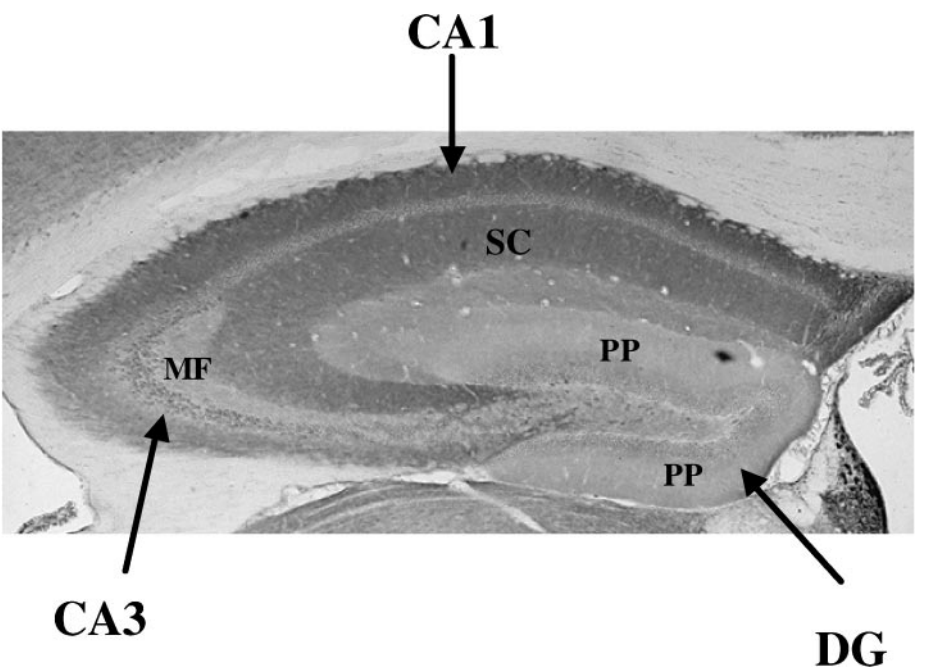

Figure 1 The hippocampal formation. The dentate gyrus (DG), area CA3 (CA3) and area CA1 (CA1) are indicated, along with the locations of the perforant path (PP) inputs from outside the hippocampus, the mossy fiber (MF) inputs from the DG into CA3, and the Schaffer-collateral (SC) inputs into CA1 from CA3. See text for additional details. Photo, courtesy of Andrew Varga, is of a mouse hippocampus stained with an antibody selective for the ERK-phosphorylated form of the potassium channel Kv4.2.

via the perforant path (Figure 1). After synapsing in the dentate gyrus, the information is moved to area CA3 via the mossy fiber path. After synapsing in area CA3, information is moved to area CA1 via the Schaffer-collateral path. After synapsing in area CA1, information exits the hippocampus and returns to subcortical or cortical structures. The connections in this synaptic circuit are retained in a fairly impressive manner in the dissection and slicing of the hippocampus for electrophysiological experiments.

Most of the electrophysiological studies of the hippocampus have focused on two phenomena, long-term potentiation (LTP) and long-term depression (LTD). LTP was first discovered by Bliss & Lomo in 1973, while looking for long-term physiological changes in the hippocampus of rabbits (17). LTP is defined broadly as a persistent increase in synaptic strength. It is usually seen experimentally as an increase in the excitatory postsynaptic potential (EPSP) response to a test stimulus following a brief, high-intensity stimulation of a presynaptic cell. While there is considerable debate about the utility of LTP as a mechanism of memory (e.g., see 18), many researchers consider LTP to be a good model of how memory might work at the cellular level (e.g., see 2).

In the 30 years since the publication of Bliss & Lomo's groundbreaking paper, many thousands of papers studying various aspects of LTP have been published. While there is disagreement in the field about many of the specifics of LTP, most researchers are in agreement about several points (reviewed in 19). First, LTP is probably dependent on both pre- and postsynaptic events. While this area is one of the more controversial areas in LTP research, most researchers will not absolutely rule out one over the other and do agree that LTP most likely depends on both pre- and postsynaptic mechanisms. Second, LTP is commonly divided into three temporal phases (Figure 2). The first stage is initial long-term potentiation, also referred to as short-term potentiation. Initial LTP is typically defined as lasting up to 30 min; its expression is both protein-kinase and protein-synthesis independent. The second phase is early LTP, or E-LTP. This phase lasts from 30 min to a few hours; its expression is mediated by the persistent activation of various protein kinases and the insertion of glutamate receptors into the postsynaptic membrane. The third phase is late LTP, or L-LTP; it lasts from a few hours to a few weeks, depending on the type of tissue preparation (20–25). L-LTP is dependent on changes in gene expression. Changes in gene expression presumably lead to changes in synaptic strength through changes in the levels of various housekeeping proteins, structural proteins, and various proteins and enzymes that mediate synaptic transmission itself.

While many different LTP induction protocols exist that vary in their closeness to naturally occurring patterns of neuronal activity, studies have shown hippocampal LTP can be induced with physiologic stimulation protocols that mimic endogenous hippocampal firing patterns. One such protocol is theta-burst stimulation, or TBS. A theta rhythm is seen in in vivo recordings of rat hippocampi while they are in the process of exploring (26–29); stimulation of hippocampal area CA3 axons with a stimulation protocol that mimics this rhythm is able to

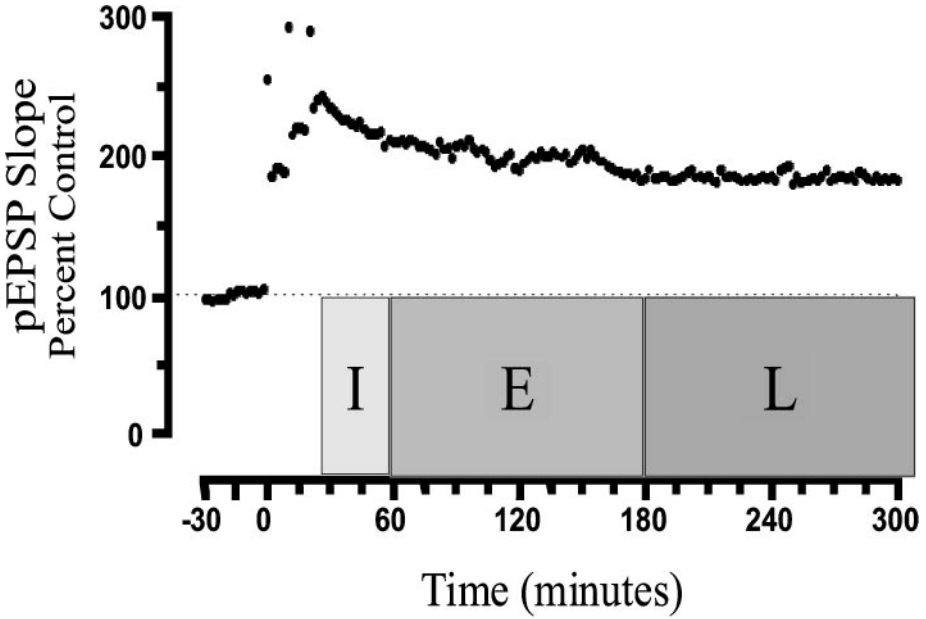

Figure 2 Electrophysiological results from a typical LTP experiment. Each circle represents the slope of the population excitatory post-synaptic potential (pEPSP) recorded at that time point. The major temporal phases of LTP are indicated. Data are from E. D. Roberson (unpublished observations).

potentiate hippocampal area CA1 dendrites in the mouse and rat (e.g., see 30, 31). LTP induced with this stimulation protocol can be modulated by neurotransmitters such as norepinephrine and epinephrine in the mouse (31, 32).

While no direct link proving or disproving a causal relationship between LTP and LTM has been shown, several lines of evidence exist to support the link between LTP and LTM (reviewed in 2, 33). Many experiments have shown that blocking various molecular components necessary for LTP also leads to memory impairments. For example, Morris et al. have shown that blocking NMDA receptors blocks LTP in vitro and in vivo, and it also leads to spatial memory impairments as assessed using the Morris water maze task (34, 35). In addition, some groups have shown that drugs that interfere with mGLuR functioning act to reduce LTP in vivo and also lead to spatial memory impairments (36, 37). More recently, genetic manipulations have been utilized to demonstrate a link between LTP and LTM. For example, Tsien et al. (38) showed that mutating the R1 subunit of the NMDA receptor in hippocampal area CA1 of the mouse leads to a deficit in LTP induced at the Schaffer-collateral synapse as well as in spatial memory. Mayford et al. (39) found that transgenic mice that inducibly expressed a constitutively active form of CaMKII showed deficits in both LTP and spatial memory only when the transgene

was expressed; when it was turned off, both hippocampal LTP and spatial memory returned to normal.

Kinases in Long-Term Potentiation

One area of LTP research that has garnered much attention in the last two decades is the mediation of the induction, expression, and maintenance of LTP by various protein kinases. Several protein kinases have turned out to be critical in the induction and expression of LTP. These include the calcium and calmodulin-regulated protein kinase CaMKII, the cAMP-dependent protein kinase PKA, PKC, protein tyrosine kinases, and the most recent addition to the field, ERK. In the next section, we explore the role that ERK plays in synaptic plasticity in the hippocampus.

The first direct evidence that the MAPK cascade is involved in synaptic plasticity came from English & Sweatt in 1996 (40). The authors first showed that ERK2 is activated in rat hippocampal area CA1 following NMDA receptor stimulation in the hippocampus, as had previously been reported for cultured embryonic hippocampal neurons in vitro (41). The authors then showed that LTP-inducing high frequency stimulation (HFS) of the Schaffer-collateral inputs to area CA1 led to the activation of ERK2 in area CA1. This activation was NMDA receptor-dependent and not observed with test stimulation. In a subsequent paper, the authors showed that ERK activation is required for the induction of stable long-term LTP (42), making use of an inhibitor of ERK activation, PD098059 (43, 44).

In many additional studies other LTP-inducing stimulation protocols have also been shown to be ERK-dependent. For example LTP induced by theta-frequency stimulation, which consists of brief pulses of stimulation applied at 5 Hz for 30 s, is blocked by inhibition of ERK activation (31, 32). O'Connor and colleagues have shown that ERK2 is involved in both NMDA receptor-dependent (induced by HFS of the medial perforant path) and -independent (induced by application of the K^+ channel blocker tetraethylammonium chloride, TEA) LTP in the dentate gyrus of the rat (45). These data have been recently confirmed using in vivo induction of LTP in the dentate gyrus (46). Several forms of NMDA receptor–independent LTP have also been shown to require ERK in both rat hippocampal area CA1 (47) and dentate gyrus (45).

It is interesting that ERK is also involved in LTP at synapses outside the hippocampus. For instance, ERK has been implicated in LTP at amygdala-insular cortex synapses in the rat (48); this pathway is thought to be involved in conditioned taste aversion (see above). In addition, ERK has been shown to be involved in LTP induced at thalamo-amygdala synapses in the rat (5); this pathway is thought to be involved in transmitting auditory information about the tone to the limbic system during fear conditioning paradigms, which involves learning an association between a tone and an electric shock (see above).

Furthermore, the role of ERK in synaptic plasticity seems to be a conserved function across species. Crow et al. (49) found that ERK is involved in the long-term enhancement induced in sensory neurons of the invertebrate *Hermissenda* during

an in vitro analog of classical conditioning. In another invertebrate species, *Aplysia californica*, ERK has been shown to be required for long-term facilitation of excitatory transmission between a sensory neuron and a motor neuron in culture (50).

Thus, within a mere five years of the first evidence of a role for ERK in synaptic plasticity, abundant evidence exists that ERK plays a role in different types of LTP (both NMDA receptor–dependent and –independent) in area CA1 and the dentate gyrus of the mammalian hippocampus, as well as in the mammalian amygdala and insular cortex. Not only is ERK involved in mammalian synaptic plasticity, but evidence from two invertebrate species indicates that ERK plays a role in synaptic plasticity across species. In the next section we address the complex mechanisms operating to regulate ERK activation in the nervous system.

REGULATION OF ERK IN NEURONS

Introduction—General Attributes of ERK Regulation

Although MAPKs were initially identified as mediators of cell differentiation and proliferation in mitotic cells, ERK 1 and 2 are most highly expressed in the postmitotic neurons of the adult mammalian central nervous system (CNS) (51, 52). Immunohistochemistry studies have demonstrated that in neurons, ERK2 is localized to the soma and dendritic trees of neurons in the neocortex, hippocampus, striatum, and cerebellum (52).

The MAPK/ERK terminology has a long and tortuous history that is very interesting to read in retrospect, and which serves as a reminder of the excitement and confusion that can be associated with the discovery of an important signaling cascade. The scientific world was first introduced to what would later be known as the MAPK family by Cooper & Hunter in 1982 with their report of epidermal growth factor (EGF)–induced tyrosine phosphorylation of cellular proteins (53). In 1988, Ray & Sturgill first identified MAPK as microtubule-associated protein kinase, due to its ability to phosphorylate microtubule-associated protein-2 (MAP-2) (54). Later the same year, they published a report in which they postulated that MAPK was phosphorylated on both tyrosine and threonine residues, which made MAPK a very odd protein at the time (55). In 1989 came the re-identification of MAPK as mitogen-activated protein kinase, owing to its ability to be activated by a number of mitogenic stimuli (56). Finally in 1990, the first MAPKs to be cloned were ERK (extracellular signal-regulated kinase) 1 and 2 by Cobb and colleagues (57).

Several additional members of the MAPK family have subsequently been cloned, including p38/HOG (58, 59), JNK/SAPK (c-jun N-terminal kinase/stress-activated protein kinase) (60, 61), and ERK 5 (62, 63). We should note here that many scientists use the terms ERK and MAPK interchangeably. As this can lead to confusion regarding the different members of the families, it is important to differentiate among the various members of the MAPK family, which are regulated by distinct mechanisms and have different effectors, giving each pathway a

distinctive function in the cell. The term MAPK now refers to the superfamily, with ERKs, JNK/SAPKs, and p38s as the main subfamilies. In this review, we are of course focusing on the MAPK ERK. Many exciting discoveries are still being made in this continually growing field, and we may see the number of subfamilies increase in the next few years (64).

The MAPKs ERK1/2 are referred to as proline-directed serine/threonine kinases owing to the fact that they require a serine or threonine in the target protein sequence to be immediately followed by a proline in order for the target protein to be phosphorylated. This proline at the $+1$ position cannot be separated from the serine/threonine by the addition of even one amino acid; this addition drastically reduces the rate of phosphorylation of the target protein. An additional proline in the -1 or -2 positions may increase the rate of phosphorylation of the target protein (65, 66).

It is important to note distinct stimuli appear to selectively trigger the activation of a particular MAPK cascade. Although the components of these pathways are highly homologous, there appears to be little if any cross talk across individual MAPK pathways. Thus, the kinase upstream from ERK, MEK1/2, for example, is thought to be dedicated strictly to the ERK MAPKs and does not regulate either JNK or p38 HOG. Conversely, the ERK MAPKs are activated only by MEK 1/2 and are thus not substrates for MEK4/JNKK or MEK3/MKK4 (67, 68). The scaffold model postulates that cross talk among these MAPK cascades is prevented by regulatory scaffolding proteins that govern the interactions of particular MAPKs and their upstream activators. Thus, a putative ERK MAPK pathway scaffolding protein might selectively bring together Raf, MEK, and ERK, while a putative JNK MAPK pathway scaffolding protein might organize MEKK, JNKK, and JNK. In fact, evidence for these kinds of scaffolding modules exists: The scaffolding protein JIP-1 brings MLK3, MEK7, and JNK1/2 together (69, 70), while the proteins MP1 and Grb10 bring some parts of the ERK pathway together (MEK1/ERK1 and Raf-1/MEK, respectively) (71, 72). The protein JSAP1/JIP3 not only binds members of the JNK pathway to increase JNK activation, but also additionally binds Raf1 and MEK1 and functions to inhibit the activation of ERK (73, 74). Thus it seems that evidence for inhibition of cross talk by the scaffold proteins does exist.

A Superfamily of MAPK Signaling Cascades

As new members of the MAPK family have been discovered, common themes among the pathways have emerged. One common theme is that each family is organized around a core cascade consisting of a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAPK. The prototypical ERK pathway has a first step activation by an extracellular signal such as a growth factor (Figure 3). In this instance, binding of the growth factor to its receptor leads to dimerization of the receptor and subsequent internal phosphorylation of tyrosine residues on the cytoplasmic tails of the receptor. This internal phosphorylation

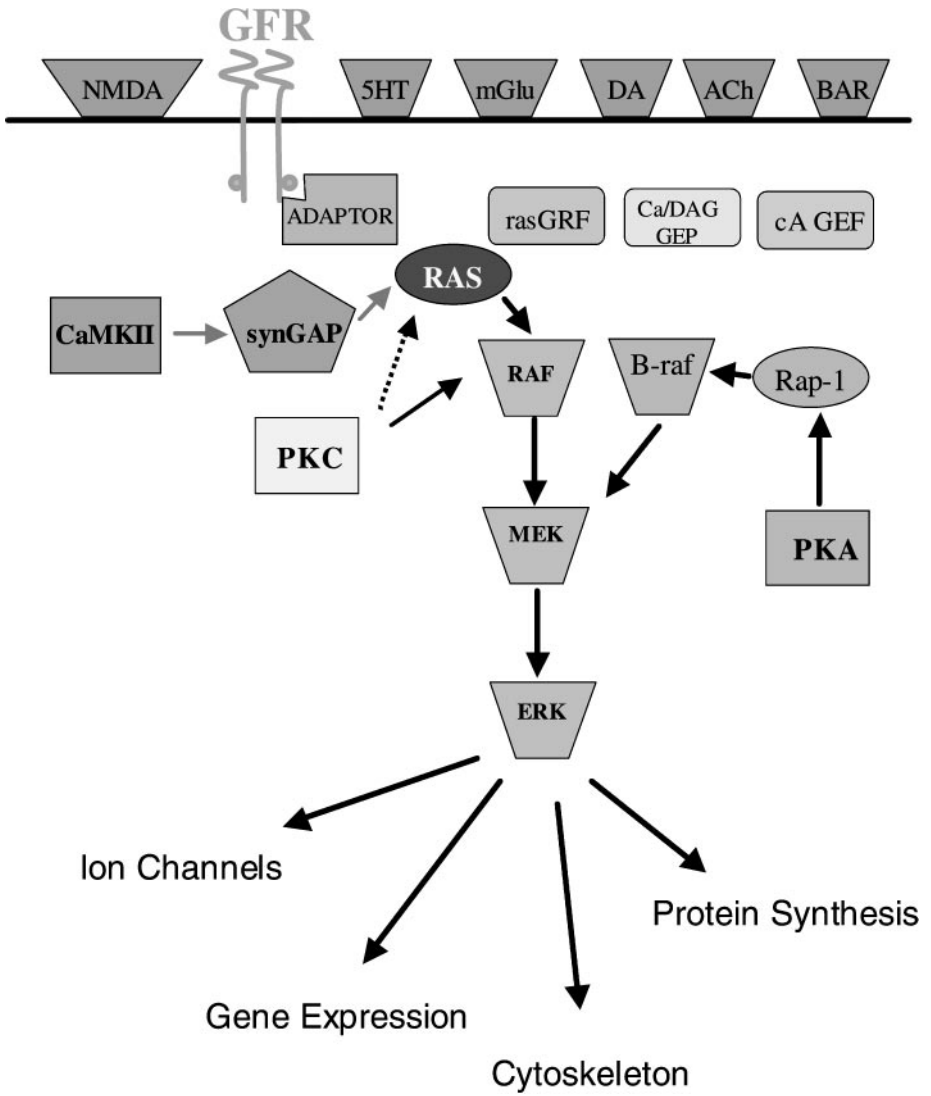

Figure 3 Regulation of ERK in neurons. See text for discussion.

leads to recruitment of several small adaptor proteins to the membrane, which bind to the receptors via their SH2 (src-homology 2) domains. These small adaptor proteins recruit guanine nucleotide exchange factors (GEFs), which bind to the adaptor proteins via proline-rich SH3 domains. The GEFs promote the attachment of membrane-associated G proteins such as Ras to GTP; in this state, Ras can activate the MAPKKK in this pathway, the kinase Raf-1. Raf-1 can then phosphorylate the MAPKK, MEK 1/2 (MAPK/ERK Kinase 1/2). Once activated, MEK 1/2 can

phosphorylate its effectors, the MAPKs ERK 1 and 2, on tyrosine and threonine residues.

While this particular MAPK pathway from growth factor to ERK activation is fairly well established, the specific pathway to the activation of ERK by a myriad of other factors is not as clear. The list of factors able to stimulate activation of ERK in various cell types is long and includes several mitogenic factors such as EGF, PDGF (platelet-derived growth factor), thrombin, and angiotensin (reviewed in 75); cytoplasmic protein tyrosine kinases such as src kinases and PYK2 (76–78); activation of certain G protein–coupled serpentine receptors, including the α-adrenergic, muscarinic, D2 dopaminergic, and A1 adenosine receptors (79–81); and activation of AMPA and NMDA receptors (82–84). More research is needed to clearly delineate the steps from these various stimuli to final ERK activation in cells.

ERK in Neurons

While most of the mechanisms listed above apply to ERK regulation in neurons, additional mechanisms appear to be especially relevant in neuronal cells. For example, neuroscientists are particularly interested in the ability of Ca^{2+} to initiate ERK activation. Several proteins may be involved in the transduction of the signal from Ca^{2+} influx to ERK activation. In PC12 (pheochromocytoma) cells, a membrane-associated form of the G protein Ras is required for Ca^{2+}-stimulated activation of ERK (85). The recently discovered Ca^{2+}/calmodulin-activated GEFs—RasGRF, RasGRP, and Ca^{2+}/DAG GEF—and/or the cytoplasmic protein tyrosine kinase PTK2 may play a role in this activation (76, 86). Another interesting way in which Ca^{2+} may lead to ERK activation is through activation of CaMKII, resulting in an inhibition of an inhibitor of ERK activation, SynGAP (reviewed in 87).

Martin et al. (50) observed that the adenyl cyclase activator forskolin elicited ERK phosphorylation and nuclear translocation in hippocampal neurons. These observations prompted the hypothesis that the PKA and MAPK systems might be serially coupled in area CA1 of hippocampus. In most systems the PKA cascade inhibits ERK activation (cf. Figure 3); however, in some cell types PKA is positively coupled to ERK through Rap-1 and B-Raf, and via these intermediaries elicits MEK activation and ERK phosphorylation (88). Both Rap-1 and B-Raf are expressed in area CA1 of rat hippocampus, and Roberson et al. found that activation of PKA in the hippocampus leads to activation of ERK2 in area CA1 [(89), and see Figure 4]. In addition, activation of PKC in the hippocampus by the phorbol ester PDA leads to activation of ERK2 in area CA1, and stimulation of several neurotransmitter receptors known to be coupled to PKA and PKC in the hippocampus can lead to ERK activation via PKA or PKC. These neuromodulatory neurotransmitter receptors include the dopamine and β-adrenergic receptors (linked through PKA), the muscarinic acetylcholine and metabotropic glutamate receptors (linked through PKC), and NMDA receptors, serotonin receptors (90, 91), and alpha7 nicotinic acetylcholine receptors (92). Several of these receptor subtypes are implicated not only in synaptic modulation, but also as modulators of LTP induction (93–99). One

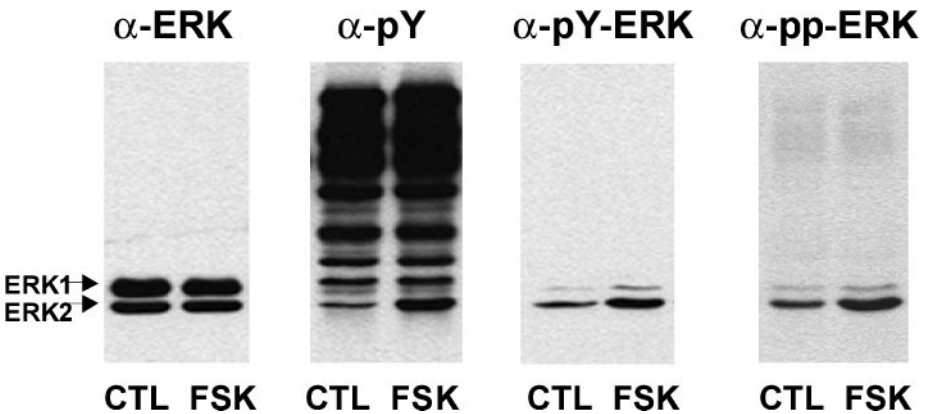

Figure 4 PKA coupling to ERK2 in area CA1. Data are presented showing that activation of PKA in area CA1 results in robust activation of ERK2. In these experiments PKA was activated using bath application of forskolin (FSK), previously observed to be efficacious in eliciting PKA activation in area CA1 (134). ERK activation was evaluated using anti-phosphotyrosine (α-pY) western blots for ERK phosphorylation, as previously described (40). To confirm that forskolin application resulted in ERK activation, two additional antibodies that selectively recognize phosphorylated activated ERK [one raised against tyrosine 204-phosphorylated ERK (pY-ERK) and one raised against threonine and tyrosine 204 dually phosphorylated ERK (ppERK)] were employed. These phosphorylation events correlate with ERK activation in a number of studies and as such are routinely used as a measure of ERK activation. With these methods, it was found that application of forskolin resulted in a substantial activation of ERK2 in area CA1 ($236 \pm 22\%$ of control, $n = 23$, $p < 0.0001$). This effect was not mimicked by the inactive forskolin analog dideoxyforskolin ($112 \pm 6\%$ of control, $n = 7$). In addition, forskolin application had little effect on ERK1; this is consistent with previous observations of selective activation of ERK2 by various stimuli in area CA1 of the hippocampus (40, 42).

interesting implication of all these findings is that modulation of synaptic plasticity by various neurotransmitter systems converging in the hippocampus may not be due directly to the PKA and PKC activation, but instead secondary to PKA or PKC activation of ERK.

It is interesting to note that in some cases in neurons, the two isoforms of ERK, ERK1 and ERK2, seem to be differentially regulated (see Figure 4, for example). Several groups have found that in neurons, both glutamate receptor activation and activation of PKC can lead to the selective activation of ERK2 (40–42, 100). The most likely reasons for selective activation are specific activation by upstream kinases, localization, and scaffolding. It does not seem likely based on in vitro evidence that the selective activation of ERK2 is due to selective phosphorylation by MEK1 or 2. For example experiments using purified MEK1 (the major MEK isoform in neurons; see below), ERK1 and ERK2 have demonstrated that MEK1 phosphorylates and activates both ERK1 and ERK2 with similar kinetics (101).

Another possibility is that ERK1 and ERK2 have distinct cellular distributions in neurons and that MEK1 is either localized globally or compartmentally via scaffolding proteins with ERK2. While these ideas are exciting, no evidence exists to date for specific localization of ERK1 and 2 in different cellular compartments. However, genetic evidence clearly points to some important differences between ERK1 and ERK2: ERK1 knockout mice are viable and appear to be neurologically normal (102), while ERK2 knockout mice are embryonic lethal (G. Landreth, personal communication). Clearly these two isoforms must have some differential functions such that a knockout of ERK1 can be compensated for, while a knockout of ERK2 is unable to be overcome and is so detrimental that the animals do not survive even to birth.

Finally, while the activation of ERK is of course important, these kinases do need to be turned off. The inactivation of ERKs seems largely to be mediated by dual-specific threonine/tyrosine phosphatases in much the same way that activation is mediated by the dual-specific kinases MEK1/2 (103–107). The dual-specific threonine/tyrosine phosphatase MKP-3 appears to be the most prominent regulator of ERK 1 and 2 dephosphorylation in neurons (106, 108). Regulation of the level of ERK activation at any time is probably determined by a balance between the activity of dual-specific kinases and dual-specific phosphatases. As an interesting note it seems that an increase in the activity of the ERK may play a role in its own regulation: In several cell types, ERK activation leads to an increase in the transcription of these dual-specific phosphatases (103, 107). Thus the ERK pathway can initiate its own inactivation via this system of negative feedback.

EFFECTORS OF ERK

Previously well-characterized effectors of ERK include a diverse set of targets that can be divided into roughly three groups: cytoskeletal proteins, nuclear proteins, and signaling proteins (reviewed in 65, 75). The cytoskeletal proteins include MAP-2 and Tau. The nuclear proteins include the transcription factors c-Myc, c-Fos and c-Jun, Elk-1, C/EBP β (CREB/Elk binding protein), and ATF-2. The signaling proteins include signaling molecules such as phospholipase A_2, as well as other kinases such as RSK (ribosomal S6 kinase). Many of these ERK substrates are likely effectors in ERK in the context of neuronal plasticity and memory. However, in the next section we focus on two potential effectors of ERK we think likely to be particularly important in neurons: the transcription factor CREB and the potassium (K^+) channel Kv4.2.

The Transcription Factor CREB

The cAMP response element binding protein, CREB, has been implicated in memory in organisms from invertebrates to mammals. For example, transgenic mice

containing a null mutation of CREB have deficits in long-term memory but not short-term memory. In these experiments, hippocampal long-term memory was assessed using contextual fear conditioning and the Morris water maze tasks (109, 110). Guzowski et al. also performed experiments based on more direct inhibition of CREB. They injected antisense oligonucleotides to CREB bilaterally into the hippocampus. When tested using the Morris water maze, these animals were shown to have defective long-term memory (111). This approach is a nice complement to gene knockout studies in that it rules out several confounds owing to developmental and compensatory expression problems.

CREB is involved in plasticity at the cellular level. Impey et al. have shown that LTP is associated with an increase in CREB-driven gene expression (112). Additionally, experiments using antibodies against CREB phosphorylated at serine 133 have shown that LTP can produce increases in CREB phosphorylation in cultured hippocampal neurons as well as in area CA1 (112, 113). Further experiments using these antibodies in immunocytochemical techniques have shown that CREB phosphorylation increases in the dentate gyrus of the rat 24 hours after induction of LTP, following high-frequency stimulation of the perforant path in vivo (114).

As first reported by Frey and confirmed by many other laboratories, application of protein synthesis inhibitors to hippocampal slices blocks the expression of late-phase LTP in hippocampal area CA1 (115). Similarly, LTM also requires new protein synthesis (reviewed in 116). A likely hypothesis, and one born out of experimental data, is that CREB is a necessary component of the mechanisms operating to increase the synthesis of certain proteins necessary for the formation of LTP and LTM. As it is known that ERK is required for the induction of stable, late-phase LTP and is required for LTM as assessed by several behavioral tasks, it seems a likely hypothesis that ERK might lie upstream of CREB phosphorylation in the hippocampus (reviewed in 117, 118).

Regulation of the transcription factor CREB is both complex and simple. It is complex in the sense that CREB can be activated and phosphorylated via several kinases, including PKA, RSK2, and CaMKI and IV. Furthermore, the kinase CaMKII can be either a poor activator of CREB or an inhibitor when it phosphorylates additional sites besides serine 133 (119–122). It is simple in the sense that each of these kinases leads to phosphorylation of CREB on the same moiety, serine 133 (120, 122, 123). Phosphorylation of serine 133 recruits the CREB binding protein, CBP, to the complex and promotes transcription of downstream genes (124). A final twist is that CREB is an indirect effector of ERK—the activation of CREB by ERK is accomplished via activation of an intervening kinase such as RSK2 (125, 126). Many genes are activated by CREB, including the transcription factor *c-fos* and the immediate early gene *zif/268*, through which CREB signaling can indirectly activate an expanded range of genes (127, 128). CREB phosphorylation at serine 133 and subsequent binding to the CRE also leads to an increase in the transcription of other proteins including synapsin-1 (129) and the alpha subunit of CaMKII (130).

Regulation of CREB Phosphorylation in the Hippocampus

In biochemical studies investigating CREB phosphorylation in the hippocampus, CREB was determined to be a downstream target of ERK in hippocampal area CA1 following PKA and PKC activation (89, 131). The observation that ERK contributes to the regulation of CREB phosphorylation by the PKA pathway was rather surprising, as clearly PKA is capable of directly phosphorylating CREB (reviewed in 132). However, the MEK inhibitor U0126 essentially completely blocks forskolin-induced CREB phosphorylation in hippocampal area CA1, which indicates that PKA must use ERK as an intermediate in this reaction (89). These observations, coupled with prior findings of a necessity for ERK activation in LTP and a transient activation of PKA in LTP (42, 133, 134), suggests that ERK activation may play an obligatory intermediate role in PKA regulation of gene expression in late-phase LTP or other forms of long-term synaptic plasticity.

Moreover, the PKC system was also observed to regulate hippocampal CREB phosphorylation via ERK. This observation suggests a new role for PKC in the hippocampus: regulation of CREB-mediated alterations in gene expression. Moreover, as PKC has been observed to regulate ERK activation in a variety of cells, it will be interesting to determine if a general role of PKC in various systems is to control CREB phosphorylation (135). It should be noted that application of the MEK inhibitor U0126 did not block all of the phorbol-ester–induced CREB phosphorylation in the hippocampus. It is interesting to speculate that activation of PKC may lead to CREB phosphorylation via ERK and through a more direct route.

In more recent studies Wu et al. examined the kinetics of CREB phosphorylation following K^+ depolarization of hippocampal pyramidal neurons in culture (136). The authors found that the kinetics of the depolarization-induced, ERK-mediated CREB phosphorylation were slow, increasing slowly over the course of an hour. These data suggest that ERK contributes to a late-phase of CREB phosphorylation, and it will be interesting to see how this "delayed" pathway might uniquely contribute to triggering long-term changes in gene expression and thus long-term synaptic plasticity.

Although the CREB protein is located in the nucleus, CREB mRNA is also found in isolated dendrites from primary neuronal cultures generated from embryonic rat hippocampi (137). An interesting issue is whether ERK, RSK2, or both need to translocate to the nucleus to induce phosphorylation of CREB, or whether CREB is transcribed and phosphorylated locally in the dendrite prior to its translocation to the nucleus. Translocation of ERK from the cytosol to the nucleus is seen in cultures of different cell lines (reviewed in 138), and in mouse hippocampal area CA1 pyramidal cells (50). Chen et al. additionally reported that the ERK target RSK was also phosphorylated and translocated to the nucleus following the growth factor stimulation of HeLa cells (139). So it seems that in hippocampal neurons, ERK, RSK, or CREB may all translocate from the cytosol of the cell body to the nucleus, where they are available to mediate CREB-based transcription. How

exactly these translocation events might occur is a mystery and an important area for future exploration.

The K^+ Channel Kv4.2

It has recently been discovered that A-type voltage-dependent K^+ channels localized to the dendrites of hippocampal pyramidal neurons play a critical role in shaping the electrical responses of these neurons. The probable functions of A-type channels in general are to repolarize the membrane, contribute to the resting potential, and regulate firing frequency (140). Concerning the specific roles of A-type channels in neurons, Johnston and colleagues have proposed a model in which A-type channels in distal dendrites of the hippocampus are critical regulators of back-propagating action potentials, which regulate LTP induction through controlling voltage-dependent NMDA receptor activation.

Johnston and colleagues found that application of activators of PKA or PKC shifts the activation curve of A-type K^+ currents recorded in hippocampal area CA1 dendrites in the depolarizing direction, leading to increases in dendritic excitability and increased back-propagating action potentials in dendrites (141). They also found that application of the MEK inhibitors PD098059 or U0126 significantly shifts the activation curve of A-type K^+ currents in hippocampal area CA1 dendrites in the hyperpolarizing direction (Johnston et al., unpublished observations). Moreover, they have found that application of U0126 blocks the increase in back-propagating action potential amplitude caused by application of PKA, PKC, or β-adrenergic receptor activators (L. Yuan & D. Johnston, personal communication). As described above, activation of PKA and PKC, as well as stimulation of β-adrenergic receptors, leads to ERK activation in hippocampal area CA1. These data suggest that the modulation of dendritic A currents by PKA and PKC may be due to ERK phosphorylation of A-type potassium channels.

One particular subtype of A-type potassium channel protein, Kv4.2, is localized to subsynaptic compartments of somas and dendrites in area CA1 of the hippocampus, and is likely the pore-forming subunit of dendritic A-type channels in CA1 pyramidal neurons (142). It is interesting that examination of the Kv4.2 amino acid sequence reveals several possible MAPK phosphorylation sites located in the C-terminal domain of the channel.

In a recent series of studies (143), we tested the idea that Kv4.2 might be a target for ERK and we found that Kv4.2 is a good substrate for ERK in vitro. We identified the amino acids at which ERK phosphorylates Kv4.2 in vitro, and then used this knowledge to develop tools with which to study the phosphorylation of Kv4.2 by ERK: phosphoselective antibodies. Using one of these phosphoselective antibodies, JPA170, we assessed the phosphorylation of Kv4.2 by ERK in a cell expression system as well as in the hippocampus. We found that Kv4.2 is an ERK substrate in these various cell types, and that ERK-phosphorylated Kv4.2 exhibits an interesting pattern of synaptic input-specific labeling in the hippocampus and other brain regions [see Figure 1 and (144)]. Overall these data are very

supportive of the idea that the potassium channel Kv4.2 is a target of ERK in neurons.

In additional recent studies, we have found that activation of PKA and PKC, as well as stimulation of β-adrenergic receptors, leads to ERK activation and Kv4.2 phosphorylation by ERK in hippocampal area CA1 (J. Adams & D. Sweatt, unpublished observations). These data suggest that the modulation of A currents by PKA and PKC may be due to ERK phosphorylation of Kv4.2. Our working hypothesis is that ERK phosphorylation of Kv4.2, the K^+ channels thought to mediate A currents in hippocampal dendrites, decreases the probability of current passing through the channel. Once a population of these channels in a particular dendrite is essentially nonfunctional owing to phosphorylation, the ability of a back-propagating action potential to invade that particular dendrite increases. This will increase further the likelihood of NMDA receptor activation and Ca^{2+} influx locally and enhance the possibility of inducing LTP at that synapse.

A variety of available physiologic data support this hypothesis. Several of these experiments have used an LTP-inducing protocol consisting of stimuli delivered at 5 Hz, a protocol designed to be similar to the theta rhythm recorded in the rodent hippocampus during some exploring tasks (see 31). This 5-Hz stimulation protocol, often termed "theta frequency stimulation" (5 Hz stimulation presented for 5 seconds to 3 minutes) or "theta burst stimulation" (bursts of HFS delivered at 5 Hz) causes complex spike bursting in area CA1 cells, which can back-propagate into the dendrites and depolarize synapses. These complex spike bursts are seen during in vivo recordings from area CA1 pyramidal cells (145). Several groups have shown that blocking ERK activation with PD098059, U0126, or SL327 in the mouse blocks not only the complex spike bursting seen in the theta-frequency stimulation protocol, but also the LTP that is so induced (31, 32). It seems likely that blocking ERK in these experiments decreases the phosphorylation of Kv4.2, leading to an increase in the probability of current flux through these channels.

Overall, while investigations of the roles of ERK regulation of K^+ channels in general, and Kv4.2 in particular, are at an early stage, it seems likely that this target of ERK may play a particularly important role in ERK signaling in neurons.

BIOCHEMICAL INFORMATION PROCESSING BY ERK

In the next section we turn our attention to considering why the ERK cascade might be particularly suited to the role of regulating neuronal plasticity and memory formation, approaching the question from a biochemical perspective. As the material above makes clear, regulation of the ERK cascade is quite complex in general, and ERK regulation appears to be especially complex in neurons. Why must the system be so complicated? What is the advantage to the cell that such a complex system may afford? In the following paragraphs we speculate on some potential ways the neuron might capitalize on the complexity of ERK regulation, with a particular emphasis on possible mechanisms for information processing at the cellular and biochemical level.

ERK Can Function as a Biochemical Switch

One question that has intrigued MAPK biochemists for a decade is why the system is set up as a serial three-kinase cascade. The question arises from the fact that linking three enzyme catalysts in series confers the potential for enormous signal amplification, seemingly more than the cell would ever possibly require. In this context it is interesting that a hallmark of highly amplified systems is that they can operate in an essentially all-or-none fashion. As has been elegantly explored by Ferrell & Machleder (146), the ERK cascade has this capacity. Their studies in oocytes have shown that ERK can function digitally—rapidly converting from a state of very low activity to one of essentially maximal activation. In neurons this capacity might be capitalized upon to serve as an all-or-none biochemical switch for triggering plasticity.

ERK Can Function as a Coincidence Detector

Recent seminal work by O'Dell's group has shown that the neuronal ERK cascade can operate as a biochemical coincidence detector (39). These investigators showed that the simultaneous presence of beta-adrenergic receptor stimulation plus muscarinic acetylcholine receptor stimulation led to ERK activation in hippocampal neurons when the presence of either stimulus alone gave essentially no effect. This is a compelling specific example of what may be a general role of the neuronal ERK cascade: intracellular integration of diverse cell-surface signals (reviewed in 118).

In these same studies Watabe et al. also provided a beautiful example of a functional consequence for this coincidence detection in regulating the induction of synaptic plasticity (39). They showed that the coincident presentation of beta-adrenergic receptor agonists and muscarinic receptor agonists promoted the activity-dependent triggering of hippocampal LTP that was blocked by inhibition of ERK activation. Thus in this system the ERK cascade is participating in a mechanism for three-way coincidence detection (at a minimum), wherein potentiation is selectively triggered by the simultaneous presence of two cell-surface signals when coupled with synaptic activity.

As described above, we and others hypothesize that one likely site of action of ERK is dendritic potassium channels, where ERK may regulate back-propagating action potentials and thereby control NMDA receptor activation. Incorporating this hypothesis with the observations of Watabe et al. and others allows the formulation of a model for potential four-way coincidence detection as a gate for triggering plasticity. Briefly stated, ERK regulation of action potential back-propagation could allow for LTP to be selectively triggered under very specific conditions: when activation of two neuromodulatory inputs is coupled with glutamate at the synapse plus firing of an action potential in the cell body. Such sophisticated control over the triggering of long-term synaptic change would confer powerful information-processing capacity in the context of the behaving animal. In fact, while the mechanisms are unclear at present, Schafe et al. have found evidence

for one specific part of this model in the behaving animal–specific coincidence-dependent ERK activation in the amygdala with Pavlovian fear conditioning in rats (5).

ERK Can Store Information

Several groups, using a variety of approaches in vivo, in vitro, and in silico have shown that under some circumstances ERK can be persistently activated (147, 148). Thus, a brief environmental or cell surface signal can lead to long-lasting (theoretically permanent) ERK activation in specific brain regions or cells. This is of course biochemical information storage, and mechanisms of this sort must underlie memory at some level in the animal. At the present time it is unclear what the biochemical mechanisms for the persistent activation are, in those cases where the phenomenon has been observed experimentally in neurons. The most intriguing possibility is positive-feedback regulation of upstream activators of ERK, mediated through ERK-dependent regulation of the expression of genes for these activators. A positive feedback loop of this sort is a potential self-reinforcing information storage mechanism (149).

ERK Can Function as a Temporal Integrator

One attribute of most forms of learning is that repetitive spaced stimulation tends to elicit the most robust and long-lasting memories. Wu et al. recently reported an analogous biochemical effect in hippocampal neurons—repetitive spaced membrane depolarization selectively elicited persistent ERK activation and ERK-dependent structural changes in the dendrites of hippocampal neurons in culture (147). Thus in this system, by mechanisms yet to be worked out, ERK appears to be serving as a read-out of temporal integration of cell signals. It will be very interesting to determine the biochemical basis of this unique mode of ERK regulation, and to determine if indeed such a mechanism contributes to temporal integration of spaced stimuli in the behaving animal.

Blockade of ERK Activation May Serve as a Synaptic Lock

Finally, it is worth noting that typical transcriptional targets of ERK are genes encoding MAP kinase phosphatases (MKPs). MKPs are dual-specificity phosphatases that dephosphorylate ERK and thereby inactivate the enzyme. The MKPs are proposed to thereby serve a negative feedback function to turn off ERK after it is activated, acting as an inhibitory feedback response mechanism. While speculative at present, it is possible that ERK-dependent induction of MKPs might serve to lock the synapse or cell in an aplastic state for some period of time, at least as far as further ERK-dependent plasticity is concerned. This may serve a function in the consolidation of memory by protecting a given neuronal site from alteration by subsequent signals.

CONCLUDING REMARKS

A wide variety of psychopharmacologic studies makes it clear that subtle chemical changes can have profound cognitive effects in humans. In Alzheimer's disease, overproduction of a single peptide is hypothesized to lead to profound memory disorder. Understanding the chemical basis of cognition is key to our understanding of both normal function and the pathobiology of cognitive disorders. Concerning one form of cognition, learning and memory, it is clear that ERK is a key player. This key role for ERK necessitates understanding the regulation of ERK in the neuron and the neuronal targets of ERK that mediate its functions. The information obtained from studies of ERK regulation and its targets is sure to be useful because the behavioral and physiologic role of ERK is clear from numerous studies in vitro and in vivo. What is likely to come out of further biochemical studies of the ERK cascade in the nervous system is an understanding of information-processing at the biochemical level, and an understanding of how the cell utilizes signal transduction mechanisms to perform complex logical operations. The hope is that by understanding these biochemical issues, and incorporating the information into the appropriate cellular and anatomical context, we will ultimately achieve an understanding of the molecular basis of memory.

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