Research

Foreground contextual fear memory consolidation requires two independent phases of hippocampal ERK/CREB activation

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Fear conditioning is a popular model for investigating physiological and cellular mechanisms of memory formation. In this paradigm, a footshock is either systematically associated to a tone (paired conditioning) or is pseudorandomly distributed (unpaired conditioning). In the former procedure, the tone/shock association is acquired, whereas in the latter procedure, the context/shock association will prevail. Animals with chronically implanted recording electrodes show enhanced amplitude of the extracellularly recorded field EPSP in CA1 pyramidal cells for up to 24 h after unpaired, but not paired, fear conditioning. This is paralleled by a differential activation of the ERK/CREB pathway in CA1, which is monophasic in paired conditioning (0–15 min post-conditioning), but biphasic (0–1 h and 9–12 h post-conditioning) in unpaired conditioning as revealed by immunocytochemistry and Western blotting. Intrahippocampal injection of the MEK inhibitor U0126 prior to each phase prevents the activation of both ERK1/2 and CREB after unpaired conditioning. Block of any activation phase leads to memory impairment. We finally reveal that the biphasic activation of ERK/CREB activity is independently regulated, yet both phases are critically required for the consolidation of long-term memories following unpaired fear conditioning. These data provide compelling evidence that CA1 serves different forms of memory by expressing differential cellular mechanisms that are dependent on the training regime.

From invertebrates to mammals, long-term memory, but not short-term memory, requires the induction of new mRNA and protein synthesis for the development or reorganization of networks that support the engram (Dudai and Eisenberg 2004). Since the pioneering work of Grecksch and Matthies (1980), more recent data confirmed that memory consolidation might rely on two protein-synthesis-dependent phases (Freeman et al. 1995; Rose 2000; Igaz et al. 2002). In fear conditioning, this biphasic activation is a dynamic process as the development of one or two waves of protein synthesis is dependent on the level of training (Bourtchouladze et al. 1998). Different forms of memories may also engage differential molecular mechanisms, and it is therefore indicated to examine the molecular pathways upstream to protein synthesis.

This has been recently highlighted by genetic and behavioral studies showing that different types of hippocampus-dependent memories can differ in their underlying molecular mechanisms (Mizuno and Giese 2005). As a corollary, one might expect that each learning paradigm implicating hippocampal function could result in a specific “molecular signature” that could be characterized either by the signaling pathway involved or by the kinetics of activation of a critical signaling pathway.

Upstream to protein synthesis, the cAMP/PKA/CREB pathway has been proposed as a major candidate sustaining the two waves of transcription (Bernabeu et al. 1997; Bourtchouladze et al. 1998; Stanciu et al. 2001). Extracellular signal-regulated kinase

(ERK1/2, a MAPK family member) cascade, however, has been described to be a common converging route for many signaling pathways like AMPc/PKA and PKC to activate CREB and other transcription factors involved in the regulation of novel protein synthesis for long-term synaptic plasticity (Impey et al. 1999; Roberson et al. 1999; Vanhoutte et al. 1999; Wu et al. 2001a) and stabilization of new memories (Bourtchouladze et al. 1994; Bernabeu et al. 1997; Taubenfeld et al. 1999; Kida et al. 2002; Pittenger et al. 2002). Interestingly, although ERK1/2 phosphorylation has been described in different brain regions with several learning paradigms (Atkins et al. 1998; Blum et al. 1999; Schafe et al. 2000), only one study reports a biphasic activation of the MAPK/ERK pathway in the insular cortex after novel taste learning (Swank and Sweatt 2001). Because some data revealed the coincident activation of both ERK1/2 and CREB (Cammarota et al. 2000; Swank and Sweatt 2001; Wang et al. 2004), we investigated the common/differential functions of ERK/CREB in consolidation of fear memory.

We used two versions of Pavlovian fear conditioning that differentially involve the hippocampus. One protocol promotes an elemental association between the conditioned tone stimulus (CS) and the unconditioned stimulus (US footshock) (paired conditioning) and requires minimal hippocampal contribution. In contrast, the second protocol favors hippocampal processing to support contextual conditioning by systematically minimizing CS/US contingency (unpaired conditioning) (Kim and Fanselow 1992; Desmedt et al. 1998, 2003). Interestingly, both types of conditioning induce fear to the context, but they are known to result in distinct contextual processing (background vs. foreground contextual conditioning) (Phillips and LeDoux 1994; Calandreau et al. 2005). We mainly focused on hippocampal CA1

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because especially when using contextual conditioning protocols, a dominant role of CA1 is proposed based on dorsal hippocampal lesion and pharmacological interventions (Hall et al. 2001; Bast et al. 2003; Lee and Kesner 2004).

In this study, we asked whether the two fear conditioning protocols induce distinct “molecular events” within the hippocampus such as differential kinetics of activation of the ERK/CREB pathway. We first examined whether the selected training protocols result in different forms of synaptic plasticity in CA1. In parallel, ERK1/2 and CREB phosphorylation was assessed at multiple time points post-conditioning in order to identify the distinct phases of activation for the two training protocols. Once this was established, the MEK (ERK1/2 kinase) inhibitor U0126 was locally administered to reveal the functional requirement of these phases in memory consolidation.

Results

CA1 field EPSPs are enhanced after unpaired, but not paired, conditioning

This experiment was aimed at demonstrating the differential involvement of the hippocampus in both forms of conditioning. Mice with chronic recording electrodes placed in the pyramidal layer of CA1 of the dorsal hippocampus were divided into two groups according to the conditioning protocol. When compared to baseline, unpaired conditioning induced an immediate and long-lasting increase in synaptic efficacy in CA1 field EPSPs. In contrast, paired conditioning produced a weak, if any, synaptic potentiation (Fig. 1). One-way ANOVA with repeated measures confirmed a significant main effect of conditioning protocol ($F_{1,11} = 5.65$, $P = 0.037$) and a significant interaction with time ($F_{9,99} = 6.53$, $P = 0.0006$). A further analysis of the time course of the field potentials in animals trained to the unpaired protocol showed a main effect of time point ($F_{9,63} = 8.89$, $P < 0.0001$) with an immediate post-training increase of the evoked field potential amplitude that started to be significant at 15 min, continued to increase over the first 9 h of recording, and lasted for at least 24 h. In contrast, the effect of time points in the paired group was not significant when compared with baseline level ($F_{9,36} = 1.24$, $P = 0.30$). Thus, these findings reveal a clear dissociation in hippocampal long-term synaptic plasticity with respect to the type of conditioning.

Monophasic and biphasic increase in pERK1/2 and pCREB in CA1 following paired and unpaired conditioning, respectively

Given that unpaired and paired conditioning induced differential modifications of synaptic plasticity in the hippocampo-septal pathway (Desmedt et al. 1998), we reasoned that these changes in hippocampal plasticity are accompanied by molecular alterations in intracellular cascades. Toward this end, the ERK/CREB pathway is of particular interest as proposed for other forms of neural plasticity (Davis et al. 2000). Hippocampal tissue harvested at different time points post-conditioning was examined with immunohistochemistry using antibodies recognizing phosphorylated forms of ERK1/2 (pERK1/2) and CREB (pCREB) (Fig. 2). It was found that both pERK1/2 and pCREB were increased in a monophasic manner immediately after paired conditioning in the CA1 of the dorsal hippocampus (Fig. 2B). However, the activation was biphasic when the unpaired protocol was applied; an early phase was identified 0–1 h post-training, and a second and also transient phase occurred 9–12 h post-conditioning (Fig. 2A,B). Analyses of variance with conditioning protocol and time (excluding 12 h of the unpaired group as this was not studied in the paired protocol) as factors yielded main effects of condition-

Figure 1. Enhancement of synaptic efficacy in the CA1 of the dorsal hippocampus after unpaired fear conditioning. Mice were chronically implanted with stimulating electrodes in the ventral hippocampal commissural pathway and recording electrodes in the contralateral pyramidal layer of the CA1. Field potentials evoked by a single-pulse stimulation of the ventral hippocampal commissural pathway were recorded at different time points either before conditioning to determine the baseline level, or after each type of conditioning (unpaired or paired conditioning). (A) Kinetic of the effect of each type of conditioning on the evoked field potential amplitudes. At different time intervals after either conditioning protocol, evoked field potentials were recorded in a neutral context. Unpaired conditioning ($n = 12$) induced a significant increase in evoked field potential amplitude in the CA1 of the hippocampus at any analyzed time points after conditioning. Paired conditioning ($n = 8$) induced no variation as revealed by statistical analyses. (B) Examples of recording curves obtained just before or 1 h after unpaired conditioning. Dashed lines represent the evoked field potential amplitudes used to establish the quantifications represented in A. Statistics: $*P < 0.05$.

ing protocol for both pERK1/2 ($F_{1,78} = 60.05$, $P < 0.0001$) and pCREB ($F_{1,78} = 6.62$, $P < 0.05$), of time (pERK1/2: $F_{6,78} = 31.78$, $P < 0.001$; pCREB: $F_{6,78} = 7.39$, $P < 0.001$), and interactions (pERK1/2: $F_{6,78} = 7.71$, $P < 0.001$; pCREB: $F_{6,78} = 7.76$, $P < 0.001$), confirming a differential pattern of ERK1/2 and CREB activation depending on the conditioning protocol (Fig. 2B). Changes in immunoreactivity of pERK1/2 and pCREB were significant after both unpaired and paired conditioning (all $F_s > 5$, $P_s < 0.001$) and a planned post hoc analysis confirmed the mono- versus biphasic nature of the activity increase in the two training regimes (see asterisks in Fig. 2B). This analysis was confirmed by Western blot experiments carried out on the hippocampus (Fig. 2C). Again, we obtained the biphasic activation pattern of CREB and ERK1/2 (mainly on pERK1) typical for unpaired conditioning, further supporting a so-far-unknown learning-associated biphasic coactivation of ERK1/2 and CREB.

The biphasic activation of ERK1/2 and CREB appeared somewhat weaker in the Western blot experiments compared to immunohistochemistry because the measures were carried out on the whole dorsal hippocampus. Indeed, further immunohistochemical analyses performed on the hippocampal CA3 and dentate gyrus also revealed a biphasic pattern of ERK1/2 activation in CA3 with a slightly different time course, or an early transient activation in the dentate gyrus (data not shown). Thus, the specific pattern of activation observed in the CA1 could not be strictly reproduced by Western blot analysis since this requires more tissue and is therefore a reflection of an overall activation of the dorsal hippocampus.

Pharmacological investigation of the functional role of the patterns of ERK1/2 activation

An unresolved issue pertains to the question whether the different phases of ERK1/2 activation induced by the two types of conditioning also have a differential role in memory consolidation. To address this issue, infusion of the MEK inhibitor U0126

in the dorsal hippocampus was performed at various time points before or after unpaired and paired conditioning in order to selectively block either the early or late phase of ERK1/2 activation (see Fig. 3A). In comparison to a non-operated control group, intrahippocampal injection of vehicle did not alter performance in either unpaired or paired conditioning ($F_{1,51} < 2.6$, $P_s \geq 0.12$) (data not shown). Thus, we pooled these groups in one control group for further analyses.

Blockade of ERK1/2 activation disrupts retention of unpaired conditioning

See Figure 3B. U0126 was infused either 30 min before or 7.5 h after unpaired conditioning to interfere with the two phases of ERK1/2 activation and at 2 h post-training to examine the effect on memory consolidation when ERK1/2 was inactive.

Given that tone and footshock were not associated during unpaired conditioning, mice showed only a weak freezing response to the tone during retention testing, and this was not reliably different from the pre-training freezing response ($F < 1$). Independent of the time of injection, U0126 treatment did not affect retention of the tone in mice ($F < 1$) (Fig. 3B, left).

In contrast, context-dependent freezing revealed pronounced differences depending on the time of U0126 injection (Fig. 3B, right). This impression was confirmed statistically (time of injection ($F_{3,49} = 12.46$, $P < 0.001$), a significant block effect ($F_{2,98} = 36.19$, $P < 0.001$), and an interaction ($F_{6,98} = 3.70$, $P < 0.01$). Post hoc planned comparisons of all treatment groups against controls yielded a reliable impairment induced by U0126 administered 30 min before or 7.5 h after conditioning ($F_s \geq 17.28$, $P_s < 0.01$), whereas U0126 did not block the retention of contextual memory when injected 2 h after conditioning ($F_{1,33} = 2.01$, $P > 0.1$). It follows that blockade of either of the two ERK1/2 activation phases impairs memory for contextual freezing.

Blockade of ERK1/2 activation disrupts retention of paired conditioning

See Figure 3C. In contrast to the unpaired conditioning protocol, paired conditioning induced significant freezing to the tone during retention testing (Fig. 3C, left). A 4×2 factorial analysis of variance with time of injection and test session revealed a marginal effect of injection time ($F_{3,48} = 2.72$, $P = 0.055$), a reliable effect of test session ($F_{1,48} = 215.65$, $P < 0.001$), and an interaction ($F_{3,48} = 4.56$, $P < 0.01$). Mice treated with the U0126 30 min before training, but not at any time afterward,

Figure 2. (Legend on next page)

Figure 3. Transient inhibition of the MAPK/ERK1/2 pathway induced long-term memory deficits depending on the type of conditioning. Means $\pm$ SEM. (A) The MEK inhibitor U0126 or vehicle (DMSO 2% in aCSF) was infused bilaterally in the CA1 area of the dorsal hippocampus, 30 min before (U0126e), 2 h (U0126i), or 7.5 h (U0126l) after conditioning. Memory retention measured as freezing was evaluated 24 h later by re-exposing animals to the tone in a neutral context (tone test), and 2 h later to the context. Sham and vehicle-infused animals, which were spread out according to the different time points of injection, were pooled into one control group (paired: $n = 26$; unpaired: $n = 27$) since no differences were detected between groups ($P \geq 0.12$). (B) U0126 infusion 30 min before (U0126e, $n = 8$) or 7.5 h after (U0126l, $n = 10$), but not 2 h after (U0126i, $n = 8$), conditioning blocked freezing responses to the context normally observed. Freezing to the tone was absent and not altered by drug. (C) Mice trained in paired conditioning exhibited a high freezing response to the tone when tested 24 h later. U0126 infused 30 min before the conditioning (U0126e, $n = 8$) impaired the retention to the tone, whereas the injection at later time points had no effect (U0126i, $n = 8$; U0126l, $n = 10$). Moreover, although these animals displayed a high level of freezing when re-exposed to the context of conditioning, U0126 had no effect on this response. Statistics: $*P < 0.05$; $***P < 0.001$.

exhibited a significantly lower freezing response to the tone in the retention test relative to controls ($P < 0.05$). In addition to the unpaired conditioning results, present data confirm that blockade of CA1 ERK1/2 activation during training in a paired conditioning task disrupts memory formation.

Exposure to the context induced a freezing response that revealed the overshadowing effect of the context by the tone classically observed after tone-US conditioning. However, this behavior was not affected by U0126 administration (time of injection, $F < 1$) (Fig. 3C, right).

The interesting issue, therefore, is that despite the ERK1/2 inhibition during the same time window in the unpaired and paired task, contextual freezing is impaired only in the former, but not the latter task. This seems to reflect a task-specific change in ERK1/2 activation within CA1 of the hippocampus.

Inhibition of ERK1/2 activation does not affect short-term memory

Memory impairments induced by inhibition of ERK1/2 activation phases suggest a blockade of molecular mechanisms underlying consolidation of long-term memory. However, it could also be argued that pre-training drug administration may interfere with the learning/encoding process and not with consolidation per se. A corollary of such an interpretation is that both long-term and short-term memory should be impaired in animals treated with U0126 during training. We used unpaired and paired conditioning paradigms to assess this prediction and exposed both control and U0126 groups to a short-term memory test 1 h after conditioning. U0126 did not affect freezing to the context or to the tone in this test session (Fig. 4) ($F_s < 1$), suggesting that the ERK1/2 signaling pathway in CA1 is not involved in encoding and in retrieval of short-term memory. This supports our initial contention that activation of ERK1/2 is crucially involved in molecular processes underlying long-term memory consolidation.

Functional coupling between ERK1/2 and CREB

Given the similar time course of activation for both ERK1/2 and CREB in hippocampal CA1 following conditioning, we suggested that ERK1/2 may be functionally associated to CREB activation. This was assessed further by intrahippocampal injection of U0126 performed 30 min before unpaired conditioning, followed by measurement of ERK1/2 and CREB phosphorylation 15 min after the conditioning. Figure 5, B and C, shows that conditioning-induced activation of ERK1/2 and CREB was abolished by the MEK inhibitor U0126 (Student t -test: respectively, $t_3 = 4.61$, $P < 0.01$; and $t_3 = 3.24$, $P < 0.05$) (Fig. 5C). There was a near complete disappearance of pERK1/2 labeling in both dendritic and somatic compartments and a return to near basal levels for pCREB within the hippocampal CA1 (Fig. 5C, compared with control group in Fig. 2B). In contrast, the injection of U0126 carried out 2 h after unpaired conditioning did not affect the expression of the late activation phase ($P \geq 0.1$) (Fig. 5D,E), which is consistent with our behavioral data. When U0126 was

Figure 2. ERK1/2 and CREB phosphorylation induced by unpaired compared to paired conditioning. (A) Immunocytochemical analyses of pERK1/2 (left) and pCREB (right) at different time intervals following context conditioning. pERK1/2 labeling was prominent in both the dendritic and somatic compartments with peak levels attained at 15 min and 9 h post-training. At baseline (home cage animals; ctrl), all somata were pCREB positive, but the intensity increased at 15 min and 9 h post-training. (B) Quantitative analyses of immunocytochemical labeling of pERK1/2 (left) and pCREB (right) revealed different activation patterns in paired (top row) and unpaired conditioning (bottom row). Paired conditioning induced an early transient activation phase of both pERK1/2 and pCREB in the CA1 of the dorsal hippocampus ($n = 6$ for each experimental group, $n = 13$ for the control group) with no further activation at later time points. Unpaired conditioning, in contrast, produced two peaks of pERK1/2 and pCREB at 0–1 h and around 9 h post-training ($6 < n < 10$ for each experimental group). Mean $\pm$ SEM. (C) Representative Western blots showing pERK1/2 and ERK1/2 (left) as well as CREB and pCREB levels (right) from hippocampal extracts from mice sacrificed at different time intervals after unpaired fear conditioning. The biphasic pattern of phosphorylation as seen in immunocytochemistry was confirmed (for ERK1/2, mainly on the ERK1 [p44] isoform). Note that total ERK1/2 and CREB protein levels were not affected at any delay. Statistics: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Figure 4. Short-term memory processes were not affected by U0126 administered 30 min before paired (left, $n = 4$) or unpaired (right, $n = 4$) conditioning. Short-term memory was tested 1 h after training. Means $\pm$ SEM.

injected 7.5 h after conditioning and mice were killed at the time point of 9 h, the MEK inhibitor blocked both pERK1/2 and pCREB (t -test: respectively, $t_{10} = 2.86$, $P < 0.05$; and $t_{10} = 10.85$, $P < 0.001$) (Fig. 5D,E). In addition, U0126 effect appears to be restricted to the CA1 area, as pERK labeling was not affected in the CA3 at the two studied delays (15 min and 9 h) (data not shown). Together, these findings suggest that the CA1 activation of ERK1/2/CREB pathway is a necessary step required for long-term memory formation of contextual representations when the context is in the foreground.

Relationship between the two activation phases

In the last experiment, we addressed the issue whether the late activation of both ERK1/2 and CREB is dependent on the occurrence of the early phase. U0126 was infused 30 min prior to unpaired conditioning, and pERK1/2 and pCREB were measured 9 h after training. Interestingly, the blockade of the early phase did not affect the levels of pERK1/2 and pCREB that were normally observed 9 h after unpaired conditioning (t -test: both $t_s \geq 0.33$, both $P_s > 0.7$) (Fig. 5D,E).

Although we have demonstrated that both early and late phosphorylation of ERK1/2 are required for long-term memory consolidation, the latter finding indicates that the late phase occurs independently of the early one and, thus, is not merely a molecular consequence of the early phase.

Discussion

The present report provides immunochemical and pharmacological evidence that the ERK1/2 activation in CA1 is required for long-term memory formation of foreground contextual conditioning and, to a lesser extent, of paired conditioning. Although some data have already hinted at the existence of two critical protein synthesis activation periods for memory consolidation, our results are the first to firmly establish that the second phase of ERK1/2 and CREB activation observed after unpaired conditioning is independent of the occurrence of the earlier activation phase, yet both are necessary for long-term memory formation.

Dorsal hippocampus and fear conditioning

There is strong support in favor of a prominent role for the dorsal hippocampus in contextual fear conditioning (Phillips and LeDoux 1992; Anagnostaras et al. 2001; Matus-Amat et al. 2004). However, some findings apparently weaken this contention by showing that lesions of the dorsal hippocampus induce normal levels of conditioned freezing to the context (Phillips and LeDoux 1994; Maren et al. 1997). To address this question, recent studies in our laboratory have shown that hippocampal lesions were more detrimental to the processing of the contextual cues when the CS and the US were unpaired as opposed to when they

were paired (Desmedt et al. 2003). Thus, the conditioning procedure may modulate the hippocampal-dependent processing of contextual cues as a function of the salience of the contextual stimuli with respect to the discrete tone-CS (Desmedt et al. 2003). On this basis, our electrophysiological data provide evidence that long-term synaptic plasticity within the CA1 depends on the relation of contingency between the discrete CS and the US. When the CS was randomly distributed with regard to the US, thus allowing contextual cues as the primary stimuli that enter into association with the US, a marked long-lasting strengthening was observed in CA1 synaptic efficacy. This suggests strong contribution of the hippocampus to the consolidation processes of context-US associations. On the contrary, pairing the CS with the US fostered elemental associations. This procedure produced no noticeable change in CA1 synaptic plasticity, suggesting that, when background contextual cues are overshadowed by the discrete cue, hippocampal contribution to memory consolidation may be marginal. Thus, these electrophysiological data advocate a prominent role for hippocampal CA1 in memory consolidation of contextual representations as long as the contextual cues are the primary fear-associated stimuli. This is also substantiated by our immunohistochemical experiment showing a biphasic pattern of ERK1/2/CREB activation triggered by unpaired conditioning but only an early monophasic activation induced by paired conditioning. Our pharmacological experiments with the MEK inhibitor further confirm this result. Indeed, blockade of ERK1/2 activation during paired conditioning was shown to spare conditioning to the background context. However, this blockade resulted in a slight impairment of the freezing response to the tone. Thus, this suggests that, in addition to its role in contextual fear consolidation, the hippocampus also participates in cued fear consolidation (Maren et al. 1997; Bast et al. 2001; Ahi et al. 2004). Taken as a whole, these data indicate that the hippocampus contributes to the most prevalent conditioned association depending on the training procedure: tone-CS association in the paired conditioning and foreground context-US association in the unpaired conditioning.

Functional coupling between ERK1/2 and CREB

Different signals mediated by the activation of several kinase cascades including PKC and PKA (Roberson et al. 1999) converge at ERK1/2. Since CREB is a downstream target of these cascades, the coincident activation of ERK1/2 and CREB induced by fear conditioning was expected. However, available data so far only revealed the coincident activation of both ERK1/2 and CREB (Cammarota et al. 2000; Swank and Sweatt 2001; Wang et al. 2004), or failed to demonstrate any simultaneous activation (Schulz et al. 1999; Mizuno et al. 2002) or functional coupling after learning (Kanterewicz et al. 2000). More consistent was the result that inhibition of ERK1/2 activation leads to the blockade of CREB activation as well (Davis et al. 2000; Zhang et al. 2003). Our finding shows that the injection of U0126 into CA1 prior to both activation phases blocked the phosphorylation of both ERK1/2 and CREB and resulted in an impairment of foreground contextual conditioning. Given that other kinases like CaMKIV have the capacity to phosphorylate CREB (Lonze and Ginty 2002), the dramatic reduction of p-CREB induced by U0126, which is a selective MEK inhibitor (Roberson et al. 1999), leaves open questions on the mechanisms of the regulation of CREB activity. Our data suggest that conditioning-induced coincident phosphorylations of both ERK1/2 and CREB are functionally coupled events.

The first phase of ERK1/2/CREB cascade activation

Our immunochemical data revealed a differential temporal dynamic of molecular events in CA1 depending on the condition-

ing protocol. Accordingly, after unpaired conditioning, ERK1/2 and CREB phosphorylation occurred in a biphasic manner at 0–1 h and 9–12 h post-conditioning. In contrast, paired conditioning only induced a transient early wave of ERK1/2 and CREB activation immediately post-training. Our results are congruent with data showing with different behavioral paradigms a learning-induced activation of ERK1/2 (Atkins et al. 1998; Blum et al. 1999; Cammarota et al. 2000; Sananbenesi et al. 2002; Runyan et al. 2004; Wang et al. 2004) in hippocampus. Considering that these studies revealed different time courses of activation, we cannot ascertain that the early activation we found in both conditioning protocols is driven by common molecular mecha-

nisms. Some data obtained from cultured hippocampal neurons have revealed that the duration of ERK1/2 activation depends on the level of stimulation and that the early peak and the sustained phase of this activation are regulated by different biochemical events (Wu et al. 2001b). This report also showed that PKA signaling was critical for stabilization of ERK1/2 phosphorylation, even though PKA was not involved in its initial activation (Wu et al. 2001b). The assumption that PKA might be responsible for the longer activation of ERK1/2 observed here after unpaired conditioning is substantiated by data showing an early requirement of PKA activity in protein-dependent fear memory consolidation (Bernabeu et al. 1997; Bourchouladze et al. 1998). Hence, we

speculate that because of the limited involvement of the hippocampus in paired conditioning, the brief ERK1/2 activation observed in this structure might correspond with the early peak described by Wu et al. (2001a,b). On the contrary, unpaired conditioning requires recruitment of sustained neuronal activity in the hippocampus that is accompanied by a prolonged ERK1/2 phosphorylation possibly triggered by the PKA signaling pathway.

In both conditioning situations, the first wave of phosphorylation of ERK1/2 is sufficient to stimulate various cellular targets, such as CREB and Elk-1, leading to transcriptional modulation of immediate early genes that can initiate long-lasting forms of synaptic plasticity (Sgambato et al. 1998; Vanhoutte et al. 1999; Horgan and Stork 2003). Our results provide further evidence that inhibition of the first wave of ERK1/2 activation dramatically impaired long-term retention without affecting encoding and short-term memory processes. In contrast, blockade of the second wave of pERK1/2 also resulted in retrograde amnesia for contextual fear conditioning, suggesting that the first peak of phosphorylation is necessary but not sufficient for long-term memory formation of contextual fear.

The second phase of ERK1/2/CREB activation

To date, few reports that have demonstrated a biphasic molecular activation of ERK1/2 or CREB after learning have interpreted the second wave as a downstream molecular consequence of the first one (Bernabeu et al. 1997; Swank and Sweatt 2001). Our data, however, are supporting another view. Not only do we show that the second phase of ERK/CREB activation is required but not sufficient for fear conditioning consolidation, but we also demonstrate that this activation occurs independent of the first one and is therefore induced by a mechanism independent of the first phase of ERK/CREB phosphorylation. Thus, cross-talk interactions between

Figure 5. CREB phosphorylation depends on the phosphorylation of ERK1/2, but the second peak of ERK1/2 is independent of the first phase. (A) Microphotography showing the inhibitory effect of U0126 (left panel) intrahippocampal infusion on pERK1/2 labeling compared to vehicle (right panel) in the CA1 of the dorsal hippocampus 15 min after unpaired conditioning. (B) U0126 infusion into the dorsal hippocampus 30 min before unpaired conditioning reduced pERK1/2 immunoreactivity (bottom left) compared to control (top left) 15 min post-training. Similarly, immunoreactivity for pCREB was also reduced in U0126 animals (bottom right) compared to controls (top right). Quantified data (Mean ± SEM) are depicted in C confirming the reduction in pERK1/2 and pCREB (right) after U0126 treatment. (D) Immunoreactivity for pERK1/2 (left column) and pCREB (right column) after U0126 infusion 30 min before U0126, $n = 4$; vehicle, $n = 3$), 2 h after U0126, $n = 4$; vehicle, $n = 3$), and 7.5 h after U0126, $n = 4$; DMSO, $n = 3$) conditioning. Animals were sacrificed at 9 h post-training. Both pERK1/2 and pCREB labeling was reduced when U0126 was administered 7.5 h post-training. Data are quantified (Mean ± SEM) in E for pERK1/2 (left) and pCREB (right).

different signaling pathways may account for the onset of the second phase. Possible upstream regulators for the late ERK/CREB phosphorylation include the cAMP/PKA cascade, although the time windows of cAMP/PKA activation do not overlap with the second peak of ERK/CREB phosphorylation (Bernabeu et al. 1997; Bourtochouladze et al. 1998). Alternatively, strong interactions have been demonstrated between the brain-derived neurotrophic factor and the ERK/CREB pathway (Tyler et al. 2002; Ying et al. 2002; Alonso et al. 2004; Ou and Gean 2005). Recent studies have clearly demonstrated the critical role of ERK1/2 in the regulation of local translation (Kelleher III et al. 2004) and the formation of stable changes in dendritic architecture (Wu et al. 2001b). Thus, the second wave of ERK/CREB phosphorylation may be responsible for a late synthesis of structural proteins triggered by the induction of transcription factors like CCAAT enhancer binding protein (Taubenfeld et al. 2001). However, an alternative interpretation has been offered very recently based on the exploitation of the catFISH method on *Arc* mRNA and protein expression. In this study, the behaviorally induced second wave of *Arc* expression was seen in neurons that were a subset of the originally activated neuronal ensemble. However, this reactivation was not interpreted as being a cell-autonomous mechanism but rather as reflecting a network property (Ramirez-Amaya et al. 2005). Fueling this view, we recently found after fear conditioning, biphasic patterns of ERK1/2 and CREB activation in other structures than the hippocampus, namely, the amygdala and the medial prefrontal cortex (mPFC) (P. Trifilieff, L. Calandreau, C. Herry, N. Mons, and J. Micheau, unpubl.). It is thus possible that the second phase of ERK/CREB activation may reflect the interplay between several brain structures including the hippocampus, the amygdala, and the mPFC that are strongly interconnected (Ishikawa and Nakamura 2003). This may constitute the distributed neural substrates of fear conditioning. However, this assumption deserves to be strengthened by more investigations.

Materials and Methods

Subjects and surgery

Adult male C57Bl/6J mice (3–5 mo) were purchased from IFFA Credo. The animals were individually housed in standard Macrolon cages with ad libitum access to food and water with a 12-h light/12-h dark cycle. All experiments took place during the animals' light cycle. Three days before the experiments, mice were handled for 2–3 min/day. Animal care and behavioral and electrophysiological tests were conducted in accordance with the standard ethical guidelines (European Communities Guidelines on the Care and Use of Laboratory Animals: 86/609/EEC).

For guide cannulae implantation in dorsal hippocampus, animals were anesthetized with avertin (tribromoethanol 10 mL/kg) and secured in a stereotaxic frame. Stainless steel guide cannulae (30 gauge, 8 mm long) were implanted bilaterally at the following coordinates: 2 mm posterior to Bregma, 1.4 mm lateral, and 0.9 mm below the skull surface; and fixed in place with dental cement.

Using avertin as anesthetic and conventional surgery techniques, mice were implanted with electrodes made of twisted platinum-iridium wires (90 μ m diameter) insulated except at the tip. In order to reduce cell damage, the tips of the wires were sectioned at an angle. The stimulating electrodes were positioned in the ventral hippocampal commissural pathway (0.5 mm posterior to bregma and 0.3 mm lateral to midline), whereas the recording electrodes were in the contralateral pyramidal layer of CA1 (1.8 mm posterior to bregma, 1.3 mm lateral to midline) at a location generating a maximal amplitude of the hippocampal field potential (Etchamendy et al. 2001). The entire miniature system was fixed in place onto the skull with dental cement.

After surgery, subjects were allowed to recover in their home cages in the animal room for at least 7 d.

Intracerebral injections

U0126 (Cellsignal) was dissolved in DMSO (100%) and stored at -20°C . Animals were given intrahippocampal injection of either 0.3 μ L of DMSO (2%) per site in artificial cerebrospinal fluid (aCSF: 5 mM glucose, 1 mM CaCl_2 , 125 mM NaCl, 1 mM MgCl_2 , 27 mM NaHCO_3 , 0.5 mM KCl, 0.5 mM Na_2SO_4 , 0.5 mM NaH_2PO_4 , 1.2 mM Na_2HPO_4) (vehicle), or 0.6 μ g of U0126 (2 μ g/ μ L) in DMSO (2%) in aCSF. Injections were performed in freely moving mice at a constant rate of 0.08 μ L/min via an injection cannula (8.9 mm long) connected to a 1- μ L Hamilton syringe by flexible polyethylene tubing.

Behavioral procedure

The behavioral procedure has been described in detail elsewhere (Desmedt et al. 1998).

Aversive conditioning

Experiments were performed in a conditioning chamber comprised of clear Perspex (30 $\times$ 24 $\times$ 22 cm high) and placed on a table in an experimental room with plenty of extra-maze cues. The floor of the chamber consisted of 60 stainless steel rods (2 mm diameter) connected to a shock generator (Imetronic). The box was cleaned with 70% ethanol before each trial. Animals were submitted to either unpaired or paired fear conditioning. In unpaired conditioning, animals were placed in the conditioning chamber for a total of 7 min during which they received five pseudorandomly occurring tones (1000 Hz, 65 dB, 15 sec) that were not contingent on delivery of the five shocks (0.6 mA, 50 Hz, 3 sec). In contrast, paired conditioning was attained by pairing of five tones with footshocks that coterminated. Each animal was returned to its home cage after the conditioning procedure.

Retention test

One or 24 h after conditioning, mice were examined for freezing behavior as an index of conditioned fear defined as the absence of all movements except respiratory-related movements (Blanchard and Blanchard 1969). Animals were submitted to the auditory cue first: they were kept in their home cage and this was placed into a gray Perspex chamber to prevent access to visuospatial cues in the experimental room. The 4-min session was divided into two successive recording periods of the behavioral response: prior to (first 2 min) and during (next 2 min) tone presentation. Then mice were returned to the animal room for 2 h before being submitted to the context test: they were exposed to the conditioning chamber for 6 min, but no shocks were administered. The behavior of the animals was videotaped for off-line scoring of freezing. In unpaired conditioning, freezing was continuously scored and calculated as the percentage of the total time spent freezing during every 2-min period of the retention test. In paired conditioning, freezing was calculated as the ratio of the percentage increase during the 2 min of tone presentation relative to the 2 min prior to presentation of the tone.

In vivo electrophysiology

Electrophysiological recordings were performed in a square transparent Plexiglas box (18 cm side $\times$ 23 cm high) with a gray plastic floor. The Plexiglas box (recording context) was placed inside a sound-attenuating and temperature-regulated Plexiglas cubicle located in a room that was separated from the main room (where the experiment was guided via computer). The recording context was washed with 70% alcohol before each session. Electrophysiological activity was recorded through JFET operational amplifiers connected to the headstage to minimize artifacts due to head movement. Cables from the JFET were relayed at the top of the recording chamber by a multichannel rotating connector. This system allowed the animal free movement within the recording chamber. Hippocampal field potentials were evoked by single-pulse commissural stimulation (0.1-msec rectangular biphasic pulses were sent to an amplifier [gain 1000 $\times$; bandpass 1–10 kHz] and recorded [pClamp6 software; Texas Instruments] for off-line analysis). Stimulus intensity was chosen (from the

baseline input/output curves: 60–600 μ A) to obtain a response amplitude ~40% of the maximal level.

Following recovery from surgery, mice were habituated to being transported (from the animal house to the experimental room) and to connection and disconnection of the miniature headstage over a 3-d period. After habituation, baseline electrophysiological responses were established over a 2-d period (one recording session per day; each recording corresponding to an average of seven field potentials recorded at 0.2 Hz). Six days later, a third recording session was made in order to test the stability of responses prior to conditioning. Mice were then divided into two groups. Each mouse was placed into the conditioning chamber; mice of the two groups were submitted to pairing and unpairing conditioning, respectively.

Immunochemical procedures

Immunohistochemistry

All immunohistochemical studies were performed after conditioning. Mice were anesthetized at specific time points after conditioning by an overdose of avertin and perfused intracardially with 100 mL of 4% paraformaldehyde (PFA) in 0.1 M $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer (pH 7.5). Brains were quickly removed, post-fixed in the same fixative for 12 h, and sectioned in a vibratome (Leica), and the sections were immediately processed for immunohistochemistry. Briefly, floating sections were rinsed four times in tris-buffered saline (TBS/NaF; 0.1 M Tris and 0.5 M NaCl, 0.5 g/240 mL NaF at pH 7.5), incubated for 30 min in TBS/NaF containing 0.5% H_2O_2 , and rinsed four times in TBS/NaF. After 1 h of incubation in 0.2% Triton X-100, 3% bovine serum albumin (BSA) in TBS/NaF, sections were incubated for 48 h at 4°C in primary antibody anti-phospho-ERK1/2 (anti-phospho-Thr202–Tyr204, rabbit polyclonal antibody, 1:500; Cell Signaling), or anti-phospho-CREB (anti-phospho-Ser133–CREB, rabbit polyclonal antibody, 1:3000; Upstate Biotechnology) in TBS/NaF–0.2% Triton X-100–3% BSA. After four rinses in TBS, sections were incubated for 2 h with the secondary biotinylated antibody (anti-rabbit IgG, 1:2000; Jackson ImmunoResearch) in TBS. Sections were rinsed four times and incubated at room temperature for 2 h in avidin–biotin–complex solution (ABC Solution; Vector Laboratories) in TBS. Sections were rinsed in TBS and then in TB, and the peroxidase reaction end product was visualized in 0.05 M TB (pH 7.6) containing diaminobenzidine tetrahydrochloride (DAB, 0.025%), 0.03% H_2O_2 , and 5% nickel ammonium sulfate for 10 min. After processing, immunolabeled sections were washed in phosphate buffer, mounted onto gelatin-coated slides, and dehydrated through alcohol to toluen for light microscopical examination. The quantification of pERK- and pCREB-positive cells was carried out at $10\times$ magnification, which yielded a field of view of $849 \times 637 \mu\text{m}$. At least three serial sections were digitized and analyzed using a computerized image analysis system (Visiolab 2000, V4.50; Biocom). Structures were defined according to the Franklin and Paxinos atlas (1997). Labeled cells above a threshold determined from control animals were counted. Except for the control group, the experimenter was blind to experimental grouping.

Western blotting

Mice were decapitated at different time points after unpaired conditioning. Brains were rapidly removed and frozen, and 300- μm sections were cut with an ice-cold microtome. Hippocampi were then dissected on adjacent sections, and lysed in a solubilization buffer (10 mM Tris-HCl, 50 mM NaCl, 1% Triton X-100, 30 mM sodium pyrophosphate, 50 mM NaF, 5 μM ZnCl_2 , 100 μM Na_3VO_4 , 1 mM DTT, 5 nM okadaic acid, 2.5 μg of aprotinin, 2.5 μg of pepstatin, 0.5 μg of PMSE, 0.5 mM benzamide, 2.5 μg of leupeptin). Insoluble material was removed by centrifugation (13,000 rpm for 20 min at 4°C). Cell lysates (30 μg per lane for the detection of pERK1/2 and pCREB) were separated by 10% SDS-PAGE before electrophoretic transfer onto polyvinylidene difluoride membranes (0.45 μm Hybond P; Amersham Biosciences).

Blots were treated as previously described (Vanhoutte et al.

1999). Briefly, membranes were saturated for 1 h at room temperature with 5% BSA (Fraction V; Sigma) and incubated overnight at 4°C with the anti-active antibodies. On the second day, blots were incubated for 2 h at room temperature with goat anti-rabbit-horseradish peroxidase-conjugated antibodies before exposure to the ECL substrate (Amersham Biosciences) and photographic processing. Then the blots were stripped (glycine-HCl at pH 2.8, two times for 30 min at 55°C, 10 min in 2% SDS), saturated for 2 h at room temperature in 5% non-fat dry milk, and incubated overnight with the non-active antibodies. Polyclonal antibodies were raised against the double-phosphorylated Thr/Glu/Tyr region within the catalytic core of the active form of ERK1 and ERK2 (anti-phospho-Thr202–Tyr204 ERK1/2s, dilution 1/5000; Cell Signaling), and a phospho-Ser133 peptide corresponding to residues 129–137 of CREB (dilution 1/500; Upstate Biotechnology). The total amount of ERK1/2s proteins was detected with a polyclonal anti-ERK2 antibody (dilution 1/5000; Santa Cruz). Western blotting quantification was conducted by measuring optical density directly on photographic films (Scion image).

Histology

After completion of the behavioral studies, animals were given an overdose of avertine (15 mL/kg) and transcardially perfused with saline (0.9% NaCl in H_2O_2), followed by 10% buffered formalin. Brains were post-fixed in formalin-saccharose 30% solution for 1 wk, frozen, cut coronally on a sliding microtome into 50- μm sections, and mounted on gelatine-coated slides, and stained with thionine in order to verify cannulae placements.

Data analysis

Data are presented as the mean $\pm$ SEM. Statistical analyses were performed using an analysis of variance (ANOVA: Statview 512+) followed by an appropriate post hoc test (Tukey/Kramer) set to a reliability level of $P = 0.05$.

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References

- Ahi, J., Radulovic, J., and Spiess, J. 2004. The role of hippocampal signaling cascades in consolidation of fear memory. *Behav. Brain Res.* **149**: 17–31.
- Alonso, M., Medina, J.H., and Pozzo-Miller, L. 2004. ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons. *Learn. Mem.* **11**: 172–178.
- Anagnostaras, S.G., Gale, G.D., and Fanselow, M.S. 2001. Hippocampus and contextual fear conditioning: Recent controversies and advances. *Hippocampus* **11**: 8–17.
- Atkins, C.M., Selcher, J.C., Petraitis, J.J., Trzaskos, J.M., and Sweatt, J.D. 1998. The MAPK cascade is required for mammalian associative learning. *Nat. Neurosci.* **1**: 602–609.
- Bast, T., Zhang, W.N., and Feldon, J. 2001. Hippocampus and classical fear conditioning. *Hippocampus* **11**: 828–831.
- . 2003. Dorsal hippocampus and classical fear conditioning to tone and context in rats: Effects of local NMDA-receptor blockade and stimulation. *Hippocampus* **13**: 657–675.
- Bernabeu, R., Bevilacqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., Izquierdo, I., and Medina, J.H. 1997. Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proc. Natl. Acad. Sci.* **94**: 7041–7046.
- Blanchard, R.J., and Blanchard, D.C. 1969. Passive and active reactions to fear-eliciting stimuli. *J. Comp. Physiol. Psychol.* **68**: 129–135.
- Blum, S., Moore, A.N., Adams, F., and Dash, P.K. 1999. A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial memory. *J. Neurosci.* **19**: 3535–3544.
- Bourtchouladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., and Silva, A.J. 1994. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* **79**: 59–68.
- Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K., and

- Kandel, E.R. 1998. Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. *Learn. Mem.* **5**: 365–374.
- Calandrea, L., Desmedt, A., Decorte, L., and Jaffard, R. 2005. A different recruitment of the lateral and basolateral amygdala promotes contextual or elemental conditioned association in Pavlovian fear conditioning. *Learn. Mem.* **12**: 383–388.
- Cammarota, M., Bevilaqua, L.R., Ardenghi, P., Paratcha, G., Levi de Stein, M., Izquierdo, I., and Medina, J.H. 2000. Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hippocampus after a one-trial avoidance learning: Abolition by NMDA receptor blockade. *Brain Res. Mol. Brain Res.* **76**: 36–46.
- Davis, S., Vanhoutte, P., Pages, C., Caboche, J., Laroche, S., Barnier, J.V., Guibert, B., Besson, M.J., Hipkind, R.A., Sgambato, V., et al. 2000. The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. *J. Neurosci.* **20**: 4563–4572.
- Desmedt, A., Garcia, R., and Jaffard, R. 1998. Differential modulation of changes in hippocampal-septal synaptic excitability by the amygdala as a function of either elemental or contextual fear conditioning in mice. *J. Neurosci.* **18**: 480–487.
- Desmedt, A., Marighetto, A., Garcia, R., and Jaffard, R. 2003. The effects of ibotenic hippocampal lesions on discriminative fear conditioning to context in mice: Impairment or facilitation depending on the associative value of a phasic explicit cue. *Eur. J. Neurosci.* **17**: 1953–1963.
- Dudai, Y. and Eisenberg, M. 2004. Rites of passage of the engram: Reconsolidation and the lingering consolidation hypothesis. *Neuron* **44**: 93–100.
- Etchamendy, N., Enderlin, V., Marighetto, A., Vouimba, R.M., Pallet, V., Jaffard, R., and Huguier, P. 2001. Alleviation of a selective age-related relational memory deficit in mice by pharmacologically induced normalization of brain retinoid signaling. *J. Neurosci.* **21**: 6423–6429.
- Franklin, K.B.J. and Paxinos, G. 1997. *The mouse brain in stereotaxic coordinates*. Academic Press, New York.
- Freeman, F.M., Rose, S.P., and Scholey, A.B. 1995. Two time windows of anisomycin-induced amnesia for passive avoidance training in the day-old chick. *Neurobiol. Learn. Mem.* **63**: 291–295.
- Grecksch, G. and Matthies, H. 1980. Two sensitive periods for the amnesic effect of anisomycin. *Pharmacol. Biochem. Behav.* **12**: 663–665.
- Hall, J., Thomas, K.L., and Everitt, B.J. 2001. Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: Selective activation of hippocampal CA1 neurons during the recall of contextual memories. *J. Neurosci.* **21**: 2186–2193.
- Horgan, A.M. and Stork, P.J. 2003. Examining the mechanism of Erk nuclear translocation using green fluorescent protein. *Exp. Cell Res.* **285**: 208–220.
- Igaz, L.M., Vianna, M.R., Medina, J.H., and Izquierdo, I. 2002. Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. *J. Neurosci.* **22**: 6781–6789.
- Impey, S., Obrietan, K., and Storm, D.R. 1999. Making new connections: Role of ERK/MAP kinase signaling in neuronal plasticity. *Neuron* **23**: 11–14.
- Ishikawa, A. and Nakamura, S. 2003. Convergence and interaction of hippocampal and amygdalar projections within the prefrontal cortex in the rat. *J. Neurosci.* **23**: 9987–9995.
- Kanterewicz, B.I., Urban, N.N., McMahon, D.B., Norman, E.D., Giffen, L.J., Favata, M.F., Scherle, P.A., Trzskos, J.M., Barrionuevo, G., and Klann, E. 2000. The extracellular signal-regulated kinase cascade is required for NMDA receptor-independent LTP in area CA1 but not area CA3 of the hippocampus. *J. Neurosci.* **20**: 3057–3066.
- Kelleher III, R.J., Govindarajan, A., Jung, H.Y., Kang, H., and Tonegawa, S. 2004. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* **116**: 467–479.
- Kida, S., Josselyn, S.A., de Ortiz, S.P., Kogan, J.H., Chevere, I., Masushige, S., and Silva, A.J. 2002. CREB required for the stability of new and reactivated fear memories. *Nat. Neurosci.* **5**: 348–355.
- Kim, J.J. and Fanselow, M.S. 1992. Modality-specific retrograde amnesia of fear. *Science* **256**: 675–677.
- Lee, I. and Kesner, R.P. 2004. Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. *Hippocampus* **14**: 301–310.
- Lonze, B.E. and Ginty, D.D. 2002. Function and regulation of CREB family transcription factors in the nervous system. *Neuron* **35**: 605–623.
- Maren, S., Aharonov, G., and Fanselow, M.S. 1997. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behav. Brain Res.* **88**: 261–274.
- Matus-Amat, P., Higgins, E.A., Barrientos, R.M., and Rudy, J.W. 2004. The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. *J. Neurosci.* **24**: 2431–2439.
- Mizuno, K. and Giese, K.P. 2005. Hippocampus-dependent memory formation: Do memory type-specific mechanisms exist? *J. Pharmacol. Sci.* **98**: 191–197.
- Mizuno, M., Yamada, K., Maekawa, N., Saito, K., Seishima, M., and Nabeshima, T. 2002. CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. *Behav. Brain Res.* **133**: 135–141.
- Ou, L.C. and Gean, P.W. 2005. Regulation of amygdala-dependent learning by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol-3-kinase. *Neuropsychopharmacology* **31**: 287–296.
- Phillips, R.G. and LeDoux, J.E. 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* **106**: 274–285.
- . 1994. Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. *Learn. Mem.* **1**: 34–44.
- Pittenger, C., Huang, Y.Y., Paletzki, R.F., Bourtochouladze, R., Scanlin, H., Vronskaya, S., and Kandel, E.R. 2002. Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. *Neuron* **34**: 447–462.
- Ramirez-Amaya, V., Vazdarjanova, A., Mikhael, D., Rosi, S., Worley, P.F., and Barnes, C.A. 2005. Spatial exploration-induced arc mRNA and protein expression: Evidence for selective, network-specific reactivation. *J. Neurosci.* **25**: 1761–1768.
- Roberson, E.D., English, J.D., Adams, J.P., Selcher, J.C., Kondratik, C., and Sweatt, J.D. 1999. The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. *J. Neurosci.* **19**: 4337–4348.
- Rose, S.P. 2000. God's organism? The chick as a model system for memory studies. *Learn. Mem.* **7**: 1–17.
- Runyan, J.D., Moore, A.N., and Dash, P.K. 2004. A role for prefrontal cortex in memory storage for trace fear conditioning. *J. Neurosci.* **24**: 1288–1295.
- Sananbenesi, F., Fischer, A., Schrick, C., Spiess, J., and Radulovic, J. 2002. Phosphorylation of hippocampal Erk-1/2, Elk-1, and p90-Rsk-1 during contextual fear conditioning: Interactions between Erk-1/2 and Elk-1. *Mol. Cell. Neurosci.* **21**: 463–476.
- Schafe, G.E., Atkins, C.M., Swank, M.W., Bauer, E.P., Sweatt, J.D., and LeDoux, J.E. 2000. Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of Pavlovian fear conditioning. *J. Neurosci.* **20**: 8177–8187.
- Schulz, S., Siemer, H., Krug, M., and Holtt, V. 1999. Direct evidence for biphasic cAMP responsive element-binding protein phosphorylation during long-term potentiation in the rat dentate gyrus in vivo. *J. Neurosci.* **19**: 5683–5692.
- Sgambato, V., Pages, C., Rogard, M., Besson, M.J., and Caboche, J. 1998. Extracellular signal-regulated kinase (ERK) controls immediate early gene induction on corticostriatal stimulation. *J. Neurosci.* **18**: 8814–8825.
- Stanciu, M., Radulovic, J., and Spiess, J. 2001. Phosphorylated cAMP response element binding protein in the mouse brain after fear conditioning: Relationship to Fos production. *Brain Res. Mol. Brain Res.* **94**: 15–24.
- Swank, M.W. and Sweatt, J.D. 2001. Increased histone acetyltransferase and lysine acetyltransferase activity and biphasic activation of the ERK/RSK cascade in insular cortex during novel taste learning. *J. Neurosci.* **21**: 3383–3391.
- Taubenfeld, S.M., Wiig, K.A., Bear, M.F., and Alberini, C.M. 1999. A molecular correlate of memory and amnesia in the hippocampus. *Nat. Neurosci.* **2**: 309–310.
- Taubenfeld, S.M., Wiig, K.A., Monti, B., Dolan, B., Pollonini, G., and Alberini, C.M. 2001. Fornix-dependent induction of hippocampal CCAAT enhancer-binding protein β and δ co-localizes with phosphorylated cAMP response element-binding protein and accompanies long-term memory consolidation. *J. Neurosci.* **21**: 84–91.
- Tyler, W.J., Alonso, M., Bramham, C.R., and Pozzo-Miller, L.D. 2002. From acquisition to consolidation: On the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn. Mem.* **9**: 224–237.
- Vanhoutte, P., Barnier, J.V., Guibert, B., Pages, C., Besson, M.J., Hipkind, R.A., and Caboche, J. 1999. Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices. *Mol. Cell. Biol.* **19**: 136–146.

- Wang, H., Ferguson, G.D., Pineda, V.V., Cundiff, P.E., and Storm, D.R. 2004. Overexpression of type-1 adenylyl cyclase in mouse forebrain enhances recognition memory and LTP. *Nat. Neurosci.* **7**: 635–642.
- Wu, G.Y., Deisseroth, K., and Tsien, R.W. 2001a. Activity-dependent CREB phosphorylation: Convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proc. Natl. Acad. Sci.* **98**: 2808–2813.
- . 2001b. Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. *Nat. Neurosci.* **4**: 151–158.
- Ying, S.W., Futter, M., Rosenblum, K., Webber, M.J., Hunt, S.P., Bliss, T.V., and Bramham, C.R. 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**: 1532–1540.
- Zhang, J.J., Okutani, F., Inoue, S., and Kaba, H. 2003. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathway leading to cyclic AMP response element-binding protein phosphorylation is required for the long-term facilitation process of aversive olfactory learning in young rats. *Neuroscience* **121**: 9–16.

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