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ERK phosphorylation is required for retention of trace fear memory

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Abstract

The extracellular signal-regulated kinase (ERK) has been previously associated with long-term memory formation. Earlier studies have demonstrated a role for phospho-ERK in delay fear conditioning and it has been shown to disrupt trace fear memory when inhibited after training. cAMP response element binding protein (CREB) is a key transcription factor that has been implicated in long-term memory formation across different species. It has also been shown to be modulated by ERK. In our study, we used the drug SL327 to prevent ERK phosphorylation. Two groups of Fischer 344 male rats (2–4 months) were injected intraperitoneally with 100% DMSO (2 ml/kg) or SL327 (100 mg/kg/2 ml dissolved in DMSO) 45 min before 10 trials of trace fear conditioning. Each trial consisted of a tone paired with a footshock with a 30-s interval separating the stimuli. Twenty-four hours later, rats were tested for fear to the tone. Our results showed that SL327-treated rats displayed memory deficits 24 h after training. Western blot analyses of total hippocampal protein revealed a significant increase in phosphorylated ERK immediately after training. There were also decreases in phosphorylated ERK at 45 and 90 min post-injection of SL327-treated rats as compared to DMSO-treated rats, but levels of phosphorylated CREB remained the same. These findings indicate that ERK phosphorylation is increased immediately after trace fear conditioning and inhibiting this increase is correlated with memory deficits in trace fear conditioning 24 h later. These findings support a role for ERK phosphorylation in the formation of trace fear memories.

Keywords: Trace fear conditioning; Extracellular-signal regulated kinase; cAMP response element binding protein; Learning and memory

1. Introduction

The mitogen-activated protein kinases (MAPK) are a family of kinases that take part in signal transduction pathways from the membrane to the nucleus. They participate in a variety of cellular programs which include cell division, cell differentiation, cell movement, and cell death. The family members include the extracellular signal-regulated kinases 1 and 2 (ERK 1 and ERK 2), c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPK), p38 MAPK, ERK 3, and ERK 5 (English et al., 1999; Schaeffer & Weber, 1999; Seger & Krebs, 1995).

Although classically studied in cellular growth processes, ERK signaling pathways are now being implicated in regulation of neuronal function. The ERK cascade is composed of three kinases that are consecutively phosphorylated. The first kinase is Raf-1, which in turn phosphorylates the second kinase, MEK (mitogen-activated, ERK-activating kinase) by serine/threonine phosphorylation. MEK then activates ERK through dual phosphorylation of both a threonine and tyrosine residue (English et al., 1999; Seger & Krebs, 1995). Interestingly, immunohistochemical studies show that ERK is prominently found in the neocortex, hippocampus, striatum, amygdala, and cerebellum, which are all areas involved in learning and memory processing (Fiore et al., 1993; Flood et al., 1998; Ortiz et al., 1995). More specifically, ERK is found in the cell bodies and dendrites of the dentate gyrus, CA3, and CA1 hippocampal areas (Fiore et al., 1993; Flood et al., 1998).

Because ERK is found in several brain regions associated with learning and memory, numerous studies are

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now focusing on ERK in the regulation of neuronal function and memory processing. Numerous studies have shown that the ERK cascade is important in both invertebrate and vertebrate memory systems. Long-term facilitation in *Aplysia* is inhibited with the addition of antibodies against phosphorylated ERK or MEK inhibitors in the presynaptic cell (Martin et al., 1997). Crow, Xue-Bian, Siddiqi, Kang, and Neary (1998) also show that in vitro one-trial conditioning and multi-trial Pavlovian conditioning in *Hermissenda* results in the activation of ERK. More recently, phosphorylation of extra-nuclear ERK in the crab Chasmagnathus was important for long-term memory consolidation (Feld, Dimant, Delorenzi, Coso, & Romano, 2005). Additionally, levels of phosphorylated ERK increase in the insular cortex by a taste experience that produces a long-term taste memory. Equally important, inhibiting ERK phosphorylation in the insular cortex before exposure to a novel taste in a conditioned taste aversion task causes long-term taste memory deficits (Berman, Hazvi, Rosenblum, Seger, & Dudai, 1998). Hippocampally associated tasks such as the Morris water maze, contextual fear conditioning, and inhibitory avoidance require ERK phosphorylation as well. Using a specific inhibitor (SL327) of MEK to prevent phosphorylation of ERK, studies show that mice are impaired in contextual learning as well as spatial learning (Selcher, Atkins, Trzaskos, Paylor, & Sweatt, 1999). Additionally, another inhibitor of MEK, PD098059, blocked long-term spatial memory in rats trained in the water maze (Blum, Moore, Adams, & Dash, 1999). Atkins, Selcher, Petraitis, Trzaskos, & Sweatt (1998) show that ERK is activated in the hippocampus after tone and contextual conditioning and is needed for consolidation of this learning. Furthermore, studies using PD098059 have shown that hippocampal ERK is involved in retention of an inhibitory avoidance task, specifically in a time-dependent manner (Walz et al., 1999, 2000). Transient increases in ERK phosphorylation can also be seen in the amygdala after delay fear conditioning, and infusion of U0126 into the lateral amygdala impairs long-term memory of this fear conditioning (Schafe et al., 2000). These studies strongly support a role for ERK phosphorylation in learning and memory processes.

cAMP response element binding protein (CREB) is a part of a large family of structurally related proteins that bind to cAMP response element (CRE)-containing promoter sites of different genes. The critical event in CREB activation is the phosphorylation of Serine133 in the kinase-inducible domain (KID), which includes concensus phosphorylation sites for protein kinase A, protein kinase C, calcium/calmodulin kinases, and RSK2. This phosphorylation can be regulated by increases or decreases in the levels of cAMP and calcium, which activate these kinases [as reviewed in (Silva, Kogan, Frankland, & Kida, 1998; Lamprecht, 1999)]. The involvement of CREB in learning and memory processes has been extensively studied across different species (*Aplysia*, *Drosophila*, rat, and mouse). Long-term facilitation in *Aplysia* cultured neurons was disrupted after injection of oligonucleotides with CRE sequences (Dash, Hochner, & Kandel, 1990). In *Drosophila*, pretraining disruption of CREB function through transgenic expression of a dominant-negative CREB protein caused long-term memory deficits in olfactory learning and memory (Yin et al., 1994). Mice with a $CREB^{\alpha\Delta}$ mutation have long-term memory impairments in contextual fear conditioning, the Morris water maze, and the social transmission of food preferences (Bourtchuladze et al., 1994; Kogan et al., 1997). Guzowski & McGaugh (1997) showed that pretraining intrahippocampal infusion of CREB antisense oligodeoxynucleotides (ODN) produce disruptions in CREB protein levels and that this disruption impaired retention in rats trained in the Morris water maze, suggesting an importance of CREB in the consolidation of memory processes initiated at the time of training.

Trace fear conditioning is a task in which the subject must learn to associate a conditioned stimulus (CS; tone) with an unconditioned stimulus (US; foot shock) when they are separated over time. This information is referred to as being temporally discontiguous. Rawlins (1985) has suggested that most tasks sensitive to hippocampal damage contain some form of temporally discontiguous information, which must be used to solve the learning task correctly. Young rats with hippocampal lesions are impaired in both trace eye blink and trace fear conditioning (McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; Weiss, Bouwmeester, Power, & Disterhoft, 1999). Thus, the hippocampus is important for connecting temporally discontiguous events.

Recently, Runyan, Moore, & Dash (2004) examined the role of the medial prefrontal cortex in trace fear conditioning through inhibition of the ERK cascade. Pre-training prefrontal cortex infusions of the MEK inhibitor, U0126, impaired trace memory retention. In subsequent experiments, post-training infusions of U0126 in the hippocampus also disrupted trace memory retention. However, their behavioral protocol differed from ours in that they used a shorter tone, a shorter trace period, and a lower shock intensity and duration. Also, freezing was measured only during the trace period of testing. Additionally, post-training hippocampal infusions of U0126 were used, which only allow for study of consolidation and does not address acquisition of trace fear conditioning. In our experiments, we used pre-training intraperitoneally (IP) injections of the MEK inhibitor SL327 to investigate acquisition and consolidation of trace fear conditioning. Furthermore, they investigated the levels of phosphorylated ERK after trace fear conditioning and found an increase at 1 h after training (Runyan et al., 2004).