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Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory

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Abstract

Learning and memory depend upon poorly defined synaptic and intracellular modifications that occur in activated neurons. Mitogen activated protein kinase-extracellular regulated kinase (MAPK-ERK) signaling and *de novo* protein synthesis are essential aspects of enduring memory formation, but the precise effector molecules of MAPK-ERK signaling in neurons are not well defined. Early growth response (Egr) transcriptional regulators are examples of MAPK-ERK regulated genes and Egr1 (zif268) has been widely recognized as essential for some aspects of learning and memory. Here we show that Egr3, a transcriptional regulator closely related to Egr1, is essential for normal hippocampal long-term potentiation (LTP) and for hippocampal and amygdala dependent learning and memory. In the absence of Egr3, the defects in learning and memory appear to be independent of Egr1 since Egr1 protein levels are not altered in amygdala, hippocampus or cortex. Moreover, unlike Egr1-deficient mice which have impairments in late phase hippocampal LTP and consolidation of some forms of long-term hippocampus- and amygdala-dependent memory, Egr3-deficient mice have profound defects in early- and late-phase hippocampal LTP, as well as short-term and long-term hippocampus- and amygdala-dependent learning and memory. Thus, Egr3 has an essential role in learning and memory processing that appears to be partly distinct from the role of Egr1.

Keywords

learning; memory; transcriptional regulation; plasticity; MAPK; Erk; mouse

Introduction

Neurons process and retain information by forming synaptic connections that are modified by the intensity and frequency of their historical activity. This remarkable capacity to regulate the

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efficacy of synaptic transmission is essential for continual remodeling of neural networks required for cognitive processes such as learning and memory. Long lasting synaptic enhancement of excitatory neurotransmission, known as long-term potentiation (LTP), is one of the best studied cellular forms of synaptic “plasticity.” Many types of synapses throughout the brain undergo LTP after appropriate patterned stimulation but the phenomenon has been best studied in the hippocampus, a brain structure that is essential for memory acquisition and consolidation (Milner et al., 1998). Enduring memory-related synaptic changes depend upon new gene transcription and translation (protein synthesis), but the specific molecular pathways involved have only been partially characterized. For example, activity dependent calcium flux into neurons through excitatory N-methyl D-aspartate (NMDA) glutamate receptors and/or voltage-gated calcium channels engages the mitogen-activated protein kinase (MAPK)-extracellular regulated kinase (ERK) signaling cascade which is essential for NMDA-dependent induction of LTP in area CA1 (English and Sweatt, 1996, 1997) and the dentate gyrus of the hippocampus *in vitro* (Coogan et al., 1999), and for regulation of LTP *in vivo* (Davis et al., 2000; Rosenblum et al., 2000). Moreover, pharmacologic inhibitors of ERK signaling impair contextual and spatial memory (Atkins et al., 1998; Blum et al., 1999). A major deficit in our understanding of MAPK-ERK signaling related to the regulation of LTP and neuronal information storage at the synaptic (LTP) and network (behavioral) level remains because the genes regulated by MAPK-ERK signaling are still poorly characterized (Thomas and Huganir, 2004).

Early growth response (Egr) transcription factors are among a relatively small number of regulatory immediate early genes (IEGs) that are expressed in response to neuronal activity and coupled to MAPK-ERK signaling. There are four structurally related Egr genes (Egr1–4) that encode proteins with highly conserved zinc finger DNA-binding domains. As MAPK-ERK effector molecules, they may regulate target gene expression required for long-term structural and/or physiologic synaptic changes associated with learning and memory. Egr1 (also known as zif268, NGFI-A, TIS8, Krox24 or ZENK) and Egr3 are the most abundant Egr proteins upregulated by synaptic activity in the brain (Li et al., 2005; O’Donovan and Baraban, 1999). Early studies correlated patterned synaptic activity required to induce LTP with *de novo* induction of Egr1 and Egr3 expression (Cole et al., 1989; Yamagata et al., 1994). Recently, Egr1 has been shown to be required for the maintenance of late phase (but not early phase) LTP, long-term memory consolidation and reconsolidation of previously formed memories (Bozon et al., 2003; Jones et al., 2001; Lee et al., 2004).

Although a role of Egr1 in learning and memory is now well established, it is not clear what role, if any, Egr3 may have in learning and memory processing. In recent studies, we showed that Egr1 and Egr3 can regulate target genes such as the plasticity associated Arc gene (Activity Regulated Cytoskeletal associated gene; also known as Arg3.1 (Link et al., 1995; Lyford et al., 1995)), suggesting that Egr1 and Egr3 may have some overlapping roles in regulating gene expression in the brain (Li et al., 2005). Similarly, other plasticity associated genes encoding the GABA receptor subunit 4 gene, GABRA4 (Roberts et al., 2005) and synaptic vesicle associated proteins, Syn1 (Thiel et al., 1994) and Syn2 (Petersohn et al., 1995) may be directly regulated by either Egr1 or Egr3. Thus, if Egr3 modulates the expression of important plasticity-associated genes in a physiologically relevant manner, it may be essential for some aspects of learning and/or memory. Here, we report that despite the fact that Egr3-deficient (Egr3^{-/-}) mice appear to have normally developed brains and normal basal synaptic transmission in CA3-CA1 hippocampal neurons where Egr1 and Egr3 are highly expressed, they have abnormal LTP in CA1 neurons, they have profound impairments in context and cued-associative learning/memory, and they have profoundly impaired short-term and long-term object recognition memory. Thus, Egr3 has an essential role in learning and memory presumably by regulating effector target genes required for memory acquisition, consolidation and/or retrieval.

Results

Normal brain development and basal synaptic transmission in *Egr3*^{-/-} mice

Egr3^{-/-} mice were previously generated but brain development was not systematically studied (Tourtellotte et al., 2001; Tourtellotte and Milbrandt, 1998). *Egr3*^{-/-} brains were compared to littermate WT to determine whether there were any detectable morphologic, pharmacologic and/or physiologic differences. Detailed examination of littermate WT and *Egr3*^{-/-} embryonic, perinatal and adult brains showed no gross or microscopic differences (data not shown). The hippocampus in particular, a brain region essential for normal learning and memory and where *Egr1* and *Egr3* are strongly induced by synaptic activity, appeared structurally normal (Fig. 1A, A'). In addition, there were no detectable differences between WT and *Egr3*^{-/-} brains in the distribution and density of neurons (Fig. 1B, B'), synaptic terminals (Fig. 1C, C'), or parvalbumin expressing interneurons (Fig. 1D, D') in the hippocampus. Similarly, Golgi-Cox staining showed no obvious alterations in neuron structure, orientation or migration in any brain regions examined (data not shown).

Dendritic synaptic spines on CA1 hippocampal neurons, which are enriched for excitatory glutamatergic receptors and which may be correlated with structural changes related to long-term memory (reviewed in (Segal, 2005)), were examined using Golgi-Cox staining. Spine structure appeared normal and spine density was similar between WT ($1.07 \pm 0.03/\mu\text{m}$) and *Egr3*^{-/-} ($1.01 \pm 0.04/\mu\text{m}$) CA1 neuron secondary dendrites ($p > 0.3$) (Fig. 2A). In addition, there was no significant difference in neuron density in CA1 (WT = $6.55 \pm 2.07 \times 10^5/\text{mm}^3$, *Egr3*^{-/-} = $6.02 \pm 2.03 \times 10^5/\text{mm}^3$, $p > 0.8$) and CA3 (WT = $3.63 \pm 1.15 \times 10^5/\text{mm}^3$, *Egr3*^{-/-} = $2.36 \pm 1.05 \times 10^5/\text{mm}^3$, $p > 0.5$) pyramidal neurons, or in the dorsal (d) (WT = $10.84 \pm 3.00 \times 10^5/\text{mm}^3$, *Egr3*^{-/-} = $6.08 \pm 2.03 \times 10^5/\text{mm}^3$, $p > 0.3$) and ventral (v) (WT = $10.22 \pm 2.86 \times 10^5/\text{mm}^3$, *Egr3*^{-/-} = $6.83 \pm 1.85 \times 10^5/\text{mm}^3$, $p > 0.4$) blades of the hippocampal dentate gyrus (dg) (Fig. 2B).

Since glutamatergic signaling is essential for normal learning and memory processing, the protein levels of several important glutamate receptor subunits were examined in WT and *Egr3*^{-/-} amygdala, hippocampus and somatosensory cortex. There were no significant changes in the NMDA (NR1, NR2A and NR2B), AMPA (GluR1, GluR2) or metabaphoric (mGluR5) glutamate receptor subunits examined (Fig. 3A). Similarly, protein levels of Erk1/2 and activated (phosphorylated) Erk1/2 (p-Erk1/2), essential mediators of short-term memory and early-phase LTP (English and Sweatt, 1997), were similar between *Egr3*^{-/-} and WT brains (Fig. 3A). *Egr1* protein, which was previously shown to be essential for late-phase LTP and long-term memory (Bozon et al., 2003; Jones et al., 2001), was not significantly different between WT and *Egr3*^{-/-} amygdala, hippocampus or somatosensory cortex (Fig. 3B).

Basal synaptic transmission was examined in CA3-CA1 synapses since both pre-synaptic and post-synaptic neurons express high levels of *Egr3* protein induced by strong CA3-CA1 neuronal activity (Li et al., 2005). Basal synaptic transmission, measured in acutely prepared hippocampal slices by varying the current pulse amplitude applied to CA3 Schaffer collaterals (20–250 μA) and by determining the amplitude of evoked excitatory post-synaptic potentials (EPSPs) in CA1 stratum radiatum, was not significantly different between WT and *Egr3*^{-/-} mice ($p > 0.5$, Fig. 4A). Similarly, there was no significant difference between WT and *Egr3*^{-/-} mice in paired pulse facilitation (PPF, a measure of presynaptic plasticity) of CA3-CA1 synapses when test pulses were administered to Schaffer collaterals at varying interpulse intervals ($p > 0.8$, Fig. 4B). Finally, the baseline Schaffer collateral synaptic EPSP initial slope was not significantly different between WT ($0.73 \pm 0.05 \text{ mV/ms}$, N=9) and *Egr3*^{-/-} ($0.65 \pm 0.05 \text{ mV/ms}$, N=9) mice ($p > 0.2$).

Hippocampal-dependent fear conditioning and novel object discrimination are impaired in *Egr3*^{-/-} mice

To examine whether *Egr3* is essential for normal learning and memory, WT and *Egr3*^{-/-} mice were tested for classical contextual and cued associative fear conditioning, and novel object discrimination. However, a series of control experiments was first performed to determine whether abnormal behaviors elicited by the tests were due to sensory and/or motor abnormalities, or whether they were attributable to memory defects. Naïve WT and *Egr3*^{-/-} mice showed similar activity when they were exposed to the experimental fear conditioning context environment (AC0, $p = 0.46$) and when they were exposed to the experimental context environment and presented with an audible tone (AT0, $p = 0.36$). Moreover, WT and *Egr3*^{-/-} mice responded to foot shock with a significant increase in locomotor activity (AS, $p < 0.0001$), and there was no difference in the induced activity response to shock between WT and *Egr3*^{-/-} mice (AS, $p = 0.6$; Fig. 5A). WT and *Egr3*^{-/-} mice also had similar behavioral responses to the foot shock. At low shock intensity, there was no significant difference between WT and *Egr3*^{-/-} mice in their flinching reflex ($p = 0.23$) and at higher shock intensity there was no significant difference in the shock intensity required to elicit a vocalization response ($p = 0.7$; Fig. 5B). In addition, there was no detectable difference between WT and *Egr3*^{-/-} mice in response to thermal nociceptive stimuli ($p = 0.62$; Fig. 5C). Thus, *Egr3*^{-/-} mice do not have any obvious defects in their ability to perceive and respond to nociceptive stimuli from shock or heat.

To examine whether *Egr3*^{-/-} mice can distinguish between different contexts and whether they have impairments in non-associative learning and/or memory, WT and *Egr3*^{-/-} mice were allowed to freely explore an open field context. Their exploratory activity was measured during a 3 minute training period and training was repeated in the same context each day for 5 consecutive days. There was no significant effect of genotype on the level of exploratory activity ($F_{1,6} = 0.2$; $p = 0.66$), but there was a significant reduction in their activity over time ($F_{4,35} = 4.4$; $p < 0.01$; Fig. 5D), suggesting they have similar non-associative learning during habituation to the context. After weak contextual fear conditioning both WT and *Egr3*^{-/-} mice exhibited significant decreased activity in response to shock-context pairing ($p < 0.001$) and the locomotor activity was significantly increased when the mice were subsequently tested in a novel context ($p < 0.001$). Thus, *Egr3*^{-/-} mice are capable of at least some forms of non-associative learning and they have at least some capacity to discriminate between two different contexts.

To examine whether *Egr3*^{-/-} mice have any capacity to learn from fear conditioning and to elicit the freezing behavior, WT and *Egr3*^{-/-} mice were trained using a high reinforcement (overtraining) paradigm. On the first day of training, the mice received 7 consecutive (reinforcement) repetitions of context/tone-shock pairing, between which freezing was assessed (see methods). Although *Egr3*^{-/-} mice showed a clear increase in freezing as the reinforcement training progressed during the first day of training, there was a significant effect of genotype ($F_{6,70} = 13.9$; $p < 0.001$; Fig. 5F) on freezing with *Egr3* mice consistently showing less freezing than WT during the reinforcement training. On the second day, after 7 rounds of reinforcement training, there was no significant difference in freezing behavior between WT and *Egr3*^{-/-} mice ($p = 0.25$; Fig. 5F). Thus, *Egr3*^{-/-} mice have some capacity for associative learning and they are capable of eliciting similar freezing behaviors as WT mice, if enough reinforcement training is provided.

If *Egr3* is involved in regulating synaptic activity dependent genes in the hippocampus that are required for normal learning and memory, *Egr3*^{-/-} mice may have learning and memory impairments in tasks that depend upon normal hippocampal function. We examined the performance of WT and *Egr3*^{-/-} mice in contextual fear conditioning and novel object discrimination, both of which depend upon intact hippocampal function (Clark et al., 2000;

Kim and Fanselow, 1992; Kim et al., 1993; Phillips and LeDoux, 1992; Zola et al., 2000). Naïve WT and *Egr3*^{-/-} mice received either single context-shock pairing (weak training) or triple context-shock pairing (strong training). Memory was assessed by measuring the conditioned response (freezing) to the same environmental context 24 hours after weak training, and 0.5 hours and 24 hours after strong training. There was a significant effect of genotype on weak ($F_{1,25} = 29.61$, $p < 0.0001$) and strong ($F_{1,25} = 48.73$, $p < 0.0001$) training (Fig. 6A). Although naïve WT and *Egr3*^{-/-} mice had a similar low frequency of freezing to context ($p = 0.86$), WT mice showed significantly increased freezing to context 24 hours after weak training relative to their naïve state ($p < 0.0001$), whereas *Egr3*^{-/-} mice showed no significant increase in freezing to context relative to their naïve state ($p = 0.16$). Thus, *Egr3*^{-/-} mice had no significant memory of the conditioned stimulus (context) 24 hours after weak training, which was manifest by significantly decreased freezing to context relative to conditioned WT mice ($p < 0.0001$; Fig. 6A). Similarly, 0.5 hours after strong training, WT mice showed markedly increased freezing to context compared to naïve WT mice and freezing remained elevated and unchanged for at least 24 hours after strong training ($p > 0.6$). By contrast, *Egr3*^{-/-} mice showed a significant freezing to context 0.5 and 24 hours after strong training relative to naïve *Egr3*^{-/-} mice ($p < 0.0001$ and $p < 0.01$, respectively), but freezing was significantly decreased both 0.5 and 24 hours after strong training compared to WT mice ($p < 0.0001$; Fig. 6A). Whereas freezing to context remained high and showed no significant change between 0.5 and 24 hours after strong training in WT mice ($p = 0.2$), the frequency of freezing to context significantly decreased between 0.5 and 24 hours after strong training in *Egr3*^{-/-} mice ($p < 0.05$) (Fig. 6A). Thus, *Egr3*^{-/-} mice have highly impaired short term (0.5 hour) and long term (24 hour) contextual fear memory, and by contrast to WT mice which retain a memory of the aversive context for at least 24 hours after strong training, in *Egr3*^{-/-} mice the memory is significantly dissipated between 0.5 and 24 hours after strong training.

WT and *Egr3*^{-/-} mice were next tested for their ability to discriminate between novel (N) and familiar (F) objects and a significant effect of genotype was observed ($F_{1,20} = 6.146$, $p < 0.05$). WT and *Egr3*^{-/-} mice were habituated over several trials to two objects within a context and they were subjected to training which showed that WT and *Egr3*^{-/-} mice spent equal time exploring both F objects ($p = 0.4$ and $p = 0.76$, respectively; Fig. 6B (Train)). After habituation training, the mice were evaluated 10 minutes and 24 hours in the same context with a single familiar object replaced by a novel object. Rodents tend to explore novel objects more frequently in this task, reflecting a memory of the familiar object which is required to discriminate the familiar object from the novel object (Clark et al., 2000). Thus, the amount of time exploring the novel object relative to the familiar object is an indirect measure of memory for the familiar object (see methods). WT mice showed a significant propensity to explore the novel object both 10 minutes ($p < 0.001$) and 24 hours ($p < 0.001$) after training. By contrast, *Egr3*^{-/-} mice showed no significant propensity to explore the novel object 10 minutes ($p = 0.17$) or 24 hours ($p = 0.15$) after training, suggesting that they had no memory of the “familiar” object encountered during prior habituation and training sessions.

Egr3 is up-regulated by synaptic activity in the amygdala and cortex, suggesting that it may have a more generalized function in regulating activity dependent gene expression outside of the hippocampus (Honkaniemi and Sharp, 1999; Yamagata et al., 1994). Thus, we examined the performance of WT and *Egr3*^{-/-} mice in cued associative fear conditioning, a task which depends upon normal amygdala function (Kim and Fanselow, 1992; Kim et al., 1993; Phillips and LeDoux, 1992). WT and *Egr3*^{-/-} mice received weak (single tone-shock pairing) and strong (triple tone-shock pairing) training and then were evaluated for their freezing response to tone in a novel context (see methods). A significant effect of genotype on weak ($F_{1,25} = 4.90$, $p < 0.05$) and strong cued associated fear conditioning ($F_{1,25} = 7.583$, $p < 0.01$) was observed. Both WT and *Egr3*^{-/-} mice showed significant freezing to auditory cue relative to naïve mice ($p < 0.0001$ and $p < 0.01$, respectively) but the cued freezing in *Egr3*^{-/-} mice was

significantly decreased relative to WT mice 24 hours after weak training ($p < 0.01$). Similarly, cued freezing was significantly increased in both WT and $Egr3^{-/-}$ mice relative to naïve mice 0.5 ($p < 0.0001$ and $p < 0.001$, respectively) and 24 hours ($p < 0.0001$ and $p < 0.001$, respectively) after strong training, but $Egr3^{-/-}$ mice showed significantly decreased cued freezing relative to WT mice 0.5 ($p < 0.01$) and 24 hours ($p < 0.01$) after strong training (Fig. 6C). There was no significant difference between the frequency of freezing either 0.5 or 24 hours after strong training in $Egr3^{-/-}$ mice. Thus, unlike contextual memory in $Egr3^{-/-}$ mice, which was completely dissipated 24 hours after weak training and nearly so 24 hours after strong training, cued memory was significantly impaired but not completely abolished 24 hours after either weak or strong training.

LTP is impaired in $Egr3^{-/-}$ hippocampal CA3-CA1 synapses

Since $Egr3$ expression is regulated by synaptic activity in CA1 neurons (Li et al., 2005; Yamagata et al., 1994) and because $Egr3^{-/-}$ mice showed clear hippocampus-dependent learning and memory defects (Fig. 6A, B), we examined whether $Egr3^{-/-}$ mice also had disrupted hippocampal LTP, a form of synaptic plasticity thought to be critical for normal learning and memory processing. LTP was reliably induced in WT and $Egr3^{-/-}$ CA3-CA1 synapses by either Schaffer collateral HFS or TBS in hippocampal slices (Fig. 7A, B). However, LTP was significantly decreased relative to WT 40 minutes after Schaffer collateral TBS (WT = $179.8 \pm 4.7\%$, $Egr3^{-/-}$ = $153.3 \pm 8.2\%$, $p < 0.02$, Fig. 7A) and similarly, LTP was significantly decreased relative to WT 30 minutes (WT = $196.6 \pm 8.4\%$, $Egr3^{-/-}$ = $132.1 \pm 9.4\%$, $p < 0.01$), 90 minutes (WT = $184.2 \pm 7.4\%$, $Egr3^{-/-}$ = $129.6 \pm 10.0\%$, $p < 0.01$) and 210 minutes (WT = $176.7 \pm 22.8\%$, $Egr3^{-/-}$ = $122.2 \pm 10.5\%$, $p < 0.05$) after Schaffer collateral HFS (Fig. 7B–D). Thus, LTP induction in Schaffer collateral synapses is similar but rapidly decreases in $Egr3^{-/-}$ mice relative to WT using two different LTP induction paradigms.

Discussion

$Egr3$ is a poorly studied member of the Egr family of transcriptional regulators that is structurally related to $Egr1$ (zif268). Like $Egr1$, $Egr3$ is regulated by synaptic activity *in vivo* and in excitatory cortical and hippocampal neurons by an NMDA receptor/MAPK-ERK signaling dependent mechanism (Li et al., 2005; Li et al., 2004; O'Donovan and Baraban, 1999; Yamagata et al., 1994). Several studies have indicated that $Egr1$ is essential for maintenance of late phase LTP, long-term memory in hippocampal dependent tasks and reconsolidation of previously memorized tasks (Bozon et al., 2003; Jones et al., 2001; Lee et al., 2004), but whether $Egr3$ has any role in learning and/or memory has not been previously examined. In this study, we showed that germline loss of $Egr3$ function has no obvious effect on the structural development of the brain, neuron architecture, the expression of several glutamatergic receptor subtypes, Erk phosphorylation, or $Egr1$ expression. Moreover, at least in the restricted context of Schaffer collateral (CA3-CA1) synaptic function, basal synaptic transmission and presynaptic plasticity are normal in $Egr3^{-/-}$ mice. However, there were defects in the magnitude of LTP induction in Schaffer collateral synapses and profound impairments in contextual fear conditioning and novel object discrimination, two memory tasks that depend upon normal hippocampal function. Since only germline $Egr3^{-/-}$ mice have been generated to date, it was not possible to exclude the possibility that some of the behavioral and physiologic abnormalities observed in $Egr3$ mutant mice may have been due to unrecognized developmental defects. Conditional mutagenesis of $Egr3$ will make it possible to selectively inactivate $Egr3$ in postnatal brain, after development is largely complete, and to bypass defects in muscle spindle morphogenesis that lead to abnormal proprioception in germline $Egr3^{-/-}$ mice (Tourtellotte and Milbrandt, 1998). In fact, the proprioception defects present in germline $Egr3^{-/-}$ mice precluded testing their behavior in other sensitive hippocampus dependent tasks that require intact motor skill, such as the Morris water maze. Nevertheless, the hippocampus

and amygdala dependent behavioral tasks that could be performed show a clear role for Egr3 in at least some forms of learning and memory, despite the fact that the precise target genes regulated by Egr3 in neurons are still not known.

Egr1 and Egr3 are non-redundant synaptic activity dependent transcriptional regulators

Egr1 and Egr3 have highly homologous c-terminal zinc finger DNA binding domains that recognize similar sequences in target gene promoters, raising the possibility that Egr proteins may have overlapping functions under some conditions (Swirnoff and Milbrandt, 1995; Tourtellotte et al., 2000). Clearly though, Egr1 and Egr3 do not have completely redundant function since they have differing expression patterns and the targeted mutant mice have differing phenotypes. For example, Egr1-deficient mice have fertility defects due to abnormal regulation of luteinizing hormone β peptide not shared by Egr3-deficient mice (Lee et al., 1996; Topilko et al., 1998) and Egr3-deficient mice have abnormalities in proprioception due to defective muscle stretch receptor development not shared by Egr1-deficient mice (Tourtellotte and Milbrandt, 1998). However, since Egr1 and Egr3 are often co-expressed in similar neurons after intense synchronous synaptic activity (i.e., seizure), drug treatment or natural behavior, it has been suggested that they may have some functional overlap in the central nervous system (CNS) (Li et al., 2005; Yamagata et al., 1994). The extent to which Egr1 and Egr3 may have overlapping function by regulating similar activity dependent target genes in the CNS is not known, but there do appear to be some differences between these two molecules. For example, Egr3 appears to have higher basal levels of expression in brains of untreated mice, Egr3 protein is more stable than Egr1 and hence remains in neurons for longer periods after activity mediated induction, and Egr1 and Egr3 have divergent activation domains which could recruit different cofactors to activate or repress the promoters of a diverse repertoire of target genes (Li et al., 2005; O'Donovan et al., 1999). The results of this study clearly highlight some functional differences between Egr1 and Egr3 in the CNS since Egr3^{-/-} and Egr1^{-/-} mice have some clear differences in learning and memory related behavior and LTP.

A role for Egr3 in Schaffer collateral-CA1 LTP

Whereas both Egr1^{-/-} and Egr3^{-/-} mice have normal basal synaptic transmission and short term plasticity (paired pulse facilitation), LTP is differentially affected. In particular, Jones et al. reported that Egr1-deficient mice have intact early LTP (up to 60 minutes following tetanic stimulation) but absent late LTP (24–48 hours) in the dentate gyrus *in vivo*. By contrast, in Egr3^{-/-} mice early LTP in CA1 is impaired relative to WT mice, a difference that occurs too rapidly to be explained by deficient LTP-induced Egr3 protein synthesis and impaired target gene regulation. Thus, Egr3 appears to be required prior to conditioning stimulation to regulate proteins that support WT levels of the early phase of LTP, whereas Egr1 is required to regulate gene expression required for late-phase but not early-phase LTP (Jones et al., 2001).

A role for Egr3 in hippocampal and amygdala dependent learning and memory

Egr1 and Egr3 also appear to have some differing roles in hippocampus and amygdala dependent memory. With contextual fear conditioning, Egr3^{-/-} mice had no memory of the aversive context 24 hours after weak training and they had highly impaired memory after strong training compared to WT mice. Interestingly however, Egr3^{-/-} mice were able to elicit the freezing behavior and they could be conditioned to freeze to context similar to WT, mice but only after a high degree of reinforcement training. Thus, Egr3-mediated gene expression is not absolutely required for context associated fear conditioning but rather, it seems to be required for the process to occur efficiently. By contrast, previous studies demonstrated that Egr1^{-/-} mice had no abnormalities in contextual fear conditioning after either weak or strong training in similar behavioral paradigms (Ko et al., 2005). Using the germline Egr3^{-/-} mice it was not

possible to determine how Egr3 protein that was present within neurons from neuronal activity prior to the learning task and Egr3 protein that was induced during the learning task contributed to task-dependent learning acquisition and/or memory retrieval. Additional molecular-genetic studies designed to manipulate Egr3 protein levels prior to acquisition, during consolidation and during retrieval will be required to more precisely define when Egr3 is required for certain stages of learning and/or memory processing. Nevertheless, since contextual fear memory was significantly dissipated in Egr3^{-/-} relative to WT mice between 0.5 and 24 hours after strong training, Egr3 protein may be required to stabilize the memory over time. Similarly, Egr3^{-/-} mice had no short-term (10 minute) or long-term (24 hour) object recognition memory, whereas Egr1^{-/-} mice were previously reported to have normal short-term but impaired long-term recognition memory in a similar behavioral paradigm (Jones et al., 2001). Thus, Egr3 is required for some forms of both short-term and long-term hippocampus-dependent memory, whereas Egr1 appears to be primarily required for long-term hippocampus-dependent memory. In amygdala-dependent memory, the differences between Egr1 and Egr3 were again apparent. Whereas, Egr3^{-/-} mice had impairments in cued fear conditioning after weak and strong training that became apparent 30 minutes after strong training, Egr1^{-/-} mice were previously shown to have comparatively mild defects in cued fear conditioning that only became apparent 3 days after conditioning (Ko et al., 2005).

Future work focused on defining the target gene networks that are differentially regulated by Egr1 and Egr3, and a controlled side-by-side comparison of Egr1- and Egr3-deficient mice, will be important for understanding how this family of MAPK-ERK coupled transcriptional regulators function in learning and memory processing. These current results identify Egr3 as a learning and memory related transcriptional regulator of considerable significance, particularly since Egr1^{-/-} mice have relatively mild impairments in learning and memory compared to Egr3^{-/-} mice.

Experimental Methods

Animals

Egr3 heterozygous mice from a BL/6-129Sv/J hybrid strain were backcrossed 4 generations to BL/6 isogenic mice and maintained as an inbred strain. The mice were generated and progeny were genotyped as previously described (Tourtellotte and Milbrandt, 1998; Whitehead et al., 2005). All experimental procedures complied with protocols approved by the Northwestern University and University of Nebraska Institutional Animal Care and Use Committees.

Histology and immunohistochemistry

Deeply anesthetized adult WT and Egr3^{-/-} mice were perfused through the heart with freshly prepared 4% paraformaldehyde/0.1 M phosphate buffer (pH=7.4). Immunohistochemistry was performed on free floating sections prepared from cryoprotected brain that had been equilibrated in 30% sucrose/PBS for 24 hours prior to freezing as previously described (Li et al., 2005). Primary antibodies that cross-reacted with NeuN (MAB377, Chemicon) to detect neurons, Synaptophysin (SC-9116, Santa Cruz) to detect synaptic terminals and Parvalbumin (MAB#235, Swant) to detect interneurons were used. After primary antibody incubation, the sections were treated with a biotinylated secondary antibody generated in the appropriate species and the antibody complexes were detected using avidin-biotin histochemistry with DAB as a chromagen, according to the manufacturer's specifications (Vector Labs).

In vitro slice preparation and field EPSP recordings

Hippocampal slices (350 μ m) from 3–4 month old male mice (TBS experiments) or male and female mice (HFS experiments) were prepared as previously described (Dong and Xiong, 2006; Yun et al., 2005). The animals were decapitated after deep anesthesia and their brains

were placed in ice-cold artificial cerebrospinal fluid (ACSF; 124 mM NaCl, 3 mM KCl, 2.4 mM CaCl₂, 1.3 mM MgSO₄, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, 10 mM glucose and saturated with 95% O₂/5% CO₂ to a final pH=7.4). Vibratome slices were equilibrated for ≥ 2 hr. at room temperature in ACSF and were transferred as needed to a submersion chamber maintained at 32°C. Field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum radiatum of area CA1 in the hippocampus in response to electrical stimulation of the Schaffer collateral/commissural axons. Each fEPSP was an average of 5 (TBS protocol) or 3 (HFS protocol) consecutive sweeps generated in response to 0.05 Hz test pulses. Electrophysiological measures of basal synaptic transmission included input/output (I/O) relationships and short-term presynaptic plasticity (paired pulse facilitation, PPF). I/O relationships were measured by comparing EPSP amplitudes as a function of stimulus intensity using a pulse width of 0.1 msec and a current range of 20–250 μ A. The current that produced approximately 35% of maximal responses (usually 20–40 μ A) was used throughout all subsequent experiments for monitoring test pulses. The same current was used to evoke PPF which was calculated as a percentage of EPSP slopes (pulse 2/pulse 1) in response to pairs of stimuli delivered with interpulse intervals of 20 msec, 50 msec and 100 msec. The data were sampled at 10 kHz and low-pass filtered at 3 kHz. The baseline EPSP amplitude was recorded in response to 0.05 Hz test pulses for 10 minutes after which LTP was induced by applying either TBS which consisted of 10 bursts with an inter-burst interval of 200 ms (5 Hz) or HFS which consisted of 2 tetani of 1 second each at 100 Hz and with a 20 second inter-tetanus interval. During TBS and HFS, the stimulus intensity was not varied. The magnitude of LTP was expressed as the percentage of baseline and compared between groups of WT and Egr3^{-/-} mice at 40 minutes after TBS and 30 minutes, 90 minutes and 210 minutes after HFS. All responses were normalized to the averaged 10 minute (TBS) or 30 minute (HFS) baseline measurements to allow comparison among slices. pClamp v. 8.0 (Axon Instruments, U.S.A.) was used for data collection and analysis.

Neuron counts

Neurons from defined anatomical regions of the: dorsal (d) and ventral (v) blade of the dentate gyrus as well as area CA1 and CA3 pyramidal neurons of the hippocampus were counted in 3 WT and 3 Egr3^{-/-} adult brains (8–12 week old). The mice were perfused through the heart with 0.1 M PB, pH=7.4 followed by 4% paraformaldehyde/0.1 M PB, pH=7.4. The brains were embedded in paraffin and serial 6 μ m coronal sections were generated and counterstained with hematoxylin and eosin. For each brain, neurons were counted from 4 consecutive sections each separated by 40 μ m. The sections spanned a total rostral-caudal distance of 160 μ m including and caudal to coordinates: interaural, 2.34 mm; bregma -1.46 mm (Paxinos and Franklin, 2001). The number of neurons within each analyzed region of the hippocampus was estimated using a statistically unbiased optical dissector protocol (StereoInvestigator, MicroBrightfield). The neuron density (number of neurons per unit volume) was compared between WT and Egr3^{-/-} brains for each of the areas of the hippocampus analyzed.

Golgi silver impregnation

Golgi-Cox silver impregnation of neurons was performed using commercially available reagents (FD Rapid Golgi Stain Kit, NeuroTechnologies). Twelve-week-old freshly dissected mouse brains were immersed in silver impregnation solution for 2 weeks, transferred to Solution C for 2 days, and cut into 100 μ m sections on a cryostat. The sections were mounted on 3% gelatin-coated glass slides, air-dried for 1 week and then stained with Solution D and E followed by alcohol dehydration and cover-slip mounting with Permount.

Spine counts

Dendritic spines were counted in Golgi impregnated CA1 neurons using oil-immersion and 1000 $\times$ total magnification. Spines were counted along a 40 μ m distance of CA1 neuron secondary dendrites starting 10–30 μ m from their point of origin from the primary dendrite. The number of individual spines in 40 μ m segments of secondary dendrites were counted in 40 CA1 neurons from each of 3 WT and 3 Egr3^{-/-} brains. The results were expressed as the number of spines per μ m length of secondary dendrite.

Behavioral Studies

Conventional fear conditioning and novel object discrimination were performed as previously described with some modification (Jones et al., 2001; Radulovic et al., 1998). WT and Egr3^{-/-} littermate male and female mice ranging in age from 8–12 weeks were tested.

Fear conditioning—Fear conditioning was performed using a computer-controlled activity monitor and shock/tone generator (TSE, Bad Homburg, Germany). The mice were exposed to two contexts: Context 1 consisted of a Plexiglas chamber (35 $\times$ 20 $\times$ 20 cm) inside of which was placed a removable shock grid made of stainless steel rods (4 mm diameter with 0.9 cm spacing). The shock grid was connected to a constant current power supply set to deliver a 2-second 0.7 mA shock. The shock grid and chamber were cleaned with 70% aqueous ethanol prior to testing each animal. Context 2 consisted of a Plexiglas chamber (35 $\times$ 20 $\times$ 20 cm) that was exposed to a constant 20-watt light source and no shock grid. The chamber was washed with 1% aqueous acetic acid before each mouse was placed into the chamber. A signal generator connected to a loudspeaker (Conrad, KT-25-DT) produced a 10 kHz, 75 dB tone and the locomotor activity (cm/s) of each mouse was automatically recorded by an infrared beam detector array in each of the experimental context chambers.

Training—Mice were subjected to two training conditions: *Weak training* (single conditioning trial) which consisted of a 3 minute exposure to context 1, followed by a tone and a single foot shock; *Strong training* (multiple conditioning trial) consisted of a 30 second exposure to context 1, followed by a tone and a foot shock with context and tone/shock pairing repeated 3 times.

Contextual fear conditioning—Contextual memory was tested 24 hours after weak training, and 0.5 and 24 hours after strong training. Testing was performed by re-exposing each mouse for 3 minutes to context 1 without the administration of either shock or tone.

Cued associative fear conditioning—Each mouse was exposed to context 2 and freezing response was measured for 3 minutes after presentation of a tone. For both contextual and cued associative fear conditioning, freezing was defined as a lack of movement associated with a crouching posture. Freezing behavior was assessed at the end of every 10 second interval by two trained observers during the 3 minute trial period (a total of 18 assessments were made during the 3 minute trial). The data were recorded as a fraction (percent) of the total measurements scored as freezing for each trial.

Novel object discrimination—The natural behavior of WT and Egr3^{-/-} mice was observed in an open 25 $\times$ 22 $\times$ 13 cm field (context) which contained two objects. Each mouse was habituated to the context and objects for 10 minutes on day 1 by allowing them to freely explore the area and the objects. Further habituation was performed on days 2 and 3, which consisted of two 10 minute trials of context and object exploration interrupted by a 10 minute period in the home cage. On day 4 each mouse was subjected to a single 10 minute training session followed by testing. Testing was performed 10 minutes and 24 hours after the training session by replacing one of the familiar (F) objects with a novel (N) object (a different novel object

was used for the 10 minute test and the 24 hour test). Measurements were made by recording the amount of time each mouse spent with both forelimbs oriented toward and within 4 cm of either object. The data were reported as the mean total time (sec.) spent exploring the novel and familiar object during the 3 minute trial period for WT and *Egr3*^{-/-} mice.

The results obtained from the performance of male and female mice on a particular task were pooled since there was no gender affect for strong contextual fear conditioning ($F_{1,72} = 0.454$, $p = 0.50$), strong cued associative fear conditioning ($F_{1,72} = 2.95$, $p = 0.1$), weak contextual fear conditioning ($F_{1,48} = 2.39$, $p = 0.14$), weak cued associative fear conditioning ($F_{1,48} = 0.20$, $p = 0.65$) or novel object discrimination ($F_{1,60} = 0.23$, $p = 0.64$).

Shock threshold testing—Adult WT (N=6) and *Egr3*^{-/-} (N=5) mice were placed inside the fear conditioning chamber with a removable shock grid floor. Individual mice were subjected to 2-second shocks with intensities ranging from 0.1 mA to 0.8 mA in 0.1 mA increments, similar to that described previously (Bourtchuladze et al., 1994). At each shock intensity, the animals were scored for their involuntary flinching or overt vocalization responses to shock. The lowest current intensity to elicit the particular behavior was recorded and the mean response thresholds were compared between WT and *Egr3*^{-/-} mice.

Nociception testing—Nociception was tested by placing adult WT (N=5) and *Egr3*^{-/-} (N=5) mice on a 55 °C hotplate and the latency to lick their hindpaw was measured.

Non-associative learning (habituation) testing—Adult WT (N=4) and *Egr3*^{-/-} (N=3) mice were placed in a 35 × 20 × 20 cm context chamber and their activity during a 3 minute training period was recorded by infrared beam crossing instrumentation (TSE, Bad Homburg, Germany). The training was repeated for each mouse for 5 consecutive days and the mean activity was recorded and compared over time between WT and *Egr3*^{-/-} mice.

Context discrimination—Adult WT (N=13) and *Egr3*^{-/-} (N=11) mice were tested for their ability to discriminate between two different contexts. Mice received weak contextual fear conditioning and were tested 24 hours after training. The testing was performed by re-exposing the mice to the original context (context 1) that was used during the context-shock fear conditioning and their freezing behavior was recorded during a 3 minute trial. Subsequently, the mice were exposed to a novel context (context 2) for 3 minutes during which time freezing was measured.

Freezing test—To examine whether *Egr3*^{-/-} mice can produce the expected freezing behavior after shock and to evaluate their capacity for associative learning, adult WT (N=5) and *Egr3*^{-/-} (N=5) mice were exposed to a highly reinforced (overtaining) fear conditioning protocol similar to that previously reported for testing PKC- β -deficient mice (Weeber et al., 2000). The mice were placed in the fear conditioning chamber containing the shock grid for 1 minute, followed by a 30 second acoustic tone (10 kHz, 75 dB) and a 2-second 0.7 mA shock at the end of the tone. Freezing behavior was measured during this 90 second context-shock interval and the process was repeated 7 times during Day 1. The entire procedure was repeated again on Day 2.

Quantitative Western blotting

Microdissected hippocampus, amygdala and somatosensory cortex were collected from WT and *Egr3*^{-/-} mice and lysed in RIPA buffer to collect total cellular protein as previously described (Li et al., 2005). Protein samples (100 μ g/lane) were electrophoresed on 10% SDS poly-acrylamide gels and the protein was immobilized onto PVDF membranes (Immobilon-P, Millipore). The membranes were incubated at room temperature 1 hour with specific

antibodies to: Egr3 (sc-191), NR1 (sc-9058), Erk-1/2 (sc-94), p-Erk1/2 (sc-16982) all from Santa Cruz Biotechnology, Santa Cruz, CA, NR2A (JH1817, R. Huganir, Johns Hopkins University, Baltimore, MD), NR2B (JH1741, R. Huganir, Johns Hopkins University, Baltimore, MD), GluR1 (AB1540, Chemicon, Temecula, CA), GluR2 (JH1707, R. Huganir, Johns Hopkins University, Baltimore, MD), mGluR5 (AB5675, Chemicon, Temecula, CA), and β -actin (A2228, Sigma-Aldrich, St. Louis, MO), followed by incubation with alkaline phosphatase-conjugated Goat anti-rabbit antibody (Jackson ImmunoResearch) and visualized using enhanced chemiluminescence (ECL) (CDP-star, Tropix) on autoradiography film (Amersham hyperfilm ECL). To ensure that the ECL readout was within the linear detection range of the film, standard curves were generated by systematically varying the protein concentration of samples and subjecting them to ECL and densitometry. The blots probed for the proteins of interest were then processed for differing lengths of time in ECL and densitometry measurements were performed using the computer software Wincam 2.2 (Cybertech, Berlin, Germany) and ensuring that the densitometry readings fell within the linear range of the standard curves. Background signals were subtracted from a film region close to the bands of interest. The densitometric values were normalized to β -actin to control for variation in protein loading.

Statistical Analyses

All data were analyzed by two-way ANOVA using Genotype (WT and Egr3^{-/-}) and Test (e.g., baseline, short-term memory and long-term memory) as grouping factors. Post-hoc analyses were performed using Scheffe's test for multiple comparisons. Since all WT animals learned the particular task, comparisons grouped by *Test* were all significant: object discrimination ($F_{2,60} = 3.704$, $p < 0.05$), strong contextual fear conditioning ($F_{2,75} = 43.968$, $p < 0.001$), strong cued associative fear conditioning ($F_{2,75} = 31.270$, $p < 0.0001$), weak contextual fear conditioning ($F_{2,75} = 36.925$, $p < 0.0001$) and weak cued associative fear conditioning ($F_{2,75} = 37.828$, $p < 0.0001$). All values were expressed as mean $\pm$ SEM and when individual groups were compared Student's unpaired t-test was used to compare the means. The I/O correlations and PPF were analyzed using repeated measures ANOVA with Genotype as the grouping variable. In all instances $p < 0.05$ was considered statistically significant.

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Figure 1.

Hippocampal development is normal in *Egr3*-deficient mice. Normal neuron distribution, synaptic terminal labeling and interneurons are identified in the hippocampus of (A, B, C, D) WT and (A', B', C', D') *Egr3*^{-/-} mice. Compared to (A) WT, the hippocampus appears structurally normal in (A') *Egr3*^{-/-} brains. Similarly, (B, B') NeuN immunohistochemistry to label neurons, (C, C') synaptophysin (Syn) immunohistochemistry to label synaptic terminals and (D, D') parvalbumin (Parv) immunohistochemistry to label interneurons shows no definitive differences in the hippocampus between WT and *Egr3*^{-/-} mice. [Scale bars: 200 μ m]

Figure 2.

Normal CA1 hippocampal neuron spine density and hippocampal neuron numbers in Egr3^{-/-} mice. (A) Golgi-cox staining shows normal CA1 pyramidal neuron morphology with typical primary apical dendrites (1°) and spine laden secondary dendrites (2°). Spine morphology on WT and Egr3^{-/-} secondary dendrites is similar (enlarged regions obtained from representative 2° dendrites similar to white outlined area), and spine density on CA1 pyramidal neuron secondary dendrites is not significantly different ($p > 0.3$) in WT and Egr3^{-/-} brains. (B) Neuron density in the (d) dorsal and (v) ventral blade of the dentate gyrus as well as in areas CA3 and CA1 are not significantly different in Egr3^{-/-} hippocampus relative to WT ($p > 0.8$, $p > 0.5$, $p > 0.5$, $p > 0.4$, Student's unpaired t-test, respectively). (results represent mean $\pm$ SEM with white bars = WT and black bars = Egr3^{-/-}) [Scale bars: 10 μm (A) and 200 μm (B)]

Figure 3.

Biochemical analysis of glutamatergic signaling molecules in WT and Egr3^{-/-} hippocampus, amygdala and somatosensory cortex. (A) Glutamate receptor subunits, Erk1/2, and activated Erk1/2 (p-Erk1/2) protein levels were analyzed by Western blotting and quantitative densitometry in amygdala, hippocampus and somatosensory cortex. There were no significant differences identified for any of the proteins and brain regions examined in WT and Egr3^{-/-} mice ($p > 0.1$). (B) The level of Egr1 protein, a transcriptional regulator closely related to Egr3 and known to play an essential role in learning and memory, was not significantly different between WT and Egr3^{-/-} in amygdala, hippocampus and somatosensory cortex ($p > 0.1$ for each comparison). For Egr1 detection, the blots and densitometry results were derived from 5

minute ECL exposures to detect low basal levels of Egr1 in unstimulated (directly from home cage) WT and Egr3^{-/-} mice. (results represent mean ratios of Egr3^{-/-}/WT protein $\pm$ SEM with black bars = amygdala, white bars = hippocampus and gray bars = somatosensory cortex)

Figure 4.

Normal basal synaptic transmission in Egr3^{-/-} hippocampal Schaffer collateral synapses. (A) CA1 excitatory post synaptic potentials (EPSPs) elicited by stimulating WT and Egr3^{-/-} Schaffer collateral synapses with varying current pulse intensity (0.1 ms pulse width, 20–250 μA) were not significantly different ($p > 0.5$). (B) Paired-pulse facilitation (PPF) was not significantly different between WT and Egr3^{-/-} Schaffer collateral synapses using pulse intervals of either 20 ms, 50 ms or 100 ms ($p > 0.8$). (results represent mean $\pm$ SEM with WT = white bars and Egr3^{-/-} = black bars).

Figure 5.

Locomotor activity, shock threshold response, thermal nociception, non-associative learning and overtraining fear conditioning behavior compared between WT and Egr3^{-/-} mice. (A) Naïve WT and Egr3^{-/-} mice showed no significant differences in baseline activity (AC0) or in their activity response to audible tone while exploring context (AT0). However, they showed a highly significant increase in activity immediately after foot shock (AS) and there was no difference in the locomotor response to shock between WT and Egr3^{-/-} mice. (B) There was no significant difference between WT and Egr3^{-/-} mice in the shock intensity threshold required to elicit either involuntary flinching behavior or a vocalization response to the shock stimulus. (C) Similarly, there was no significant difference in thermal nociception as determined by hotplate testing and hindpaw lick latency in WT and Egr3^{-/-} mice. (D) Egr3^{-/-} mice showed normal habituation to exploratory activity in a non-associative learning and memory task. (E) In addition, they were capable of discriminating between two different contexts. WT and Egr3^{-/-} mice were subjected to weak contextual fear conditioning and after 24 hours, they showed significantly decreased activity as a consequence of fear conditioned freezing. When the mice were immediately exposed to a novel context, their locomotor activity

was significantly increased back to levels that were not statistically different from WT and $Egr3^{-/-}$ naïve mice. (F) To examine whether $Egr3^{-/-}$ mice are capable of eliciting a normal freezing behavior and to determine whether they have any capacity to learn after highly reinforced training, WT and $Egr3^{-/-}$ mice received 7 consecutive rounds of context-shock pairing on Day 1 and Day 2, and freezing behavior was scored. $Egr3^{-/-}$ mice showed consistently less freezing behavior than WT mice after each reinforcement trial but by the end of training on Day 2, freezing behavior between WT and $Egr3^{-/-}$ was maximal (100%) and not significantly different. (results represent mean $\pm$ SEM with WT = white bars and $Egr3^{-/-}$ = black bars; n.s. = not significant, * = $p < 0.001$, Student's unpaired t-test)

Figure 6.

Examination of hippocampus and amygdala dependent memory in *Egr3*^{-/-} mice. (A) Naïve WT and *Egr3*^{-/-} mice showed very little freezing to context prior to training. However, 24 hours after weak training freezing to context was significantly increased in WT mice. By contrast, *Egr3*^{-/-} mice had no memory of the aversive shock 24 hours after weak training since there was no significant increase in freezing to context compared to naïve *Egr3*^{-/-} mice. Similarly, *Egr3*^{-/-} mice showed significantly less freezing to context than WT mice 0.5 and 24 hours after strong training. Whereas freezing to context remained high and did not significantly change between 0.5 and 24 hours after strong training in WT mice, freezing to context significantly dissipated between 0.5 and 24 hours in *Egr3*^{-/-} mice. (B) Habituated WT

and $Egr3^{-/-}$ mice spent equal time exploring two familiar (F) objects presented in the context (Train). WT mice showed a highly significant preference to explore a novel (N) object when it was substituted for a familiar object, whereas $Egr3^{-/-}$ mice showed no such preference either 10 minutes or 24 hours after habituation training. $Egr3^{-/-}$ mice apparently had no memory of the remaining familiar object after one of the familiar objects was replaced with a novel object either 10 minutes or 24 hours after training since in either case there was no significant preference to explore one object relative to the other. (C) Naïve WT and $Egr3^{-/-}$ mice showed minimal freezing to tone. However, 24 hours after weak cued associative training both WT and $Egr3^{-/-}$ mice showed significantly increased freezing to tone compared to naïve mice, but freezing to tone was significantly decreased in $Egr3^{-/-}$ compared to WT mice. Similarly, 30 minutes and 24 hours after strong training, $Egr3^{-/-}$ mice showed significantly less freezing to tone compared to WT mice. (results represent mean $\pm$ SEM with WT = white bars and $Egr3^{-/-}$ = black bars; n.s. = not significant, * = $p < 0.001$, Student's unpaired t-test).

Figure 7.

Defective LTP in Schaffer collateral synapses in Egr3^{-/-} mice. LTP was reliably induced in Schaffer collateral synapses using either (A) TBS or (B) HFS in WT and Egr3^{-/-} hippocampal slices. However, LTP decreased in Egr3^{-/-} mice shortly after induction relative to WT and remained significantly decreased, at least up to 210 minutes after induction. (C) Representative EPSP traces at three time points after LTP induction by HFS. In WT slices, EPSP magnitude was markedly increased after LTP induction (thick line trace) relative to EPSP magnitude prior to LTP induction (thin line trace) 30, 60 and 210 minutes after HFS. In Egr3^{-/-} slices however, the CA1 EPSP magnitude progressively declined over the 30–210 minute interval after LTP induction by HFS. (D) LTP in Schaffer collateral synapses was significantly decreased in Egr3^{-/-} slices compared to WT at 30, 60 and 210 minutes post-LTP induction. Dashed line represents EPSP baseline (before HFS). (results represent mean $\pm$ SEM with WT = white and Egr3^{-/-} = black; A, average of 9 slices from 9 animals each genotype; B, D, average of 5 slices from 5 WT animals and average of 6 slices from 5 Egr3^{-/-} animals; * = $p < 0.05$, ** = $p < 0.01$, Student's unpaired t-test). [Scale bars: 10 msec, 0.1 mv]