A Requirement of Nuclear Factor-κB Activation in Fear-potentiated Startle*

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Previous studies have shown that biochemical changes that occur in the amygdala during fear conditioning in vivo are similar to those occur during long term potentiation (LTP) in vitro. Electrophoretic mobility shift assay of nuclear extracts from startle-potentiated rats showed a selective increase in the amygdala of nuclear factor-κB (NF-κB) DNA binding activity. Super-shift experiments further indicated that p65 and p50 subunits but not c-Rel were involved in DNA binding. The protein levels of IκB-α were reduced by treatments that reliably induced LTP in this area of the brain. This was accompanied by a decrease of NF-κB in the cytoplasm concomitant with an increase in the nucleus. Quantitative analysis of IκB kinase activity demonstrated that fear training led to an increase in kinase activity, and this effect was blocked by thalidomide. Paralleled behavioral tests revealed that thalidomide inhibited fear-potentiated startle. Intra-amygdala administration of κB decoy DNA prior to training impaired fear-potentiated startle as well as LTP induction. Similarly, NF-κB inhibitors blocked IκB-α degradation and startle response. These results provide the first evidence of a requirement of NF-κB activation in the amygdala for consolidation of fear memory.

It is generally believed that consolidation of long term memory in mammalian brain and long term facilitation in Aplysia require new protein synthesis (1-3). Newly synthesized proteins are thought to deposit at the synapses that have been tagged by prior activity to encode enduring changes in synaptic strength (4, 5). However, despite the importance of new protein synthesis for memory consolidation, little is known about signaling pathways leading to protein translation in neurons.

NF-κB, originally identified as a regulator of immunoglobulin κ light chain gene expression, is a DNA-binding factor that functions as a dimer. Five mammalian members of the family have been identified; p50/NF-κB1, p65/RelA, c-Rel, RelB, and p52/NF-κB2 (6). NF-κB was localized mainly to the cytoplasm in an inactive form bound to an inhibitory protein termed IκB (7). Upon stimulation by extracellular inducers, IκB was rapidly phosphorylated by the IκB kinase (IKK) complex on the serine residues 32 and 36. This phosphorylation led to the ubiquitination and subsequent degradation of IκB by the proteasome followed by nuclear translocation of NF-κB (8-11). Once translocated to the nucleus, NF-κB bound cognate DNA sequences and activated transcription of specific target genes, the majority of which encoded proteins important in immunity and inflammation (12-15). Apart from its role in hematopoiesis, accumulated evidence indicates that NF-κB is involved in neuroprotection or neurodegeneration, depending on the particular system under investigation (16-18). Furthermore, a recent study implicated that NF-κB played an important role in the synaptic plasticity, because pretreatment of hippocampal slices with κB decoy DNA prevented induction of long term depression (LTD)1 and significantly reduced the magnitude of LTP (19). We have recently demonstrated that acquisition of fear was associated with an activation of phosphatidylinositol 3-kinase (PI 3-kinase) and its downstream target Akt in the rat amygdala (20). PI 3-kinase and Akt were also activated in response to LTP-inducing tetanic stimulation (TS). In parallel, PI 3-kinase inhibitors interfered with TS-induced LTP as well as long term fear memory formation. Akt has been shown to induce the activation of NF-κB family of transcriptional factors (21, 22). Therefore, it is of interest to investigate whether NF-κB plays any role in synaptic plasticity and memory formation. Here we show that NF-κB is activated in the amygdala following fear-potentiated startle, and disruption of this signaling pathway impairs fear memory.

EXPERIMENTAL PROCEDURES

Surgery—Rats (4-5 weeks old) were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and subsequently were mounted on a stereotactic apparatus. Two cannula made of 22-gauge stainless steel tubing (C313G; Plastic Products) were implanted bilaterally into the lateral (LA) or basolateral (BLA) amygdala (anteroposterior, -2.8 mm; mediolateral, ±4.5 mm; dorsoventral, -7.0 mm) (23). A 28-gauge dummy cannula was inserted into each cannula to prevent clogging. Three jewelry screws were implanted over the skull, serving as anchors and the whole assembly was affixed on the skull with dental cement. The rats were monitored and handled daily and were given 7 days to recover. NF-κB inhibitors were administrated bilaterally in a volume of 0.5-0.8 μl at a rate of 0.5 μl/min. κB decoy and scrambled DNA were prepared and administered based on the method of Blondeau et al. (24). In brief, double-stranded κB decoy DNA was prepared by annealing complementary single strands with the sequences of 5′-GAGGG-GACCTTCCCT-3′. Control DNA with a scrambled sequence was prepared by annealing oligonucleotides of the following sequence: 5′-GAT-GGCTCTGTGGCGCA-3′. Stocks of double-stranded DNA were prepared at

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1 The abbreviations used are: LTD, long term depression; CREB, cAMP-response element-binding protein; EMSA, electrophoretic mobility shift assay; IKK, IκB kinase; LTP, long term potentiation; D-APV, D-2-amino-phosphonovalerate; PI 3-kinase, phosphatidylinositol 3-kinase; TPCK, N-tosyl-l-phenylalanine chloromethyl ketone; TLCK, N′-p-tosyl-l-lysine chloromethyl ketone; TS, tetanic stimulation; LA, lateral; BLA, basolateral; ACSF, artificial cerebrospinal fluid; PMSF, phenylmethylsulfonyl fluoride.
IWestern blots were developed in the linear range used for densitometry. A chemiluminescence kit (Amersham Biosciences) was used for detection.

60 mM KCl, 1 mM NaCl, 0.25 M sucrose, 5 mM EDTA, 1 mM EGTA, 1 mM

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*NF-κB and Fear-potentiated Startle*

**Fear Conditioning**—Fear conditioning was measured using the potentiated startle paradigm in a startle apparatus (San Diego Instrument, San Diego, CA) as described previously (20, 23). Briefly, the animal was constrained in a Flexiglass cylindrical tube (length, 20 cm; diameter, 10 cm) with an accelerometer sensor attached on the base. The whole set-up was enclosed in a ventilated, soundattenuating cabinet (length, 38 cm; width, 38 cm; and height, 55 cm). The acoustic startle stimulus was a 50-ms white noise at an intensity of 95 dB. The visual conditioned stimulus was a 3.7-s light produced by an 8-watt fluorescent bulb attached to the back of stabilimeter. The unconditioned stimulus was as the aversive component necessary for the startle response. Conditioning, was a 0.6-millamp foot shock with a duration of 0.5 s. Startle amplitude was defined as the maximum accelerometer voltage that occurred during a 200-ms period after the onset of the startle stimulus. Seven days after surgery, each rat was exposed to habituation sessions over two consecutive days. In these sessions, rats were placed in the startle box and 3 min later presented with 10 startle stimuli at 2-min intertrial intervals. On the basis of their mean startle amplitudes in the second of these two sessions, rats were matched into groups with similar response levels. On the training day, rats were placed in the startle chamber and received 10 light-foot shock pairings. Unpaired controls received the same number of light and foot shock presentation but in a pseudo-random fashion in which the unconditioned stimulus could occur at any time except at 3.2 s following the conditioned stimulus.

**Slice Preparation and Extracellular Recordings**—Male Sprague-Dawley 4–6-week-old rats were decapitated, and their brains were rapidly removed and placed in cold oxygenated artificial cerebrospinal fluid (ACSF) solution. Subsequently, the brain was hemisected, and transverse slices of 450-μm thickness were made. ACSF solution had the following composition: 117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 25 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 11 mM glucose. The ACSF was bubbled continuously with 95% O₂, 5% CO₂ and had a pH of 7.4.

Extracellular field potentials were made by electrical stimulation of the external capsule, which contained fibers from the auditory cortex to the lateral amygdala, with a concentric bipolar stimulating electrode (SNE-100; Kopf Instruments, Bern, Germany). Electrical stimuli (150 μs in duration) were delivered at a frequency of 0.05 Hz. Base-line field potentials were adjusted to ~30–40% of the maximal responses. LTP was elicited by four trains of tetanus (100 Hz, 1 s at 3-min intervals) at the same stimulation intensity used for baseline. For *κB decoy DNA experiments, amygdala slices from each rat were divided into two groups. Slices in the treatment group were incubated with 50 μM *κB decoy DNA for at least 3 h before recordings were made. Control slices were incubated for a similar length of time with scrambled DNA or non Encoding the NF-κB subunit of IKK, kinase activity was determined in kinase buffer (20 mM Hapes (pH 7.7), 2 mM MgCl₂, 2 mM MnCl₂, 10 μM ATP, 1–3 μCi of [γ-32P]ATP, 10 mM β-glycerophosphate, 10 mM NaF, 10 mM NP40, 300 μM Na₃VO₄, 1 mM benzamidine, 2 μM PMSF, aprotinin at 10 μg/ml, and 0.5 μM dithiothreitol) for 30 °C for 30–60 min in the presence of the indicated substrate. The kinase reaction was stopped by the addition of sample buffer, subjected to SDS-PAGE analysis, and visualized by autoradiography. IκB-α (1–317) substrate (Santa Cruz Biotechnology) was expressed and purified from Escherichia coli with glutathione-agarose affinity chromatography.

**RESULTS**

**Fear-potentiated Startle Induces Activation of NF-κB**—We determined whether conditioning was associated with activation of NF-κB by using EMSA in which nuclear extracts from the amygdala of paired or unpaired rats were incubated with radiolabeled NF-κB consensus DNA sequence. Fig. 1A shows that fear training significantly increased the association of NF-κB with consensus oligonucleotide. By contrast, NF-κB DNA binding activity was not altered in unpaired controls.

Regulation of NF-κB may be a general phenomenon across the entire brain. To determine whether it was specific for the amygdala, additional experiments were performed to measure NF-κB DNA binding activity in the hippocampus and cerebellum. As illustrated in Fig. 1B, training of visual conditioned fear did not lead to an increase in NF-κB binding activity in the hippocampus and cerebellum. These results indicate that regulation of NF-κB activity occurs in the region of the brain involved in the formation of fear memory.

Previous studies have shown that biochemical changes that occur in areas LA and BLA during fear conditioning in *vivo* are similar to those occurring during LTP in *vivo* (28–30). We next determined whether forskolin or TSH, the protocols that reliably...
followed by an almost complete resynthesis of NF-κB shows that the induction of the active state of NF-κB and Fear-potentiated Startle

Slices were treated with forskolin (50 μM) for 15 min, and nuclear extracts from the LA and BLA were analyzed with EMSA using radiolabeled NF-κB probe. DNA binding activity of NF-κB was eliminated in the presence of a 100-fold excess of unlabeled NF-κB probe but was not affected by unlabeled CREB probe. Supershift experiments with anti-p65 and anti-p50 demonstrated that the protein complex interacting with the NF-κB oligonucleotide contains a p65/p50 heterodimer. Slices were given four trains of TS, and nuclear extracts were preincubated with antibodies raised against various NF-κB subunits and CREB antibody. The data shown are representative of five independent experiments.

We further examined the effect of TLCK on the NF-κB DNA binding activity. Pretreatment of TLCK before training blocked conditioning-induced binding of NF-κB (Fig. 3D). However, the direct addition of TLCK to the nuclear extracts from the conditioned rats did not affect the binding of NF-κB to the DNA. These data indicate that suppression of NF-κB binding by TLCK is not due to interference between the transcription factor and the DNA but rather that TLCK acts specifically to inhibit conditioning-induced NF-κB activity.

**Fear Training Activates IKK Activity**—IKK is the enzyme responsible for the phosphorylation of IkB-α; therefore, we examined the regulation of IKK activity in behaviorally trained rats. By using agarose-conjugated antibody against the β subunit of IKK complex, IKK was immunoprecipitated from whole cell extracts of rats that had been conditioned. Immunoprecipitates were assayed for kinase activity by incubating with IkB-α (amino acids 1–317) fusion protein in the presence of [γ-32P]ATP, electrophoresed on a polyacrylamide gel, and analyzed by autoradiography. Fig. 4 shows that fear training led to an increase in kinase activity by 10 min that continued through the 30-min time point. It has been shown that anti-inflammatory and antiangiogenic effects of thalidomide were based on its activity demonstrated that thalidomide inhibited the increase in kinase activity (33). We tested whether thalidomide produces a similar effect on the central neurons by administration of this drug (10 μg dissolved in 1.0 μl of saline, 0.5 μl per side) 30 min before training. Quantitative analysis of conditioning-induced IKK activity demonstrated that thalidomide inhibited the increase in kinase activity.
at 10 (p < 0.05) and 30 min (p < 0.01) time points as compared with that without thalidomide treatment. Paralleled behavioral tests performed 24 h after training revealed that thalidomide inhibited fear-potentiated startle. Startle potentions were 191.7 ± 25.4% in the vehicle group and 93.1 ± 9.5% in the thalidomide-treated group (t(10) = 3.85, p < 0.01).

Translocation of NF-κB to the Nucleus—Amygdala slices were given four trains of TS or were treated with forskolin (50 μM) for 15 min (B). Cytoplasmic extract proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was analyzed for degradation of IκB-α with an anti-IκB-α antibody. C, suppression of IκB-α degradation by wortmannin and TLCK. Slices were treated with wortmannin (100 nM) or TLCK (50 μM) 30 min prior to application of forskolin. Cytoplasmic extracts were analyzed for degradation of IκB-α as stated in B, **, p < 0.01; ***, p < 0.001 versus control. D, nuclear extracts from conditioned or conditioned rats given TLCK or vehicle 30 min prior to training were analyzed by EMSA for NF-κB DNA binding activity. The direct addition of TLCK (50 μM) to the nuclear extracts from the conditioned rats did not affect the binding of NF-κB to the DNA.

**Fig. 3. Induction of IκB-α degradation by TS or forskolin.** Slices were given four trains of TS (A) or were treated with forskolin (50 μM) for 15 min (B). Cytoplasmic extract proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was analyzed for degradation of IκB-α with an anti-IκB-α antibody. C, suppression of IκB-α degradation by wortmannin and TLCK. Slices were treated with wortmannin (100 nM) or TLCK (50 μM) 30 min prior to application of forskolin. Cytoplasmic extracts were analyzed for degradation of IκB-α as stated in B, **, p < 0.01; ***, p < 0.001 versus control. D, nuclear extracts from conditioned or conditioned rats given TLCK or vehicle 30 min prior to training were analyzed by EMSA for NF-κB DNA binding activity. The direct addition of TLCK (50 μM) to the nuclear extracts from the conditioned rats did not affect the binding of NF-κB to the DNA.
**NF-κB Is Required for Fear-potentiated Startle**—To investigate whether the activation of NF-κB was not only correlated but also obligatory for conditioned fear, we microinjected TLCK (0.4, 20, or 50 mM, 0.5 μl per side) bilaterally into the LA or BLA 30 min before training. Fear retention was tested 24 h after training. Fig. 6A shows that TLCK dose-dependently impaired fear memory. The analysis of variance for startle scores revealed a significant effect for dose ($F_{(4,25)} = 8.10; p < 0.001$). Post hoc comparisons (Newman-Keuls) revealed the significant differences among vehicle control and the high doses (20 and 50 mM) of TLCK ($p < 0.01$), whereas no difference was detected among control and low doses (0.4 mM; $p > 0.05$). Similarly, intra-amygdala infusion of another NF-κB inhibitor, N-tosyl-L-phenylalanine chloromethyl ketone (TPCK; 100 mM, 0.5 μl per side) blocked the acquisition of fear memory ($n = 6, p < 0.01$ versus vehicle control). The infusion cannula tip locations are shown in Fig. 6B. Only rats with cannula tips at or within the boundaries of LA and BLA were included in the data analysis.

**Fear-potentiated Startle and LTP Were Markedly Reduced in Rats Treated with κB Decoy DNA**—We administered double-stranded κB decoy DNA or control double-stranded DNA with a scrambled sequence bilaterally into the amygdala based on the method of Yu et al. (34). After conditioning, the rats were sacrificed, and nuclear extracts from the amygdala were incubated with radiolabeled NF-κB consensus DNA sequence. As shown in Fig. 7A, the conditioning-induced increase in NF-κB DNA binding activity was not seen in rats that were given κB decoy DNA. Furthermore, quantification of startle potentiation 24 h after conditioning revealed a marked depression of the degree of potentiation (48.3 ± 14.8%, $n = 6$) as compared with those receiving scrambled DNA (205.1 ± 29.6%, $n = 6, p < 0.001$) or sham-operated rats (251.3 ± 12.1%, $n = 6, p < 0.001$) (Fig. 7B). To exclude the possible toxic effect of κB decoy DNA on the amygdala neurons, rats that received
NF-κB and Fear-potentiated Startle

46725

κB decoy DNA were rested for at least 3 days and subsequently were retrained without drug treatment. Fig. 7B shows that rats that had impaired memory caused by κB decoy DNA treatment now exhibited a normal startle reflex (211.4 ± 55.1%, n = 6, p < 0.01). This result suggests that the effect of κB decoy DNA was reversible and appeared not to cause permanent damage to the amygdala function.

It has been shown that many hippocampal pyramidal neurons exhibited intense fluorescence when slices were incubated for 3 h in the presence of 50 μM κB decoy DNA, indicating penetration of the κB decoy DNA into these cells (19). Amygdala slices were incubated with 50 μM κB decoy DNA for at least 3 h before recordings were made. Control slices were incubated for a similar length of time with scrambled DNA in a different beaker. Fig. 7C shows that delivery of four sets of TS at an interstimulus interval of 3 min produced a long lasting, robust enhancement of synaptic responses in control and scrambled DNA-treated slices. The slopes of field excitatory postsynaptic potential (fEPSP) were 213 ± 18, 201 ± 15, 189 ± 16, and 178 ± 17% (n = 6), at 1, 2, 3, and 4 h after the stimulation. By contrast, the same amount and pattern of stimulation elicited a gradually decreasing long-term potentiation (LTP) in κB decoy DNA-treated slices. The fEPSP slopes at 1, 2, 3, and 4 h after the stimulation were 134 ± 23, 108 ± 26, 86 ± 14, and 88 ± 14% (n = 6) of control, respectively. The difference between control and κB decoy DNA-treated slices was statistically significant at 1 (p < 0.05), 2 (p < 0.05), 3 (p < 0.01), and 4 h (p < 0.005). We also performed control experiments to examine whether the basal synaptic transmission was changed by measuring the input-output relationship before and after incubation with κB decoy DNA. We found that the input-output relationship was not affected by κB decoy DNA (not shown).

Increase in Co-immunoprecipitation of CREB and NF-κB after TS or Treatment with Forskolin—Since CREB antibody slightly retarded the migration of proteins interacting with the NF-κB oligonucleotide (Fig. 2), we investigated possible interaction between CREB and NF-κB with co-immunoprecipitation. In the first set of experiments, slices were given four trains of TS, and nuclear extracts from the LA and BLA were immunoprecipitated with CREB antibody and immunoblotted with NF-κB antibody. As shown in Fig. 8A, TS resulted in a significant increase in NF-κB (177.0 ± 6.3% of control, n = 6, p < 0.01), which bound to CREB. Importantly, the effect was attenuated when TS was applied in the presence of d-APV (50 μM), consistent with previous reports showing that LTP in this area is dependent on N-methyl-d-aspartate receptors (41). To further verify the interaction between CREB and NF-κB, nuclear extracts were first immunoprecipitated with NF-κB antibody and then blotted with CREB antibody. A similar increase (173.0 ± 6.2% of control, n = 6, p < 0.01) in the interaction between NF-κB and CREB was observed after TS, and this effect was blocked by d-APV (50 μM).

Second, slices were incubated with forskolin (50 μM) for 15 min, and nuclear extracts from the LA and BLA were immunoprecipitated with CREB antibody and then blotted with NF-κB antibody. As shown in Fig. 8B, activation of adenyl cyclase by forskolin caused a significant increase in NF-κB (180.3 ± 13.2, n = 6, p < 0.01), which bound to CREB. The effect of forskolin was blocked by KT 5720, confirming the mediation by protein kinase A. Similarly, when nuclear extracts were first immunoprecipitated with NF-κB antibody and then blotted with CREB antibody, forskolin increased immunoreactivity of CREB (156.0 ± 7.3%, n = 6, p < 0.01), which was sensitive to KT 5720 (Fig. 8B).

**Discussion**

We have examined the potential role of NF-κB in amygdala synaptic plasticity. Here we show for the first time that fear-potentiated startle results in an activation of NF-κB and intra-amygdalectomy administration of NF-κB inhibitors, or κB decoy DNA blocks memory consolidation and LTP. Since activated NF-κB binds to cognate DNA sequences to initiate transcription of specific target genes, the present results are consistent with pharmacological studies showing an activation of new protein synthesis during fear conditioning and the late phase of LTP in the amygdala (29, 35).

The protein levels as well as the DNA binding activity of NF-κB were significantly increased in the conditioned animals. By contrast, the control group that received conditioned stimulus and unconditioned stimulus in an unpaired fashion did not present any increase, suggesting that NF-κB activation is specific to the learning component of the task. In addition, activation was restricted to the amygdala but not the hip
pocampus or cerebellum, supporting the notion that amygdala but not hippocampus or cerebellum is critically involved in auditory fear-potentiated startle. Previous reports also showed that auditory fear conditioning was associated with an activation of CREB (36) and cAMP-response element-mediated transcription (37) selectively in the amygdala. Furthermore, overexpression of CREB in the BLA using viral vectors has been shown to facilitate long term memory of fear-potentiated startle (30). Conversely, training for contextual conditioning or passive avoidance led to significant increase in cAMP-response element-dependent gene expression in areas CA1 and CA3 of the hippocampus (37). The central nucleus of the amygdala, which receives inputs from LA and BLA and in turn synapses on autonomic control cells in the hypothalamus and brain-
stem, is generally thought to mediate behavioral, autonomic, and emotional responses to stressful and fearful stimuli (38). Although we did not screen NF-κB activity in the central nucleus of the amygdala, it is very likely that its activity could be increased after training. In this respect, it has been demonstrated that auditory fear conditioning resulted in an increased in phosphorylated mitogen-activated protein kinase and phosphorylated CREB-immunoreactive cells not only in the LA and BLA but also in the central nucleus of the amygdala (28, 36).

LTP at sensory input synapses to the LA and BLA is a candidate mechanism for memory storage during fear conditioning (39, 40). Synaptic responses in the LA could be persistently enhanced after repetitive stimulation of central auditory pathways or in animals given auditory fear conditioning. In the present study, we found that TS or forskolin that reliably induced LTP in this area of the brain (20, 41) also enhanced NF-κB binding activity. In the same vein, administration of κB decoy DNA or NF-κB inhibitors resulted in an impairment of LTP and memory formation. These results reinforce the idea that memory consolidation of auditory fear and LTP in the LA may share a common molecular substrate. Supershift assays using antibodies against p65 or p50 subunits further indicated that the major NF-κB complex induced by TS or forskolin was the p65/p50 heterodimer. This finding is compatible with previous reports showing that both constitutively active and inducible NF-κB activities in the central nervous system predominantly consist of p50/p65 complexes (16, 42).

Using antibody against IκB-α, we found that protein levels of IκB-α were reduced by 20–40% at 10 min after TS or application of forskolin followed by its reappearance at a later time point (~30 min). This was accompanied by a decrease of NF-κB level in the cytoplasm concomitant with an increase in the nucleus. The rapid resynthesis of IκB-α could be due to the presence of three NF-κB binding sites in the promoter region of IκB-α gene, which was activated by NF-κB (7, 43–45). In the present study, forskolin-induced degradation of IκB-α was prevented by wortmannin, suggesting that PI 3-kinase is necessary for forskolin activation of NF-κB. A role for PI 3-kinase in the mediation of tumor necrosis factor and platelet-derived growth factor activation of NF-κB has been reported in embryonic kidney and primary fibroblast cells, respectively (21, 22).

Thalidomide, once used as a sedative and an anti-nausea medication for first trimester pregnancy, produced several developmental defects to the human fetus, leading to its removal from the market. It possesses anti-inflammatory and antioncogenic properties and has attracted considerable attention due...
NF-κB and Fear-potentiated Startle

Fear conditioning, or heightened synaptic activities in amygdala neurons induce Ca\textsuperscript{2+} influx through N-methyl-D-aspartate receptors. The synaptic activity in the amygdala also induces cAMP signaling through modulatory input such as β-adrenergic and dopamine receptors. Ca\textsuperscript{2+} and cAMP activate PI 3-kinase and its downstream target Akt, which in turn phosphorylates IKK resulting in the phosphorylation and degradation of IκB followed by nuclear translocation of NF-κB. PI 3-kinase and Akt could also induce MAPK phosphorylation, and subsequent translocation of mitogen-activated protein kinase (MAPK) into the nucleus activates CREB. In the nucleus, NF-κB and CREB may interact to fully transactivate effector genes for memory formation.

In conclusion, in an intriguing parallel, the present results show stimuli that generate LTP in slices or elicit fear-potentiated startle in animals activate NF-κB in the amygdala. NF-κB inhibitors, on the other hand, block IκB-α degradation as well as LTP and fear memory. These results provide the first evidence of a requirement of NF-κB activation in the amygdala for synaptic plasticity and memory consolidation. Furthermore, the identification of NF-κB as a molecular substrate in the formation of fear-potentiated startle suggests a potential new target for the treatment of anxiety and posttraumatic stress disorders.

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Note Added in Proof—It has come to our attention that a paper showing that IκB kinase and NF-κB activation are necessary for memory formation in the crab Chasmagnathus has recently been published in *Neuroscience* (Merlo, E., Freudenthal, R., and Romano, A. (2002) *Neuroscience* 112, 161–172).

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