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A Role in Learning for SRF: Deletion in the Adult Forebrain Disrupts LTD and the Formation of an Immediate Memory of a Novel Context

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Summary

Whereas significant insight exists as to how LTP-related changes can contribute to the formation of long-term memory, little is known about the role of hippocampal LTD-like changes in learning and memory storage. We describe a mouse lacking the transcription factor SRF in the adult forebrain. This mouse could not acquire a hippocampus-based immediate memory for a novel context even across a few minute timespan, which led to a profound but selective deficit in explicit spatial memory. These animals were also impaired in the induction of LTD, including LTD triggered by a cholinergic agonist. Moreover, genes regulating two processes essential for LTD—calcium release from intracellular stores and phosphatase activation—were abnormally expressed in knockouts. These findings suggest that for the hippocampus to form associative spatial memories through LTP-like processes, it must first undergo learning of the context per se through exploration and the learning of familiarity, which requires LTD-like processes.

Introduction

A major focus of work on the hippocampus has been to explore the relationship between synaptic plasticity, learning, and memory. The acquisition and long-term maintenance of associative spatial memories by the hippocampus are thought to be mediated through various forms of synaptic plasticity that often use long-term potentiation-like (LTP) processes (Martin and Morris, 2002). By contrast, the role of long-term depression (LTD) in learning and memory is much less clear.

Before being able to form associative spatial memories related to specific cues in an environment, an animal must first form a memory of the environment itself. Rodents respond to a novel spatial context with increased exploratory activity. As the animal forms a memory of the space, it habituates its exploration (Berlyne, 1960; Corman and Shafter, 1968; Thinus-Blanc et al., 1987). Habituation is thus the behavioral reflection of mnemonic processes and is distinct from response fatigue, can occur over the course of a few minutes, and can be maintained over long periods of time (Thompson and Spencer, 1966; Cheal et al., 1982).

Lesions of the hippocampus disrupt contextual habituation to a novel context in rats, much as they disrupt complex associative memories, producing animals that continually explore a new environment because they are unable to remember that they have previously encountered it (Gray and McNaughton, 1983; Roberts et al., 1962). Similar deficits occur following disruption in the hippocampus of either NMDA receptor-mediated synaptic transmission (Adrover et al., 2003; Vianna et al., 2000), cholinergic modulation of synaptic transmission (Lamprea et al., 2000, 2003), or CaM-kinase II-mediated intracellular signaling (Vianna et al., 2000). Consistent with these findings, in vivo microdialysis of the hippocampus has revealed that exploration of a novel environment is associated with a dramatic increase in the release of acetylcholine (Acquas et al., 1996; Giovannini et al., 2001).

Studies of neural activity associated with the processing of context-related stimuli in humans and in nonhuman primates point to a reduction in neuronal responses as novel stimuli are repeated (Rolls et al., 1993; Vinogradova, 1995; Yamaguchi et al., 2004). To probe the plasticity mechanisms leading to this response reduction, Manahan-Vaughan and Braunewell (1999) applied test pulses, which themselves did not alter synaptic efficacy, to the hippocampal CA3-CA1 Schaffer collateral pathway in vivo while the animal explored a novel environment. Exposure to novelty resulted in LTD in this pathway that was absent if test pulses were applied after the animal had habituated to the environment. Importantly, if test pulses were omitted during exposure to novelty, no LTD was induced, demonstrating that this plasticity is activity dependent. These data suggest that the encoding of a novel context, a process reflected by contextual habituation, may involve LTD-like processes in the hippocampus. Furthermore, while neurons need to be active to undergo LTD, neuronal activity even at a low frequency (like test pulses) is sufficient to induce LTD in vivo.

In this study, we report on mice carrying as adults a forebrain-restricted deletion of the transcription factor serum response factor (SRF). SRF regulates the serum response element (SRE), an enhancer site found in many immediate-early genes (Buchwalter et al., 2004; Finkbeiner and Greenberg, 1998). The genes containing an SRE are often recruited together with those

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containing a cyclic AMP response element (CRE) enhancer site, a site that has been implicated in long-term memory and the transcription-dependent late phase of LTP (Finkbeiner and Greenberg, 1998; Pittenger et al., 2002). During development of the nervous system, SRF has an important role in neuronal migration and axon guidance (Alberti et al., 2005; Knoll et al., 2006). Animals in which SRF is deleted from the forebrain beginning in late gestation have a severely hypoplastic hippocampus, are much smaller than their littermates, and die at 3 weeks postnatal (Alberti et al., 2005). We circumvented these developmental effects of SRF deletion by eliminating the gene only in adult mice.

Another recent study of an adult forebrain-restricted SRF deletion focused primarily on gene expression in knockout animals and found decreased expression of many SRE-containing genes (Ramanan et al., 2005), further supporting a role for SRF in neuronal gene transcription in vivo. Ramanan et al. suggested that these gene expression deficits reflect a role of SRF in the gene induction required for the formation of long-term memory and the late phase of LTP. However, these authors did not characterize the animals' behavior and provided limited electrophysiological analysis of LTP with no description of LTD in knockout mice.

Our forebrain-restricted SRF-deficient animals indeed had learning and memory deficits. However, these deficits were not evident as a disruption of long-term memory and the late phase of LTP. SRF knockout mice were impaired in the formation of a hippocampus-dependent immediate memory of a novel context, which prevented the learning of an associative spatial task, an effect that was specific for explicit memory processes. The deficit in habituation was also strongly associated with perturbations in the induction of LTD and not LTP. To better understand the role of SRF in LTD, we undertook gene expression studies and found altered expression of several genes regulating the release of calcium from intracellular stores and the activation of phosphatases, both processes essential for LTD.

Results

SRF Deletion in the Adult Forebrain

SRF is expressed in neurons throughout the brain, including heavily in the hippocampus (Herdegen et al., 1997). We avoided lethality related to the role of SRF in development by restricting the temporal and spatial extent of deletion of loxP-flanked *srf* using a cre recombinase transgene that was expressed only in the forebrain and turned on after development (see Experimental Procedures). Using RT-PCR, we verified that *srf* mRNA was eliminated from the hippocampi of 3-month-old knockout animals (Figure 1A). Immunostaining of brains from 3-month old knockout animals, the most sensitive method for determining the extent of SRF deletion, revealed complete elimination of SRF protein in CA1 and dentate gyrus, with nearly complete elimination in CA3 (Figure 1B). SRF protein was also eliminated in the cortex and striatum, but not in regions outside the forebrain, like the cerebellum (Figure 1B).

SRF protein levels are developmentally regulated, with upregulation occurring in early postnatal weeks (Stringer et al., 2002). As seen in Figure 1C, SRF expression was not affected in the hippocampus until animals were 5–8 weeks old, circumventing developmental deficits due to the role of SRF in brain development (Alberti et al., 2005). Finally, we found no neuroanatomical differences between knockouts and controls using either synaptophysin staining, which highlights terminals of the Schaffer collateral pathway; staining of the neuronal nuclear marker NeuN (Figure 1D); or MAP2 staining, which identifies the cellular structure of neurons (data not shown). We observed no gross changes in the number of neurons, which is consistent with a previous study on neuronal viability in another line of mice in which SRF had been deleted in the adult forebrain (Ramanan et al., 2005). In summary, we eliminated SRF selectively in the adult forebrain, and in that way avoided the neuronal migration and axon guidance deficits (and consequent hypoplastic hippocampus) that have been noted after prenatal deletion of SRF (Alberti et al., 2005; Knoll et al., 2006).

Deficits in the Encoding of a Novel Context Lead to Impaired Contextual Habituation

To examine the ability of SRF knockout animals to form an immediate memory of a novel context, we tested the ability of animals to habituate in a novel open field—a very simple form of nonaversive learning about novel contextual information. In the first 5 min after placement in a novel open field, knockout and control animals explored the space similarly (Figure 2A; $p = 0.35$). Over the ensuing hour, control animals habituated their exploratory locomotor behavior, but knockouts failed to show any habituation (Figure 2A; ANOVA effect of genotype $p < 0.001$; genotype × time interaction $p < 0.001$), even when placed back into the same context on a second day. Control animals, by contrast, displayed their memory of the context through both decreased locomotion in the first 5 min on day 2 compared to day 1 ($p = 0.005$) and a more rapid habituation on day 2 than on day 1 (ANOVA day × time interaction $p = 0.004$). These deficits could not be attributed to knockouts having altered levels of anxiety, motor deficits, a general release on locomotion, or simply the effect of the cre transgene (see the Supplemental Data available online).

If the open field habituation deficit were due to an inability of knockouts to form an immediate memory of the novel context, then the animals should be amnesic when reintroduced into a previously novel context that they had explored only moments earlier. We allowed animals to explore two arms of a Y-maze for 10 min, acquiring contextual memory by exploration in the same manner as they had in the open field. The animals were then removed and returned 2 min later with all three arms available. Control animals formed a memory of the two open arms during exploration and preferentially explored the novel arm during the test session (Figure 2C; one-sample t test $p = 0.001$). By contrast, knockout animals showed no preference ($p = 0.68$) and differed significantly from controls ($p = 0.03$).

Two days after exposure and habituation to the object-less open field, we allowed animals to explore a novel object placed in the center of the open field for 30 min. During the first 10 min, the novel object stimulated similar amounts of exploration in control and knockout animals (Figure 3A; $p = 0.54$). However, only

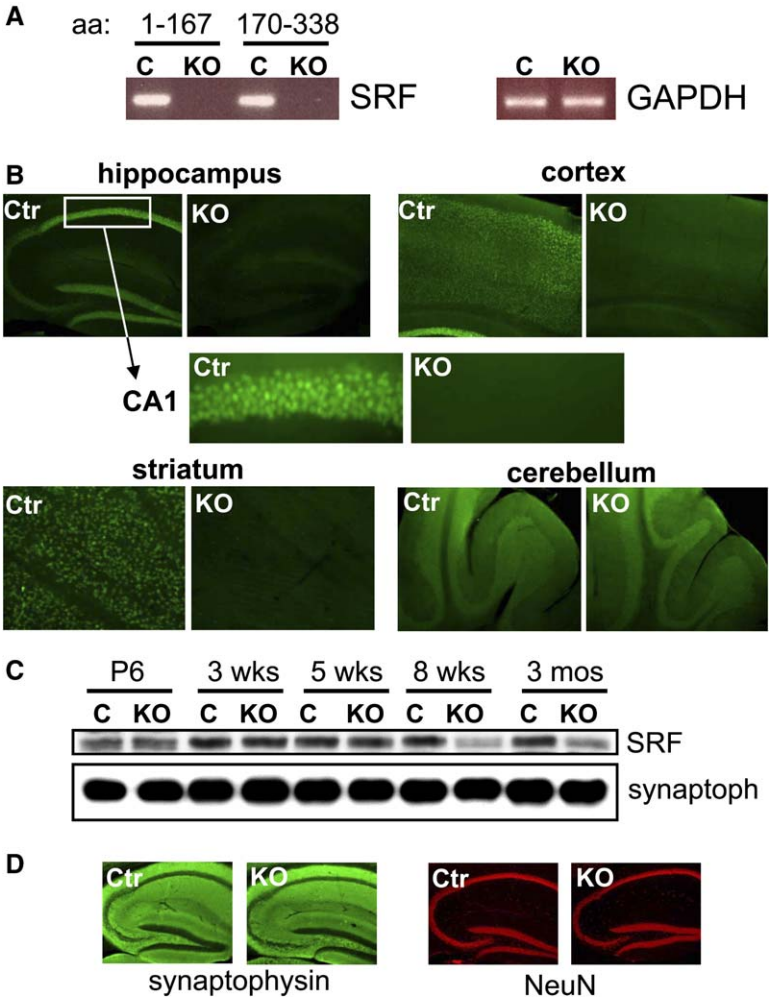

Figure 1. Conditional SRF Deficiency in the Adult Forebrain

(A) RT-PCR amplification of *srf* transcripts (amino acids 1–167 and 170–338) and a GAPDH control transcript in control and knockout hippocampi revealed elimination of *srf* mRNA in knockout brains.

(B) Immunohistochemical staining for SRF expression (immunoreactivity in green) in the hippocampus, cortex, striatum, and cerebellum of control and knockout animals. SRF elimination was seen in forebrain structures, but not in the cerebellum.

(C) Developmental time course of SRF protein expression in the hippocampus and its elimination in knockout animals. Loss of SRF was evident only after the 5 week time point.

(D) Immunohistochemical staining for synaptophysin (highlights terminals of the Schaffer collateral pathway) and NeuN (a neuronal nuclear marker), which revealed no gross morphological changes in the hippocampus of knockout animals.

the knockout animals failed to show any subsequent habituation (Figure 3A; repeated-measures ANOVA Ctr $p = 0.005$, KO $p = 0.37$; genotype × time interaction $p = 0.007$). As a control, we earlier placed the same animals into the same open field, but without adding a novel object ("mock" manipulation). Under these conditions, center exploration did not change for either genotype (Figure 3A; ANOVA genotype effect $p = 0.34$), indicating that the exploratory responses and differential habituation were due to introduction of the novel object.

In summary, SRF-deficient animals showed a selective deficit for habituation, a simple form of learning in which an animal learns about the properties of a neutral stimulus or set of stimuli, like its context. Our data strongly argue that knockout animals were unable to remember that they have experienced a previously novel context.

SRF-Deficient Animals Have Intact Noncontextual (Implicit) Habituation

We wondered whether the habituation deficit we encountered was restricted to hippocampus-dependent forms or extended to all forms of habituation. We therefore exposed animals to a novel, largely olfactory stimulus (clean bedding, see Experimental Procedures). Habituation to a novel odor does not require the hippocampus (Petrulis and Eichenbaum, 2003). The novel bedding stimulated similar increases in exploration during the first 10 min across genotypes, much as did the novel object (Figure 3B; $p = 0.99$), but now there was similar habituation in both genotypes (Figure 3B; ANOVA genotype effect $p = 0.39$).

We next examined acoustic startle reflex habituation. The neural circuitry underlying this reflex and its modification is known and well-characterized. It is located in the brain stem (where SRF was not deleted) and has been demonstrated not to require the hippocampus (Bast and Feldon, 2003). Both controls and knockouts habituated their startle responses similarly to repeated presentation of a loud startle pulse (Figure 3C; Ctr repeated-measures ANOVA $p < 0.05$; KO $p = 0.008$; ANOVA genotype effect $p = 0.99$).

The Behavioral Deficit of Mice with Forebrain-Specific SRF Deficiency Is Selective for Hippocampus-Dependent Associative Learning

We further explored SRF knockout animals' implicit memory using a simple olfactory discrimination task rather than with habituation. In this task, food-deprived animals learned to associate one of two specific novel odors with a food reward (see Experimental Procedures), a process not dependent on the hippocampus

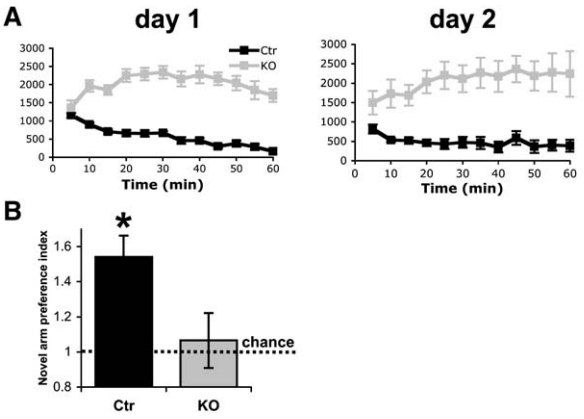

Figure 2. Knockouts Failed to Habituate to a Novel Environment

(A) Animals were placed in a novel open field for 1 hr (n = 13/13). No difference was seen between the two genotypes in the first 5 min of exploration. Whereas control animals habituated over the hour, knockouts completely failed to do so. Control animals habituated faster when re-exposed on day 2 than they did on day 1, while knockout animals still failed to show any habituation.

(B) After 10 min of exploration of two arms of a novel Y-maze, controls showed a preference for the novel third arm upon re-exposure, whereas knockouts showed no preference (n = 15/9).

Error bars signify ± SEM.

(Kaut et al., 2003). Knockouts learned the task and even made significantly fewer errors than controls (Figure 3D; p = 0.007; see also Figure S2A).

Next, we more directly compared implicit and explicit memory. The Morris water maze is a multiday learning task comprised of two related variants, one that taps implicit processes (visible platform) and is not hippocampus dependent and one that taps explicit processes (hidden platform) and is hippocampus dependent (Morris et al., 1982). Both knockout and control mice learned similarly the visible platform version of the task (Figure 3E; day 2 versus day 1 pathlengths Ctr p = 0.002; KO p < 0.001; ANOVA genotype effect p = 0.21). By contrast, during the hidden platform version, knockouts failed almost completely to learn, while control animals reached asymptotic performance by the third day of training (Figure 3E; ANOVA genotype effect p < 0.001). We performed a probe trial for platform location memory after 4 days of hidden platform training and found that while controls preferred the target quadrant over the other three quadrants (Figure 3F; p < 0.001), knockouts showed no quadrant preference (p = 0.92; ANOVA quadrant × genotype interaction p < 0.001), an effect not due simply to cre recombinase expression in knockouts (Figures S2B and S2C). Thus, knockouts were deficient only in their ability to learn the explicit version of the task.

The Hippocampus, but Not the Prefrontal Cortex, Is Required for Contextual Habituation and Immediate Memory of a Novel Context

Because in SRF knockout mice the gene has been eliminated from both the hippocampus and the prefrontal cortex and because in humans both regions show response reduction with repetition of a novel stimulus, we carried out an ibotenic acid lesion study in control mice

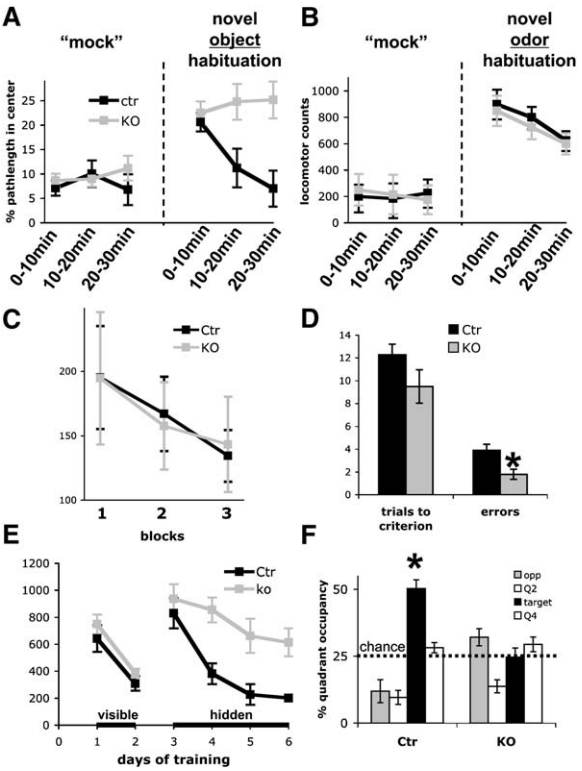

Figure 3. Specificity of the Learning Deficit in Knockout Mice for Contextual Information

(A) After 2 days of exposure to the open field, animals were reintroduced in the presence of a novel object in the center of the open field ("object") or were reintroduced to an empty open field with no object ("mock"). Exploration of the center increased similarly between genotypes and was specific to the presence of the novel object. Whereas by the end of the 30 min session control animals habituated to the novel object, knockout mice failed to show any habituation (n = 13/13). (B) After a week in the home cage, the bedding was changed to novel (clean) bedding (n = 14/11). The novel bedding specifically stimulated exploration and habituation that was similar in both genotypes. (C) Habituation to repeated acoustic startle pulses (three blocks of 120 dB pulses) was similar in both genotypes (n = 21/19). (D) Knockout animals made significantly fewer errors in an olfactory discrimination task (n = 11/10). (E) Learning curve of control and knockout animals in the visible and hidden platform version of the Morris water maze (n = 13/14). An effect of genotype was only found during the hidden platform phase, which was reflected in probe trial performance (F), where controls showed a preference for the target quadrant, while knockouts showed no preference. Error bars signify ± SEM.

(Figure 4A; see Experimental Procedures) to determine the relative contributions of both regions to the formation of an immediate memory of a novel context. We found no difference between prefrontal and hippocampal sham lesions and therefore combined them into one control group. Both sham and prefrontal-lesioned animals habituated normally in a novel open field (Figure 4B). By contrast, hippocampus-lesioned animals failed to habituate (group × time ANOVA: versus sham p = 0.001, versus prefrontal p = 0.004). Similarly, both the sham and prefrontal-lesioned animals preferred the novel arm in the Y-maze task (one-sample t test p < 0.001 for both), while the hippocampus-lesioned animals showed no preference (p = 0.48) and differed significantly from the other groups (versus sham or

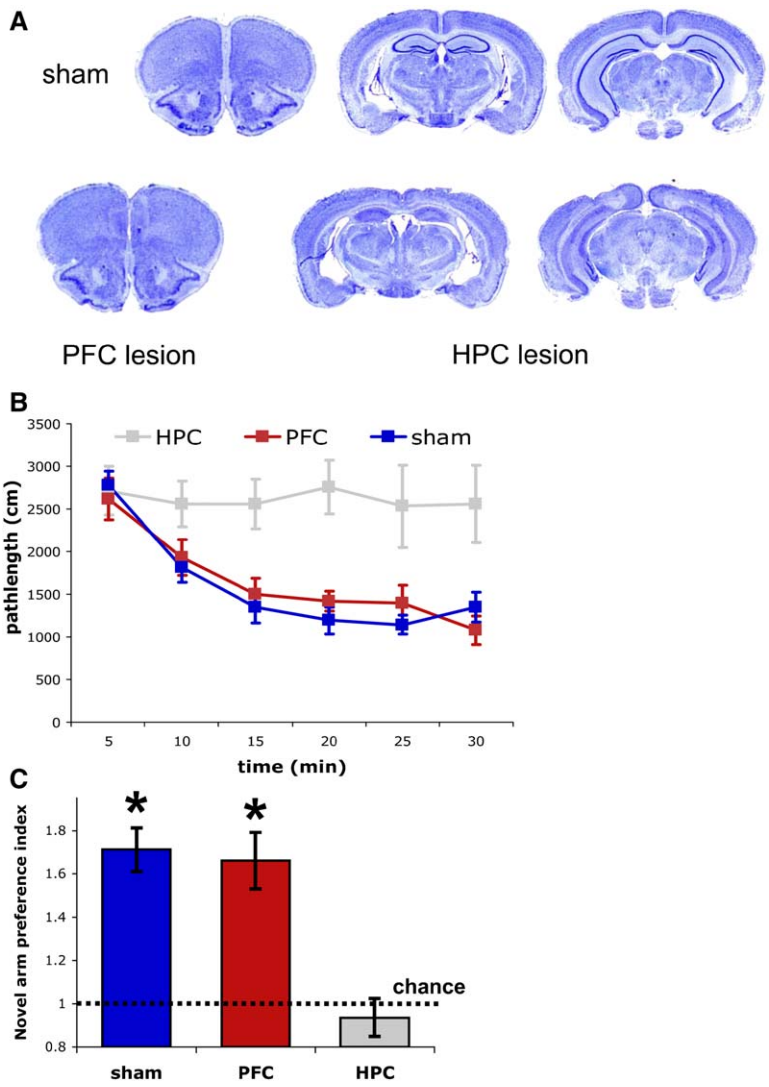

Figure 4. The Hippocampus, but Not the Prefrontal Cortex, Is Required for Contextual Habituation and Novel Arm Preference

(A) Representative sections from brains of mice that received sham hippocampal or prefrontal lesions.

(B) Only hippocampus-lesioned mice (n = 11), not prefrontal (n = 10) or sham-lesioned animals (n = 13), failed to habituate in a novel open field.

(C) Both prefrontal (n = 9) and sham-lesioned animals (n = 13) showed a preference for the novel arm in the Y-maze task, while the hippocampus-lesioned animals (n = 11) showed no preference.

Error bars signify ± SEM.

prefrontal $p < 0.001$). Thus, activity in the mouse hippocampus, but not in the prefrontal cortex, is required for contextual habituation and the formation of an immediate memory of a novel context.

Induction and Long-Lasting Deficits in LTD in Knockout Mice

In light of the results of the lesion experiment above, we focused our electrophysiological investigations on the hippocampus of knockout mice. Because novel context-induced LTD has been demonstrated in the CA3-CA1 Schaffer collateral pathway in vivo (Manahan-Vaughan and Braunewell, 1999), we first tested conventional Schaffer collateral LTD in vitro induced by a long train of low-frequency stimulation (15 min at 1 Hz) (Dudek and Bear, 1992; Malenka and Bear, 2004). This stimulation resulted in robust synaptic depression in adult control mice that slowly decayed back to baseline in ~70 min. Knockout animals failed to show as large of a depression (Figure 5A, 20–40 min $p = 0.01$). This effect was evident even at the first fEPSP after low-frequency stimulation (Figure 5A, $p = 0.007$). This difference was not due to changes in basal synaptic transmission or axonal excitability between knockout and control mice, as shown by the similar input-output relationships for each group of mice of the magnitude of the slope of the field excitatory postsynaptic potential (fEPSP; Figure S3A inset, $p = 0.78$), as well as the similar relationship between the presynaptic fiber volley and the fEPSP slope (Figure S3A, $p = 0.86$).

We next induced LTD by delivering the same number of pulses (900) at 0.5 Hz for 30 min. This form of LTD, like the 1 Hz protocol (Dudek and Bear, 1992), depends on NMDA receptors, but depends to a greater degree on release of calcium from intracellular stores (Nakano et al., 2004). This form of LTD was also significantly impaired in knockout mice (Figure 5B, 60–80 min $p = 0.02$), pointing to a reduction in both transient (1 Hz) and long-lasting (0.5 Hz) forms of LTD in knockout animals.

We then asked: will repeated trains at 1 Hz convert a short-term LTD into a long-lasting LTD, and will knockouts also be deficient in this form of LTD? This protocol resulted in robust long-lasting LTD in control mice, which was again significantly reduced in knockouts (Figure 5C; 180–200 min $p = 0.007$). Importantly, the magnitude of the first fEPSP after the second train of

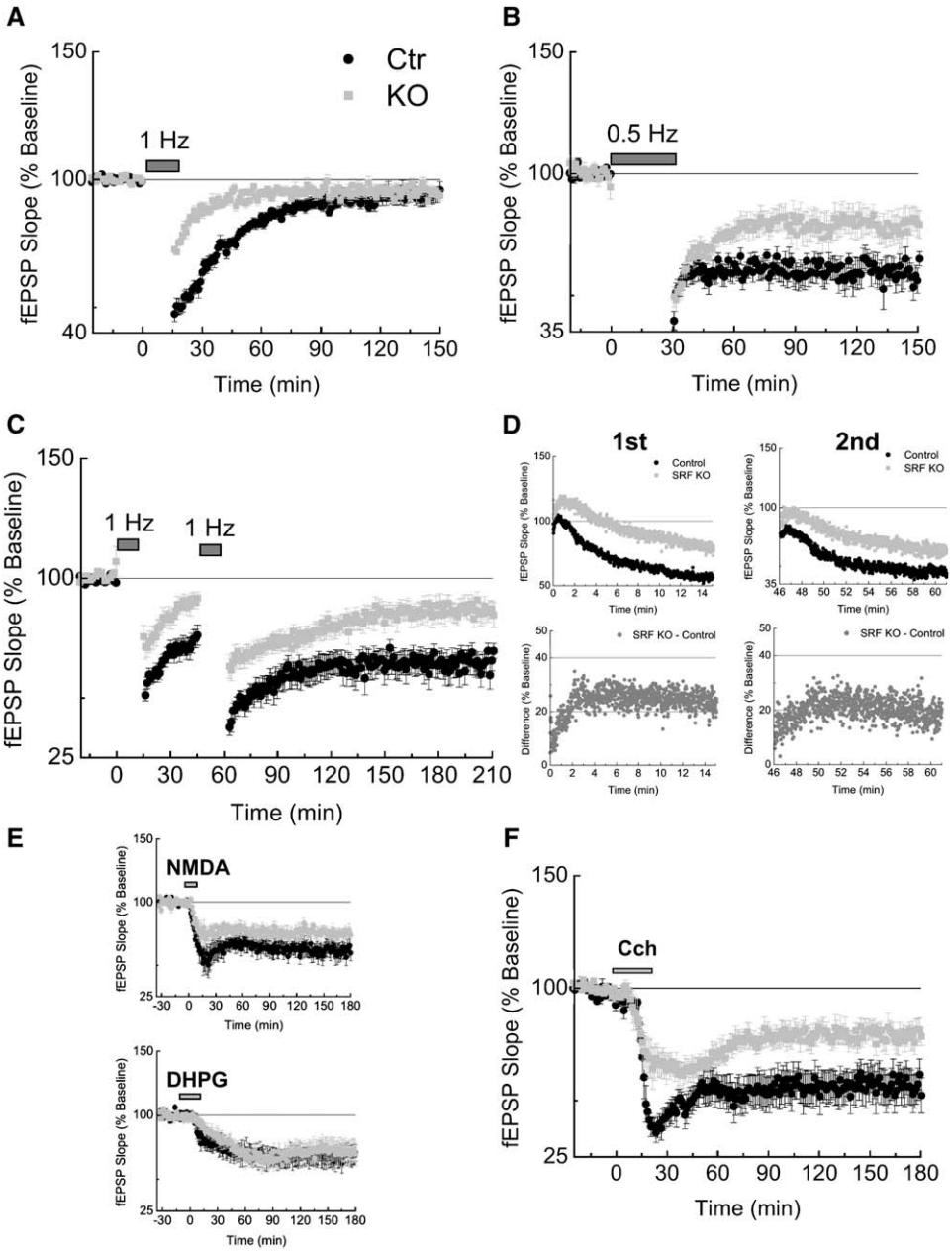

Figure 5. Impairments in Electrically Induced LTD in Knockout Mice Were Associated with a Plasticity Induction Deficit

(A) Transient LTD induced by 15 min of 1 Hz stimulation was greatly reduced in knockout mice (n = 8/8).

(B) Long-lasting LTD induced by 0.5 Hz stimulation for 30 min was smaller in knockout animals (n = 6/6).

(C) Two blocks of 1 Hz stimulation (15 min each) separated by 30 min resulted in long-lasting LTD, which was blunted in knockout animals (n = 6/6).

(D) Genotpe differences in fEPSP amplitude are already evident early in the 1 Hz stimulation train.

(E) Application of 30 μM NMDA (n = 5/5) or 50 μM DHPG (n = 5/5) led to sustained LTD. Knockouts displayed reduced NMDA-induced LTD, but did not differ in DHPG-induced LTD.

(F) Application of 50 μM carbachol (CCh), a mixed cholinergic agonist, for 20 min led to rapid and sustained LTD in control mice, which was reduced in knockout mice (n = 8/8). This deficit was already evident during the induction phase (carbachol perfusion).

Error bars signify ± SEM.

stimulation was, as was seen after one train of stimulation, significantly reduced in knockout mice (p = 0.01). Indeed, closer examination of the 15 min of 1 Hz stimulation showed a significant initial increase in fEPSP amplitude in knockout mice, as opposed to an increasing synaptic depression in control mice (Figure 5D, 1 min: Ctr 97% ± 2.5% versus KO 117% ± 3.2%, p = 0.0003).

To quantify this LTD induction deficit, we calculated genotype-related fEPSP amplitude differences by subtracting the response of control slices from knockout slices over the 15 min of stimulation (Figure 5D). We found that a steady-state difference of 25% ± 3% was reached by the third minute of stimulation. Similarly, a 20% ± 3.8% difference was seen for the second 1 Hz

train. Interestingly, knockout mice showed no deficit in the amplitude of depression after 0.5 Hz LTD induction (Figure 5B). Nonetheless, during the first few minutes of 0.5 Hz stimulation a similar deficit was seen in knockouts as was seen during that period of 1 Hz stimulation (0–3 min: Ctr 89% ± 8% versus KO 107% ± 9%, p = 0.04). This difference during 0.5 Hz stimulation disappeared by the end of stimulation (data not shown).

Thus, in knockout mice, expression of LTD was impaired due to a deficit in a mechanism for LTD induction. This deficit was most evident during the 1 Hz stimulation protocol and was already clearly evident within the first few minutes of stimulation. These LTD deficits were furthermore not attributable to cre expression, as cre-only (nonfloxed) mice showed LTD of similar magnitude as wild-type mice to 1 Hz stimulation (p = 0.2) or two trains of 1 Hz stimulation (p = 0.4, data not shown).

In slices from adult mice, electrically induced LTD is thought to involve both NMDA receptor-dependent and metabotropic glutamate receptor-dependent (mGluR) mechanisms (Kemp and Bashir, 2001; Nicoll et al., 1998; Oliet et al., 1997). To directly probe these mechanisms, we applied NMDA (30 μM) or the mGluR agonist DHPG (50 μM) to slices. As seen in Figure 5E, NMDA-induced LTD was modestly diminished in knockout mice (15–30 min: p = 0.07; 120–150 min: p = 0.04), while there was no difference between genotypes in DHPG-induced LTD (15–30 min: p = 0.2; 120–150 min: p = 0.7). Finally, we found that other forms of electrically induced synaptic depression were unaffected in knockout mice (Figures S4B–S4D), including a paired-pulse protocol (which is less dependent on NMDA receptors) and depotentiation (which also differs mechanistically from 1 Hz LTD).

LTD, Acetylcholine, and Contextual Habituation

How might failure to induce LTD relate to a lack of contextual habituation? Because acetylcholine release in the hippocampus is dramatically increased when animals are exposed to a novel environment (Acquas et al., 1996; Giovannini et al., 2001) and because cholinergic transmission is required for contextual habituation (Lalonde, 2002; Swonger and Rech, 1972; Lamprea et al., 2000, 2003; Kelly and Malanowski, 1993), we examined the effects of stimulating cholinergic pathways on synaptic efficacy. Carbachol, a mixed cholinergic agonist, has previously been shown to elicit robust activity-dependent and input-specific LTD in the hippocampus and cortex when paired with test-pulse stimulation (which by itself does not alter synaptic efficacy; Kirkwood et al., 1999). In slices, we found that carbachol (50 μM) paired with 0.017 Hz test pulse stimulation induced a rapid, robust, and long-lasting LTD in control mice that was significantly blunted in knockout mice (Figure 5F, 120–140 min p = 0.01), with no field potential oscillations observed in either genotype. Consistent with the LTD induction deficit that we found during 1 Hz tetanization, we also observed a dramatic reduction in the maximum depression achieved with carbachol in knockout mice (Figure 5F, p = 0.02). Moreover, the time to maximum depression was delayed in knockout mice (Ctr 23.5 min and KO 36.5 min). Interestingly, when carbachol was paired with 1 Hz tetanization, we found no differences between control and knockout mice (Figures S4A and S4D). It has been recently reported that, while both carbachol and 1 Hz stimulation-induced LTD require NMDA receptor activity when examined independently, their combination does not (McCutchen, 2004).

In summary, we found that in addition to an induction deficit in an NMDA receptor-dependent form of LTD in response to 1 Hz stimulation, cholinergic modulation of basal synaptic transmission was also compromised in knockout mice.

The Frequency Response Curve Is Not Shifted in Knockout Mice

LTD deficits have often been reported in the context of a metaplastic shift (enhancements in LTP). To explore this possibility, we generated frequency response curves from control and knockout mice for synaptic responses elicited by 900 pulses at 0.5, 1, 5, and 10 Hz (Bienenstock et al., 1982). We found no change in synaptic efficacy in either genotype with the 10 or 5 Hz protocols (Figure 6A, 45 min: 10 Hz p = 0.57; 5 Hz p = 0.46). By contrast, the 1 and 0.5 Hz protocols showed a blunting of the curve in knockouts (Figure 6A, 1 Hz p = 0.02; 0.5 Hz p = 0.04). Thus, LTD deficits in knockout mice were not accompanied by a metaplastic shift.

LTP Is Largely Spared in Knockout Mice

To further probe the specificity of the LTD deficits described above within the domain of synaptic plasticity, we extended our investigation using different induction protocols and examined different forms of LTP that use distinctively different molecular mechanisms (Arai et al., 1994; Castro-Alamancos et al., 1995; Huang and Kandel, 1994; Moody et al., 1998; Nguyen et al., 1994; Patterson et al., 2001; Staubli and Otaky, 1994). First, we induced "early" non-transcription-dependent LTP, using one train of 100 Hz stimulation and found similar transient potentiation in both control and knockout mice (Figure 6B, 1 min p = 0.53; 20–40 min p = 0.55). Pairing this stimulation with bath application of the D1 dopamine agonist 6-APB resulted in late-phase LTP, again with no differences between genotypes (Figure 6C, 1 min p = 0.29; 20–40 min p = 0.59). Similarly, robust forms of LTP induced by multiple trains of 100 Hz or theta burst stimulation (TBS) produced similar LTP in both genotypes (Figure 6D, 4XHFS 120–140 min p = 0.19; Figure 6E, 4XTBS 120–140 min p = 0.94). Stimulation with only one train of the theta burst protocol also led to long-lasting LTP, in which knockout mice showed a partial reduction in LTP amplitude (Figure 6F, 1 min p = 0.25; 60–80 min p = 0.02).

As indicated above (Figure 6A), LTP induced by 10 Hz was of a similar magnitude between the two genotypes. In knockout mice, this form of LTP showed a delayed onset, but this did not affect its late expression and was not due to amplitude differences after stimulation (Figure 6G, 1 min p = 0.22; 5–25 min p = 0.08; 90–110 min p = 0.27). Stimulation for 30 s at 5 Hz led to LTP in knockout mice, with barely significant delayed onset, but which was not different on the first fEPSP after stimulation or in terms of LTP expression after 1.5 hr (Figure 6H, 1 min p = 0.52; 5–25 min p = 0.049; 90–110 min p = 0.19). Finally, we also tested LTP induced by two protocols reported in a recent study of a similar SRF-deficient line

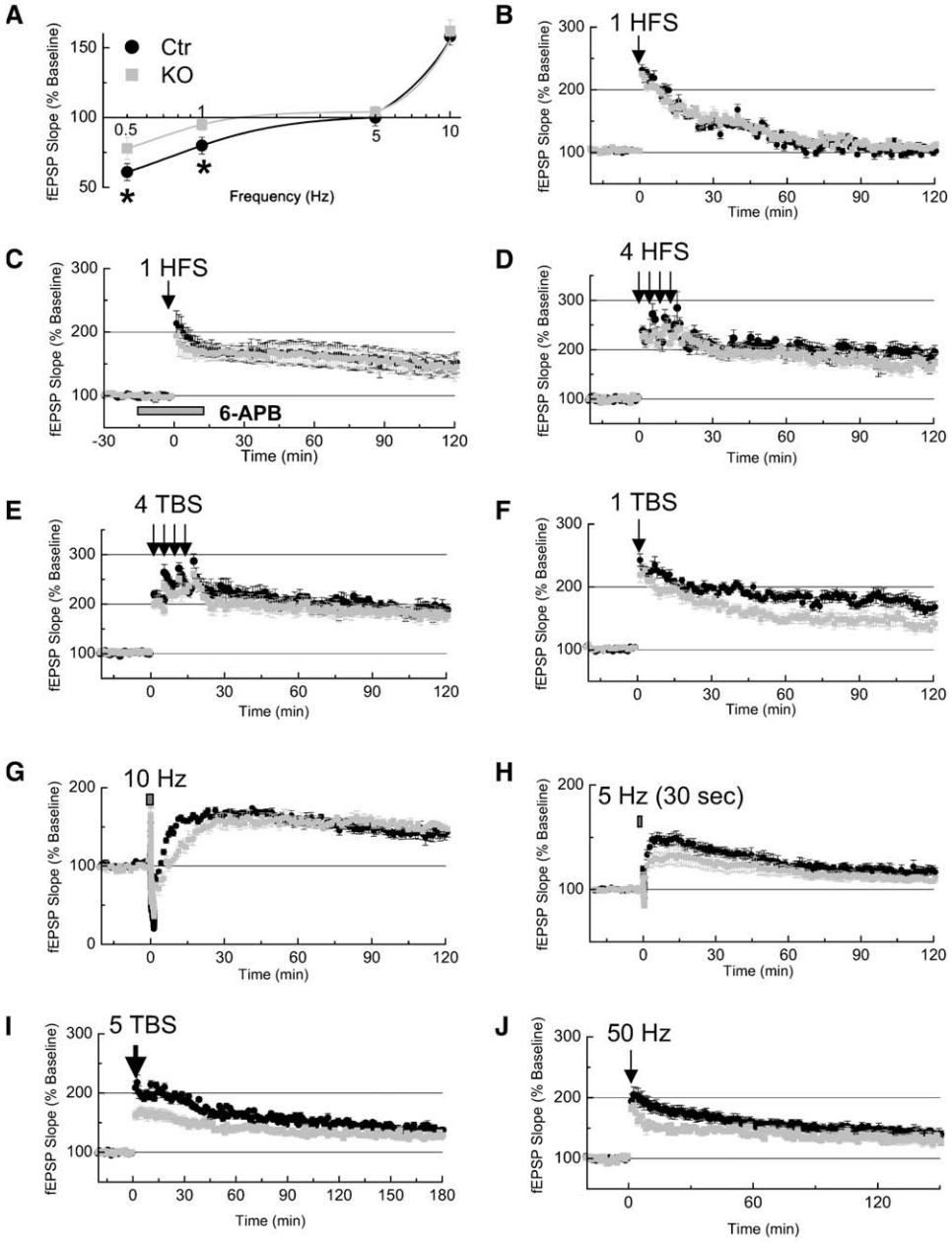

Figure 6. LTP Was Largely Unaffected in Knockout Animals

(A) Frequency-response curve (900 pulses at 0.5, 1, 5, and 10 Hz). Significant differences between genotypes were found only in the LTD portion of the curve, with no metaplastic shift toward enhanced LTP (n's = 5–8).

(B) Transient LTP induced by one train of 100 Hz stimulation was unaffected in knockout mice (n = 5/5).

(C) The same stimulation as (B) in the presence of a D1 agonist (6-APB) produced similar long-lasting LTP in both genotypes (n = 6/6).

(D and E) Four trains of stimulation at 100 Hz (D) or with a theta burst protocol (E) led to similar L-LTP in both genotypes (n = 5/5 for each experiment).

(F) LTP induced with one train of theta burst stimulation was modestly decreased in knockout slices (n = 7/7).

(G and H) No genotype differences in the magnitude of L-LTP and only modest differences in the early expression of LTP induced by 90 s of 10 Hz stimulation or 30 s of 5 Hz stimulation (n = 5/5 for each experiment).

(I) Greater LTP in control slices after induction with a massed five theta burst protocol (0–15 min, p = 0.001). LTP was only modestly decreased in knockout mice at 120–150 min (p = 0.03, n = 8/8).

(J) LTP induced by a 1 s train at 50 Hz was modestly decreased in knockouts initially (0–15 min p = 0.02), but did not differ at later time points (p = 0.3, n = 6/6).

Error bars signify ± SEM.

(Ramanan et al., 2005). Using a massed theta burst protocol (see Experimental Procedures), we found an initial deficit that became more modest over time (Figure 6I, 0–15 min p = 0.001; 120–150 min p = 0.03). Stimulation at 50 Hz for 1 s produced LTP that was initially modestly lower in knockout mice, but did not differ between

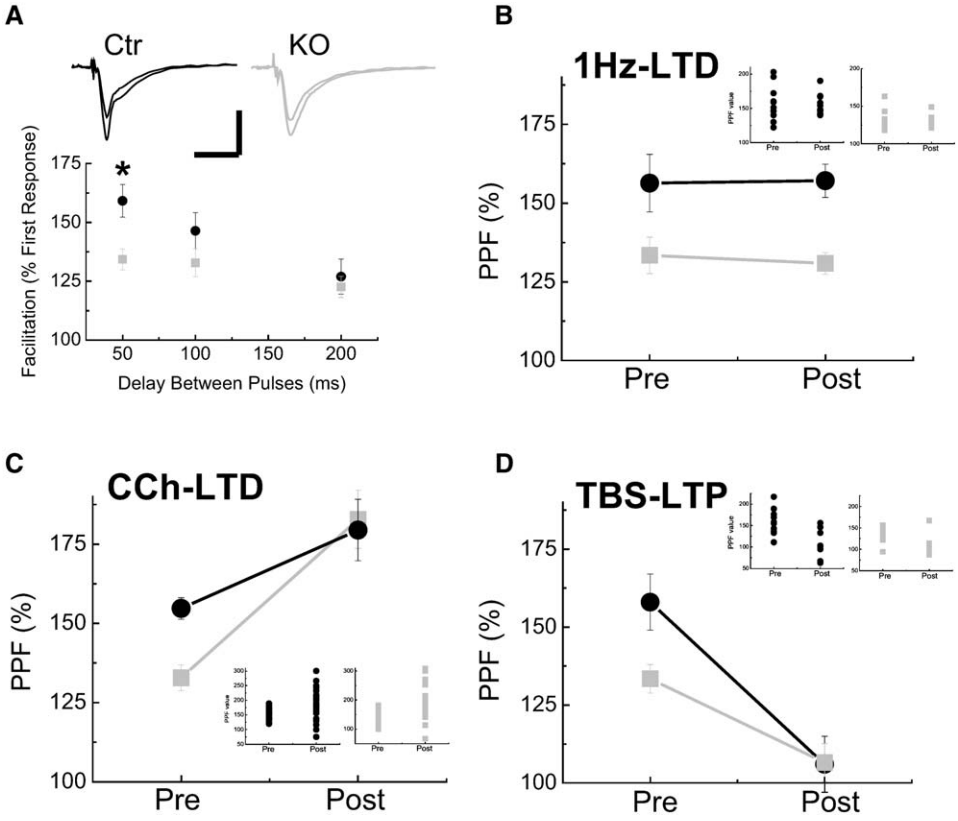

Figure 7. Basal and Plasticity-Regulated Presynaptic Function

(A) (Upper panel) fEPSP responses for the 50 ms interpulse interval. (Lower panel) Paired-pulse facilitation (PPF) was altered in knockout mice only at the 50 ms interpulse interval (n = 16/16).

(B–D) PPF was measured prior and 45 min after plasticity induction or carbachol treatment. (B) PPF values do not change in either genotype during 1 Hz-induced LTD (n = 10/7).

(C) PPF values increase to similar levels in both control and knockout slices during carbachol-induced LTD (n = 28/32).

(D) PPF values decrease to similar levels in both control and knockout slices during theta burst LTP (n = 10/12).

Error bars signify ± SEM.

genotypes at later time points (Figure 6J, 0–15 min p = 0.02; 120–150 min p = 0.3).

In summary, we found very little effect of disruption of SRF on multiple forms of LTP, with modest early deficits in LTP induced by lower-frequency stimulation (note that in TBS-LTP the short bursts of high frequency are delivered at 5 Hz), which is nearer to the 1 Hz frequency we had earlier observed a large LTD deficit in. Importantly, we found no evidence for any metaplastic shift toward enhanced LTP.

Short-Term Plasticity and Excitability of Hippocampal Neurons Are Largely Unaltered in Knockout Mice

We also investigated a number of short-term forms of plasticity and excitability in the slices. Paired-pulse facilitation (PPF) is a form of short-term synaptic plasticity that relies on the enhancement of presynaptic vesicle release due to presynaptic Ca^{2+} accumulation from two closely spaced stimuli (Dobrunz and Stevens, 1997; Emptage et al., 2001; Zucker, 1989). PPF was reduced in knockouts at one interpulse interval (Figure 7A, 50 ms p = 0.006; 100 ms p = 0.07; 200 ms p = 0.36). To further probe presynaptic function, we studied presynaptic depression (PSD), a form of short-term plasticity that reflects the extent of presynaptic fatigue (measured as fEPSP decrease) that develops during a train of 10 Hz stimulation in the presence of NMDA receptor blockers (Dobrunz and Stevens, 1997; Emptage et al., 2001; Geppert et al., 1994; Zucker, 1989), and found no deficits in PSD (Figure S3B, first 5–10 s p = 0.48). These data indicate normal presynaptic vesicle turnover in the knockouts in the context of a selective deficit in the mechanisms of PPF expression.

We next probed whether knockouts, despite their basal PPF deficit, could change transmitter release probability appropriately, as measured by changes in PPF, during LTP and LTD. 1 Hz stimulation-induced LTD did not alter PPF in either genotype (p > 0.73 for both; Figure 7B). By contrast, carbachol-induced LTD led to an increase in PPF (Figure 7C), likely reflecting a decrease in release probability, which was similar in both control (p = 0.02) and knockout slices (p < 0.001; genotype effect post-LTD p = 0.8). Similarly, theta burst LTP led to a decrease in PPF (Figure 7D), likely reflecting an increase in release probability, in both control (p < 0.001) and knockout slices (p = 0.006; genotype effect post-LTP p = 0.97). These data suggest that, despite their basal PPF deficit, knockouts can still alter transmitter release probability, and with it PPF values, in an

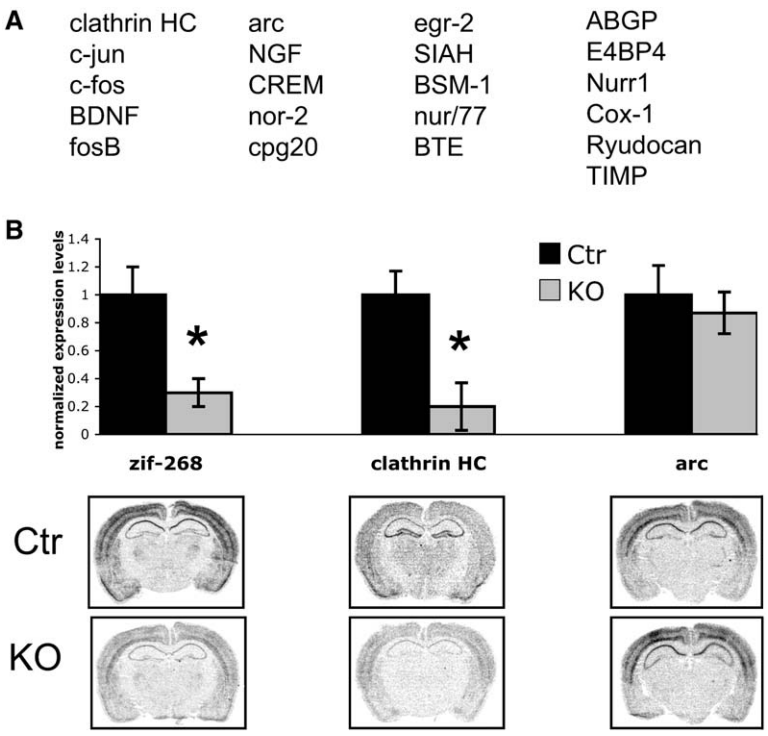

Figure 8. Zif268 and Clathrin Heavy Chain Downregulation Were Associated with SRF Inhibition

(A) Genes significantly downregulated, after correction for multiple comparisons with a false discovery rate method, in dot blot assays of dominant-negative SRF sindbis virus-infected rat primary hippocampal cultures (compared to EGFP sindbis-infected cells).

(B) Lower expression of zif-268 and clathrin heavy chain, but not arc, in the hippocampi of knockout animals, assayed using the dot blot method as in (A). In situ hybridization showed the same effects.

Error bars signify ± SEM.

appropriate manner during both LTP and LTD, reaching levels identical to control slices. As such, it is unlikely that only a basal PPF difference could account for the LTD deficit observed in knockouts.

Finally, we examined a variety of postsynaptic forms of short-term plasticity, including the depolarization induced by 1 s of stimulation at 100 Hz, EPSP-population spike coupling, and the ability to generate complex spikes. The only significant difference was a delay in complex spike generation during a stimulation train at 5 Hz, but this delay was not accompanied by a decrease in the total or average number of spikes (Figure S3).

SRF-Dependent Gene Expression

To understand the signaling pathway whereby SRF deficiency led to a deficit in LTD, we carried out a screen of target gene expression. As a first step, we established a neuronal cell culture model of SRF inhibition in which we infected 14-day-old primary rat hippocampal neurons with sindbis viruses at 85%–100% efficiency and expressed either an SRF dominant-negative construct (see demonstration of dominant-negative activity in Figure S6) or an EGFP control. Because few target genes for SRF have been described in neurons, we constructed dot blots to carry out an initial assay of gene expression in infected cultures. These dot blots contained 79 genes that we deemed most likely to be regulated by SRF (immediate-early genes, seizure-induced genes) as well as a control gene (GAPDH).

Transcripts significantly knocked down (compared to EGFP-infected cultures) in the SRF-dominant-negative expressing cultures are listed in Figure 8A, which include several genes previously found to be important for synaptic plasticity: arc, BDNF, fosB, c-*fos*, and clathrin heavy chain. Interestingly, clathrin heavy chain is an important component of clathrin-mediated endocytosis, which is essential for the internalization of AMPA receptors in LTD (Man et al., 2000). No differences were found in zif268 expression, a gene containing five SREs in its promoter, possibly because the interval between infection and RNA extraction was too short for transcript knockdown to be detectable. These experiments identified genes that may be SRF targets in vivo but may not be reflective of the effects of SRF deletion for several weeks in postnatal neurons.

To explore the role of SRF in postnatal neuronal gene expression, we examined expression of zif268, clathrin heavy chain, and arc in the brains of knockout animals. Both zif268 ($p < 0.05$) and clathrin heavy chain ($p = 0.004$) were significantly downregulated in hippocampi of SRF knockouts by the dot blot method and were subsequently confirmed by in situ hybridization (Figure 8B). We found no differences in arc expression ($p = 0.21$) by either method.

These dot blot experiments were limited, however, to a number of hand-picked genes that were thought to be likely SRF targets. The literature upon which we drew, moreover, focused on finding activity-regulated genes that may be important in the late phase of LTP. The LTD induction deficits we observed in SRF knockout mice, by contrast, are consistent with basal gene expression deficits involving genes important in LTD, not LTP. To develop a more mechanistic understanding of how SRF deletion might lead to LTD deficits, we undertook DNA microarray experiments on basal gene expression.

To identify the functional subclasses into which differentially expressed genes were clustered, we used the gene score resampling method implemented by ermineJ software (Pavlidis and Noble, 2001; Pavlidis et al., 2002). We focused on two gene ontology clusters

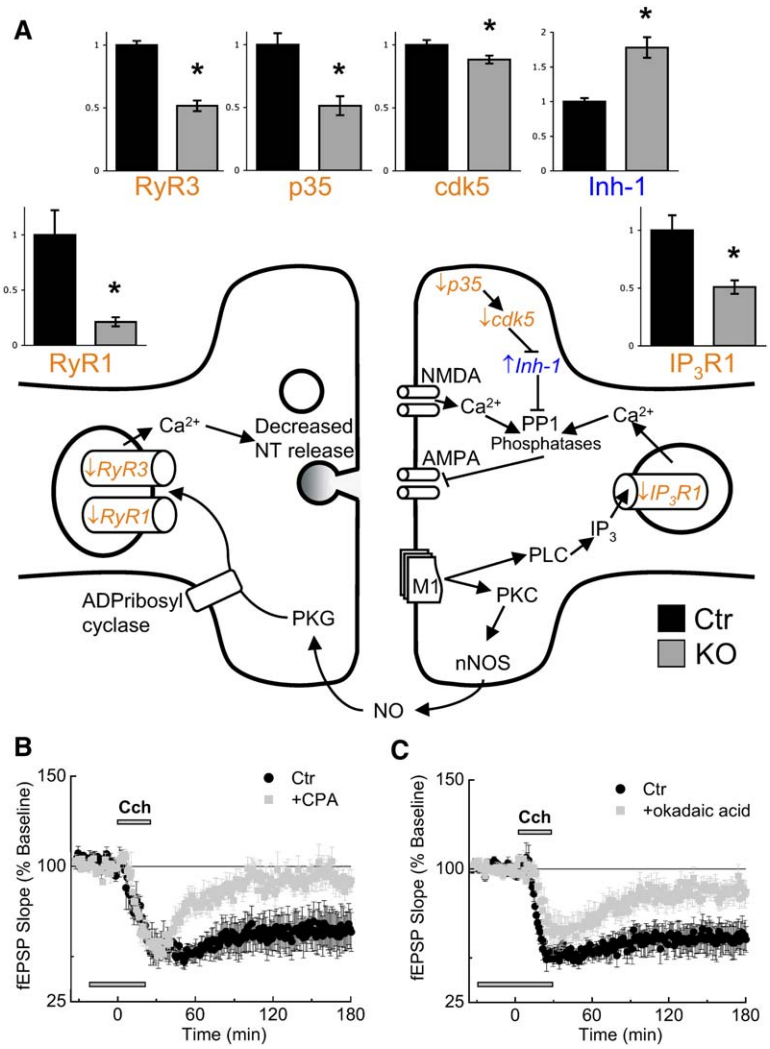

Figure 9. Several LTD-Relevant Genes Are Differentially Expressed in Knockouts and Point to Abnormalities in the Release of Calcium from Intracellular Stores and Activation of Phosphatases

(A) Fold-decrease or increase in expression (normalized to control values) of six genes using quantitative PCR (n = 7/7). These genes are depicted in a simplified diagram of signaling pathways involved in carbachol-induced LTD. Note that while ryanodine receptors, for example, are expressed in both CA3 (presynaptic) and CA1 (postsynaptic), their function is required pre- but not postsynaptically in LTD (Nishiyama et al., 2000; Reyes and Stanton, 1996).

(B) Depletion of calcium from intracellular stores by 1 μM of the Ca^{2+}-ATPase inhibitor cyclopiazonic acid (CPA) disrupts carbachol-induced LTD (n = 6/6).

(C) Inhibition of protein phosphatases 1 and 2A with 1 μM okadaic acid decreases carbachol-induced LTD (n = 6/6).

Error bars signify ± SEM.

that contained disproportionate numbers of differentially expressed genes. These clusters were identified as "calcium channel activity" ($p < 0.001$) and "protein tyrosine phosphatase activity" ($p < 0.001$). We verified gene expression using quantitative PCR on cDNAs from the hippocampi of seven new mice of each genotype. As expected, we observed downregulation of β-actin, a well-described SRF target gene (Liu et al., 2000), using both microarray and quantitative RT-PCR analysis ($p < 0.001$, data not shown). As evident in Figure 9A, we also found in knockouts significantly lower expression of several genes that regulate the release of calcium from intracellular stores, including two major ryanodine receptors (RyR1: $p < 0.02$; RyR3: $p < 0.001$) and the inositol triphosphate receptor (IP_3R1: $p = 0.009$).

In addition, we found in knockout mice that inhibitor-1 was upregulated ($p = 0.001$). Inhibitor-1 acts to inhibit protein phosphatase 1 (PP1). Moreover, protein kinase cdk5, which normally phosphorylates inhibitor-1 and thereby prevents conversion of inhibitor-1 into a PP1 inhibitor by protein kinase A (Bibb et al., 2001), was also downregulated in knockout animals, along with the cdk5 activator p35 (cdk5: $p < 0.05$; p35: $p = 0.003$).

Thus, the microarray results revealed a coordinated basal downregulation of genes critical for the release of calcium from intracellular stores, a function necessary for the electrical induction of LTD (Nishiyama et al., 2000; Reyes and Stanton, 1996). Consistent with this being part of a final common molecular signal in the pathway for LTD, we also found upregulation of inhibitor-1 and basal downregulation of its inhibitors. These data point to an overall decrease in PP1 function, a protein critical for LTD (Morishita et al., 2001; Mulkey et al., 1993).

We next tested whether the release of calcium release from intracellular stores and the activation of phosphatases, important for electrically induced LTD, also play an important role in carbachol-induced LTD. Depletion of intracellular calcium stores with 1 μM cyclopiazonic acid (CPA), a Ca^{2+}-ATPase inhibitor, resulted in a profound deficit in carbachol-LTD (Figure 9B, 120–150 min $p = 0.015$). Inhibition of PP1 and PP2A activation with okadaic acid also impaired carbachol-LTD (1 μM, 120–150 min $p = 0.012$). These data suggest that abnormalities in both intracellular calcium mobilization and PP1 activation may together lead to the LTD deficit we observed in knockout mice.

Discussion

In this study we report on the behavioral, electrophysiological, and molecular effects of SRF deletion in the adult forebrain. In the nervous system, SRF plays an important role in neuronal migration and axon guidance (Alberti et al., 2005; Knoll et al., 2006). Lack of SRF beginning in late gestation results in a severly hypoplastic hippocampus, evident on Nissl staining, and abnormal mossy fibers. These animals display phenotypic abnormalities by postnatal day 2, are much smaller than their littermates, and die by postnatal day 21. By deleting SRF only in adult mice, we did not disrupt nervous system development, which we verified in our immunohistochemical analysis of hippocampi from SRF knockout mice.

We found that mice lacking SRF in the forebrain as adults cannot form an immediate memory of a novel context. SRF knockouts failed to show habituation in a novel open field or to a novel object. These animals consequently failed to learn the hippocampus-dependent (hidden platform) version of the Morris water maze. By contrast, hippocampus-independent forms of learning, including hippocampus-independent habituation, were unaffected by this genetic manipulation. Accompanying the profound deficit in explicit learning was a profound deficit in the induction of LTD. In addition to deficits in commonly used electrical protocols for LTD, there was a deficit in the induction of LTD by a cholinergic agonist. Because acetylcholine release in the hippocampus is dramatically increased upon exploration of a novel environment and is necessary for contextual habituation, this form of LTD is especially relevant for the animal's ability to encode a novel context (Acquas et al., 1996; Giovannini et al., 2001; Lamprea et al., 2000, 2003; Kelly and Malanowski, 1993; Lalonde, 2002; Swonger and Rech, 1972). In addition, gene expression analyses in knockouts pointed to reduced release of calcium from intracellular stores and decreased protein phosphatase 1 activation, two well-described LTD mechanisms.

Previous studies in humans, nonhuman primates, and rodents have demonstrated long-lasting reductions of hippocampal responses as novel stimuli become familiar. In rats, exploration of a novel environment leads to LTD in the CA3 to CA1 Schaffer collateral pathway in vivo—the same pathway that we examined in vitro and found LTD deficits in. While we only examined plasticity in CA1, similar deficits might well be found in other glutamatergic pathways, including other subregions of the hippocampus, where LTD uses molecular mechanisms similar to those in CA1. However, our conclusions focus on the hippocampus because we found through lesion experiments that activity in the hippocampus, but not the prefrontal cortex, was required for the immediate learning of a novel context. Together with previous data, our findings therefore suggest that the formation of an immediate memory of a novel context requires an LTD-like form of synaptic plasticity in the hippocampus. Interestingly, both contextual habituation and LTD meet the parametric criteria classically described for habituation by Thompson and Spencer (see discussion in the Supplemental Data). Definitive confirmation of this hypothesis, however, will require a number of subsequent studies, using additional molecular tools and in vivo recordings.

In a similar forebrain-restricted SRF-deficient mouse, and on a somewhat different genetic background from the mice used in our study, Ramanan et al. (2005) recently reported gene induction and LTP deficits. As in our study, these authors reported decreased basal expression of zif268 in the hippocampus, though Ramanan et al. did not examine behavior or LTD. They found a large LTP deficit that began immediately after induction with a massed five theta burst stimulation protocol (5-TBS) (Ramanan et al., 2005) as well as an early LTP deficit after stimulation at 50 Hz. Using their stimulation protocols, we found a more modest LTP deficit in knockout mice. Our results thus differ quantitatively from those of Ramanan et al. (2005), and it is unclear what in addition to genetic background may account for these quantitative differences.

It is important to note, however, that numerous other studies of genetically modified mice have reported theta burst LTP deficits, including in the context of intact HFS-LTP, but all have failed to find any impairment in contextual habituation (Anagnostaras et al., 2003; Kew et al., 2000). Therefore, the most parsimonious conclusion, particularly in light of the evidence for in vivo LTD induction during novel context exploration, is that the LTD deficits correlate with the behavioral deficits in the knockout mice much more strongly than do the modest LTP differences. While this does not rule out some role for LTP in the immediate learning of a novel context, we propose that LTD, and in particular its early induction, plays a considerably larger role than LTP.

Molecular Mechanisms of LTD Deficits in SRF Knockout Mice

Previously published studies on SRF have focused on its role in neuronal activity-regulated gene transcription, which contributes to the late phase of LTP (Buchwalter et al., 2004; Finkbeiner and Greenberg, 1998). By contrast, we observed a deficit in LTD beginning early during plasticity induction, suggesting that basal gene expression changes in knockout mice are more likely to account for the phenotype than stimulus-induced gene expression changes. We found basal downregulation of the well-described SRF target genes zif268 and β-actin (Christy and Nathans, 1989; Liu et al., 2000). In addition, the expression of two major ryanodine receptors (RyR1 and 3) and one major inositol triphosphate receptor (IP_3R1) were decreased in knockout brains. These receptors are important for the regulation of calcium release from intracellular stores (Nishiyama et al., 2000; Reyes and Stanton, 1996).

Using a combination of bath application and postsynaptic (CA1) intracellular injection experiments, several previous studies have shown that release of calcium from intracellular stores is essential for electrically induced LTD (Nishiyama et al., 2000; Reyes and Stanton, 1996). Moreover, these studies found that ryanodine-regulated calcium release was required presynaptically, while IP_3R1-regulated calcium release was required postsynaptically (Nishiyama et al., 2000; Reyes and Stanton, 1996). We extended these earlier experiments by showing that depletion of intracellular calcium stores with CPA disrupts carbachol-induced LTD.

One important function for calcium release in LTD is the activation of protein phosphatases, such as PP1. We found a roughly 2-fold increase in the expression of inhibitor-1, an endogenous inhibitor of PP1. We also found downregulation of the inhibitor-1 regulators p35 and cdk5, which together suggest that knockouts have decreased PP1 activity, a function required for LTD (Morishita et al., 2001; Mulkey et al., 1993).

Others have previously shown that postsynaptic (CA1) injection of the inhibitor-1 peptide blocked electrically induced LTD (Morishita et al., 2001). Interestingly, in the limited electrophysiological and behavioral experiments reported, mice lacking p35 displayed an LTD deficit, no LTP deficit, and a learning curve in the water maze similar to SRF knockouts (Ohshima et al., 2005). In additional experiments, we found that okadaic acid, which inhibits PP1 and PP2A, diminished carbachol-induced LTD.

One result of phosphatase activation is the clathrin-dependent endocytosis of AMPA receptors during LTD (Morishita et al., 2005). We also found that clathrin heavy chain was downregulated in knockouts, suggesting that clathrin-mediated endocytosis may also be affected in these mice. In summary, our basal gene expression and electrophysiological data suggest that decreased release of calcium from intracellular stores and decreased PP1 activation may together largely account for the LTD deficits observed in SRF knockout mice.

The Hippocampus, LTD, and the Encoding of a Novel Context

When and how do LTD-like changes occur in vivo, and how is this relevant to the formation of an immediate memory of a novel environment? A portion of the population of hippocampal neurons (i.e., visually responsive neurons) fire robustly to aspects of a novel environment (Brown and Xiang, 1998; Rolls et al., 1993; Yamaguchi et al., 2004). Upon repeated exposure to the same stimuli, perhaps facilitated by the large release of acetylcholine (Acquas et al., 1996; Giovannini et al., 2001), these neurons then reduce or eliminate their firing (Brown and Xiang, 1998; Rolls et al., 1993; Yamaguchi et al., 2004). Exposure to a novel environment led to an activity-dependent and long-lasting depression of synaptic transmission, as assessed by applying test pulses (which themselves do not alter synaptic efficacy) to the Schaffer collateral pathway in vivo (Manahan-Vaughan and Braunewell, 1999). Similarly, we observed a very rapid and robust LTD during carbachol stimulation in response to test pulse stimulation. The parallels between our experiments and those of Manahan-Vaughan and Braunewell (1999) make it tempting to conclude that the novelty-induced LTD observed in vivo was mediated by the cholinergic modification of synaptic transmission in stimulated neurons, which we tapped into in vitro. The activity-dependence of novelty-induced and carbachol-induced LTD also illustrates how response decreases can be achieved specifically in those neurons responsive to aspects of the environment, as LTD-like changes will only occur if a neuron is active, even if at a low rate.

LTD likely does not occur in vivo as a consequence of 15 min of continued 1 Hz stimulation. Very few, but well-timed, spikes can induce LTD if timed to coincide with the trough of hippocampal theta frequency oscillations, a rhythm commonly observed in vivo as animals explore their environment (Huerta and Lisman, 1995; Hyman et al., 2003). Theta frequency oscillations depend on septal cholinergic innervation of the hippocampus (Vinogradova, 1995; Winson, 1978), thereby providing another potential link between novelty, the increased release of acetylcholine, and the rapid induction of LTD in the hippocampus. Consistent with the rapid decrease in neural response in the hippocampus to repeated presentations of a novel stimulus, we observed LTD deficits in knockouts very early during induction of LTD.

Our study therefore suggests that LTD in the hippocampus is not merely a negative counterpart to LTP, acting as a restraint to memory storage, but provides a unique positive learning mechanism in its own right. Because those neurons undergoing LTD-like changes in a novel environment are responsive to sensory aspects of the context, they would also be candidate neurons for undergoing subsequent LTP-like changes during the formation of an explicit associative memory related to that context. By first undergoing LTD, the subset of neurons recruited for subsequent LTP would benefit from improved signal-to-noise. We therefore suggest that LTD mediates a function in learning and memory that is distinct from that related to LTP, but that ultimately these two processes interact and affect each other. For the animal to encode complex spatial associative memories in a novel environment, such as for navigation using spatial cues, the animal must first encode that it has encountered the novel environment per se. This first step involves a distinct mnemonic process mediated by LTD-like changes and is evident behaviorally by habituation of exploration.

Experimental Procedures

Generation and Characterization of Knockout Mice

Mice carrying an allele of *srf* flanked by loxP sites (*srf*-flex1; "floxed" SRF) (Wiebel et al., 2002) and were bred with a previously reported low-expressing cre recombinase expressing line (R4Ag11; Zakharenko et al., 2003). Offspring were genotyped by Southern blotting for the cre transgene and by PCR for the floxed *srf* allele (Wiebel et al., 2002). Mice used in these experiments were ~75% C57/BL6 and ~25% 129Sv background. Animals were maintained on a 12 hr light/dark cycle in the New York Psychiatric Institute animal care facility under the supervision of Dr. Mohammed Osman and Columbia Univeristy's IACUC. Unless indicated otherwise, food and water were given ad libitum. RT-PCR was performed on first-strand cDNA derived from total hippocampal RNA from knockout and control animals. *srf* was amplified using two primer sets (amplifying amino acids 1–167 and 170–338, sequences available upon request) in the presence of 5% DMSO and 5% glycerol, with GAPDH serving as a control for cDNA loading. Immunohistochemistry and Western blotting were carried out as previously described (Alarcon et al., 2004; Mayford et al., 1996). Antibodies used were from Santa Cruz Biotech (SRF), Zymed (synaptophsyin), and Chemicon (NeuN). MK-801 and anisomycin were purchased from Sigma (St. Louis, MO).

Behavior

For all behavioral and electrophysiological tests we used adult male mice at least 3 months old, with littermates of both genotypes. The experimenters were blind to the genotype in all studies. Control animals for experiments with KO animals were mice carrying floxed *srf* but no cre transgene.

Open Field

Mice were tested for 1 hr on two consecutive days. The open field was a square chamber (43.2 × 43.2 × 30.5 cm), in which mice

were monitored with 32 infrared light sources spaced 1.25 cm apart (Med Associates, VT). The novel object test was done by securely placing a clear, brightly colored object in the center of the open field, such that it would not interfere with infrared beam operation.

Y-Maze

The Y-maze was made of three interconnected plexiglass arms (120º angle between arms) each measuring 40 × 8 × 10 cm. A plexiglass divider closed off one of the three arms during the 10 min exploration session. The animals were then removed and 2 min later replaced in the Y-maze (with all arms open and animal scents removed) for the 5 min test session. Arm entries were recorded and a novel arm preference index was calculated (novel arm entries/[other two arms/2]). An index value of 1 indicates no preference.

Home Cage Monitoring and Novel Odor Habituation

After a week of acclimatization to their home cage, the activity of singly housed mice was measured using a Stoelting monitor as described previously (Grailhe et al., 1999). The novel odor habituation task involved replacement of the "conditioned" bedding with novel (clean) bedding, with resultant exploration and habituation measured using the same Stoelting monitor apparatus.

Prepulse Inhibition, Startle Habituation, and Rotarod

Prepulse inhibition (PPI) was done as described previously (Alarcon et al., 2004). Startle habituation was determined by the startle response to 120 dB pulses in blocks at the beginning and end of the PPI paradigm (P120A, P120C) and stimuli randomly interleaved with PPI trials (P120B). The protocol for the rotarod was also previously described (Alarcon et al., 2004).

Water Maze

This task was performed as previously described (Malleret et al., 1999). Animals were given four trials (120 s maximum with a 15 min inter-trial interval) daily with a probe trial (60 s) after 4 days of hidden platform training, during which the platform was removed.

Olfactory Discrimination

Mice were single housed, food deprived, and maintained at a stable body weight of ~85% ad lib. weight. Animals were pre-exposed to the training setup and digging bowls with unscented digging medium (normal mouse bedding) for 2 days prior to training. On the training day, the mice were presented with two digging bowls, one with cinnamon-scented digging medium and the other with paprika-scented medium. Food reward was half of a Cheerio and was always buried in the cinnamon-scented bowl. The first four trials were "correction trials" where animals were allowed to make a mistake in their initial bowl choice. Mice were removed between trials so that bowls could be arranged in the proper spatial relationship, which was predetermined and pseudorandomized. Criterion was six consecutive correct choices, at which point the experiment ended and mice were refed ad lib.

Novelty-Induced Hypophagia

This test for anxiety was performed as previously described (Dulawa et al., 2004).

Lesions

Bilateral lesions of the hippocampus and the medial PFC were performed by multiple microinjections of ibotenic acid dissolved in vehicle (phosphate buffer, pH 7.2) at a concentration of 10 μg/μl, or vehicle-only sham. The injections were made using a 2 μl Hamilton syringe connected to a glass micropipette as previously described (Touzani et al., 1997). Injection sites are expressed as: mm anteroposterior/mm lateral/mm vertical/μl volume. Medial PFC: 2.9/0.3/2.2/60 and 2.7/0.3/2.3/60. Hippocampus: −1/0.7/2/60, −1.6/1/2/50, −1.6/2/2/50, −1.9/1.5/2.5/40, −1.9/2.5/2.5/40, −2.3/2/2.2/50, −2.3/3.5/2.7/40, and −2.3/3.5/3.5/40. Behavioral testing took place 3 weeks after surgery. Lesions were verified histologically for every animal by thionin staining frozen sections from perfused brains.

Electrophysiology

Transverse hippocampal slices (400 mm) from control and knockout mice were incubated in an interface chamber at 28ºC ± 1ºC, subfused with oxygenated artificial cerebrospinal fluid (ACSF, containing in mM: 119 NaCl, 4.0 KCl, 1.5 $MgSO_4$, 2.5 $CaCl_2$, 26.2 $NaHCO_3$, 1 NaH_2PO_4, and 11 glucose), and allowed to equilibrate for at least 90 min. Field excitatory postsynaptic potentials (fEPSP) were recorded at CA3/Schaffer collateral-CA1 synapses by placing both stimulating and recording electrodes in the stratum radiatum of CA1 area. The stimulation intensity (square pulse, 50 ms duration) was adjusted to give fEPSP slopes of ~40% of maximum.

Baseline and after-stimuli responses were sampled once per minute at this intensity. LTP protocol for high-frequency stimulation consisted in delivering a single train at 100 Hz (1 s, 100 pulses), four trains at 100 Hz (5 min inter-train interval), and one train at 50 Hz (1 s, 50 pulses). LTP protocol for theta burst stimulation consisted of a single train of theta bursts (nine bursts of four pulses at 100 Hz, 200 ms interbursts interval) or five trains of theta bursts (5 min inter-train interval). The massed theta burst protocol consisted of five trains of theta burst stimulation spaced by 20 s (each train consisted of 15 bursts delivered at 5 Hz and each burst was comprised of five pulses at 100 Hz). Other LTP and LTD protocols used are explained in the Results section. Carbachol, 6-APB, anisomycin, CPA, and okadaic acid were purchased from Sigma Chemical Co (St. Louis, MO). (RS) −3,5-DHPG and D-AP5 were purchased from Tocris (Ellisville, MO). NMDA was purchased from Calbiochem (La Jolla, CA). The physiologist was blind to the genotypes.

Gene Expression Studies

The SRF dominant-negative was constructed by fusing a FLAG epitope sequence to the N terminus of aa 1–272 of human SRF (similar to the aa 1–265 SRFΔC construct described in Belaguli et al. (1997)). This construct was selected after comparing its efficacy as a dominant-negative to several previously described constructs (Figure S6) by transfection into NIH3T3 cells with FuGene (Roche; Basel, Switzerland) and subsequent stimulation of serum-deprived cells (0.5% fetal-calf serum) with 15% FCS or with 50 μM forskolin. The reporter genes were composed of a basal promoter coupled to a single SRE (Clarke et al., 1998) or three CREs (Stratagene; La Jolla, CA), driving the firefly luciferase gene. Each transfection contained as an internal control a constitutive promoter driving a renilla luciferase reporter gene, and the activity of the two luciferase proteins was assayed using the dual-luciferase assay per the manufacturer's instructions (Promega; Madison, WI). Sindbis viruses were made according to manufacturer's instructions after cloning the SRF dominant-negative cDNA, or an EGFP control cDNA, into the pSinRep5 vector (Invitrogen; Carlsbad, CA). 14DIV E18 rat primary embryonic hippocampal neuronal cultures (Banker and Goslin, 1998) were infected with viruses for 1 hr and harvested for RNA 15 hr later. Oligonucleotide sequences used for in situ hybridization are available upon request and were performed as previously described (Alarcon et al., 2004; Mayford et al., 1996) .

For microarray studies, total RNA was extracted from the hippocampi of individual mice (n = 5/4) using a commercially available acid-phenol reagent (Trizol; Invitrogen Inc., Carlsbad, CA). RNA concentration was assessed by absorbance spectroscopy and RNA integrity confirmed by nondenaturing agarose gel electrophoresis. Twenty micrograms of RNA from each sample were further purified from contaminating organics and non-RNA species using a silica resin (RNEasy; Qiagen Inc., Valencia, CA) protocol according to the manufacturer's instructions. Total RNA from single animals was individually converted into biotinylated, fragmented cRNA using a labeling kit (Ambion, Inc., Austin, TX). cRNA samples derived from single animals were hybridized in recommended buffer to microarrays (Affymetrix Murine Genome Array U430Av2.0) at 45ºC for 16 hr. The samples were stained and washed according to the manufacturer's protocol on a Fluidics Station 400 (Affymetrix Inc.) and scanned on a GeneArray Scanner (Affymetrix Inc.). Primary data extraction was performed with Microarray Suite 5.0 (Affymetrix Inc.), and signal normalization across samples was carried out using all probe sets with a mean expression value of 500.

We examined gene expression only for those genes deemed "Present" by the Microarray Suite software using t tests ($p < 0.05$). To identify functionally related groups of genes with similar expression patterns, we analyzed the list of t test scores using Gene Ontology Class Scoring software (Pavlidis and Noble, 2001; Pavlidis et al., 2002).

To confirm differential expression of selected genes, first-strand cDNA was synthesized from purified total RNA from seven independent samples for each genotype using a SYBR green quantitative PCR protocol. We used the PCR SYBR Green I Quantitect Master Mix (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions in the DNA Engine Opticon (MJ Research, Waltham, MA) instrument. Relative expression values were calculated based on the reaction efficiency, and crossing thresholds of the test gene and the internal control gene Ppib (Liu and Saint, 2002). For each transcript assayed, intron-spanning primers were designed using publicly available genomic contig sequences obtained through LocusLink, the public domain primer design software, Primer3, and the DNA analysis software Vector NTI Suite Version 7 (Informax Inc., Bethesda, MD). Primer sequences are available upon request.

Statistical Analysis

For the behavioral and molecular analyses, all statistics were done in SPSS (SPSS, Inc., Chicago, IL). For the electrophysiology, statistics were done using the MICROCAL ORIGIN statistical package (Microcal Software Inc., Northampton, MA). In all electrophysiological experiments and figure legends, n indicates the number of animals in each group (i.e., n = Ctr/KO). In the text, the electrophysiological data were presented as mean ± SD. The difference between two experimental data sets was considered significant at $p < 0.05$. Unless indicated otherwise, p values represent the results of Student's t tests. In all figures, bars represent the mean ± SEM.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/50/1/127/DC1/.

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References

Acquas, E., Wilson, C., and Fibiger, H.C. (1996). Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear. J. Neurosci. *16*, 3089–3096.

Adrover, M.F., Guyot-Revol, V., Cheli, V.T., Blanco, C., Vidal, R., Alche, L., Kornisiuk, E., Epstein, A.L., and Jerusalinsky, D. (2003). Hippocampal infection with HSV-1-derived vectors expressing an NMDAR1 antisense modifies behavior. Genes Brain Behav. *2*, 103–113.

Alarcon, J.M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E.R., and Barco, A. (2004). Chromatin acetylation, memory, and LTP are impaired in CBP+/− mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron *42*, 947–959.

Alberti, S., Krause, S.M., Kretz, O., Philippar, U., Lemberger, T., Casanova, E., Wiebel, F.F., Schwarz, H., Frotscher, M., Schutz, G., and Nordheim, A. (2005). Neuronal migration in the murine rostral migratory stream requires serum response factor. Proc. Natl. Acad. Sci. USA *102*, 6148–6153.

Anagnostaras, S.G., Murphy, G.G., Hamilton, S.E., Mitchell, S.L., Rahnama, N.P., Nathanson, N.M., and Silva, A.J. (2003). Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat. Neurosci. *6*, 51–58.

Arai, A., Black, J., and Lynch, G. (1994). Origins of the variations in long-term potentiation between synapses in the basal versus apical dendrites of hippocampal neurons. Hippocampus *4*, 1–9.

Banker, G., and Goslin, K. (1998). Culturing Nerve Cells (Cambridge, MA: MIT Press).

Bast, T., and Feldon, J. (2003). Hippocampal modulation of sensorimotor processes. Prog. Neurobiol. *70*, 319–345.

Belaguli, N.S., Schildmeyer, L.A., and Schwartz, R.J. (1997). Organization and myogenic restricted expression of the murine serum response factor gene. A role for autoregulation. J. Biol. Chem. *272*, 18222–18231.

Berlyne, D.E. (1960). Conflict Arousal and Curiosity (New York: McGraw-Hill).

Bibb, J.A., Nishi, A., O'Callaghan, J.P., Ule, J., Lan, M., Snyder, G.L., Horiuchi, A., Saito, T., Hisanaga, S., Czernik, A.J., et al. (2001). Phosphorylation of protein phosphatase inhibitor-1 by Cdk5. J. Biol. Chem. *276*, 14490–14497.

Bienenstock, E.L., Cooper, L.N., and Munro, P.W. (1982). Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J. Neurosci. *2*, 32–48.

Brown, M.W., and Xiang, J.Z. (1998). Recognition memory: neuronal substrates of the judgement of prior occurrence. Prog. Neurobiol. *55*, 149–189.

Buchwalter, G., Gross, C., and Wasylyk, B. (2004). Ets ternary complex transcription factors. Gene *324*, 1–14.

Castro-Alamancos, M.A., Donoghue, J.P., and Connors, B.W. (1995). Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. J. Neurosci. *15*, 5324–5333.

Cheal, M.L., Klestzick, J., and Domesick, V.B. (1982). Attention and habituation: odor preferences, long-term memory, and multiple sensory cues of novel stimuli. J. Comp. Physiol. Psychol. *96*, 47–60.

Christy, B., and Nathans, D. (1989). Functional serum response elements upstream of the growth factor-inducible gene zif268. Mol. Cell. Biol. *9*, 4889–4895.

Clarke, N., Arenzana, N., Hai, T., Minden, A., and Prywes, R. (1998). Epidermal growth factor induction of the c-jun promoter by a Rac pathway. Mol. Cell. Biol. *18*, 1065–1073.

Corman, C.D., and Shafter, J.N. (1968). Open field activity and exploration. Psychonomic Sci. *13*, 55–56.

Dobrunz, L.E., and Stevens, C.F. (1997). Heterogeneity of release probability, facilitation, and depletion at central synapses. Neuron *18*, 995–1008.

Dudek, S.M., and Bear, M.F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc. Natl. Acad. Sci. USA *89*, 4363–4367.

Dulawa, S.C., Holick, K.A., Gundersen, B., and Hen, R. (2004). Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology *29*, 1321–1330.

Emptage, N.J., Reid, C.A., and Fine, A. (2001). Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca2+ entry, and spontaneous transmitter release. Neuron *29*, 197–208.

Finkbeiner, S., and Greenberg, M.E. (1998). Ca2+ channel-regulated neuronal gene expression. J. Neurobiol. *37*, 171–189.

Geppert, M., Bolshakov, V.Y., Siegelbaum, S.A., Takei, K., De Camilli, P., Hammer, R.E., and Sudhof, T.C. (1994). The role of Rab3A in neurotransmitter release. Nature *369*, 493–497.

Giovannini, M.G., Rakovska, A., Benton, R.S., Pazzagli, M., Bianchi, L., and Pepeu, G. (2001). Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. Neuroscience *106*, 43–53.

Grailhe, R., Waeber, C., Dulawa, S.C., Hornung, J.P., Zhuang, X., Brunner, D., Geyer, M.A., and Hen, R. (1999). Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. Neuron *22*, 581–591.

Gray, J.A., and McNaughton, N. (1983). Comparison between the behavioural effects of septal and hippocampal lesions: a review. Neurosci. Biobehav. Rev. *7*, 119–188.

Herdegen, T., Blume, A., Buschmann, T., Georgakopoulos, E., Winter, C., Schmid, W., Hsieh, T.F., Zimmermann, M., and Gass, P. (1997). Expression of activating transcription factor-2, serum

response factor and cAMP/Ca response element binding protein in the adult rat brain following generalized seizures, nerve fibre lesion and ultraviolet irradiation. Neuroscience *81*, 199–212.

Huang, Y.Y., and Kandel, E.R. (1994). Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learn. Mem. *1*, 74–82.

Huerta, P.T., and Lisman, J.E. (1995). Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. Neuron *15*, 1053–1063.

Hyman, J.M., Wyble, B.P., Goyal, V., Rossi, C.A., and Hasselmo, M.E. (2003). Stimulation in hippocampal region CA1 in behaving rats yields long-term potentiation when delivered to the peak of theta and long-term depression when delivered to the trough. J. Neurosci. *23*, 11725–11731.

Kaut, K.P., Bunsey, M.D., and Riccio, D.C. (2003). Olfactory learning and memory impairments following lesions to the hippocampus and perirhinal-entorhinal cortex. Behav. Neurosci. *117*, 304–319.

Kelly, P.H., and Malanowski, J. (1993). Cholinergic effects on spatial exploration and its memory. Can. J. Physiol. Pharmacol. *71*, 352–364.

Kemp, N., and Bashir, Z.I. (2001). Long-term depression: a cascade of induction and expression mechanisms. Prog. Neurobiol. *65*, 339–365.

Kew, J.N., Koester, A., Moreau, J.L., Jenck, F., Ouagazzal, A.M., Mutel, V., Richards, J.G., Trube, G., Fischer, G., Montkowski, A., et al. (2000). Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in the glycine binding site. J. Neurosci. *20*, 4037–4049.

Kirkwood, A., Rozas, C., Kirkwood, J., Perez, F., and Bear, M.F. (1999). Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. J. Neurosci. *19*, 1599–1609.

Knoll, B., Kretz, O., Fiedler, C., Alberti, S., Schutz, G., Frotscher, M., and Nordheim, A. (2006). Serum response factor controls neuronal circuit assembly in the hippocampus. Nat. Neurosci. *9*, 195–204.

Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. Neurosci. Biobehav. Rev. *26*, 91–104.

Lamprea, M.R., Cardenas, F.P., Silveira, R., Morato, S., and Walsh, T.J. (2000). Dissociation of memory and anxiety in a repeated elevated plus maze paradigm: forebrain cholinergic mechanisms. Behav. Brain Res. *117*, 97–105.

Lamprea, M.R., Cardenas, F.P., Silveira, R., Walsh, T.J., and Morato, S. (2003). Effects of septal cholinergic lesion on rat exploratory behavior in an open-field. Braz. J. Med. Biol. Res. *36*, 233–238.

Liu, W., and Saint, D.A. (2002). A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. Anal. Biochem. *302*, 52–59.

Liu, T., Wu, J., and He, F. (2000). Evolution of cis-acting elements in 5′ flanking regions of vertebrate actin genes. J. Mol. Evol. *50*, 22–30.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5–21.

Malleret, G., Hen, R., Guillou, J.L., Segu, L., and Buhot, M.C. (1999). 5–HT1B receptor knock-out mice exhibit increased exploratory activity and enhanced spatial memory performance in the Morris water maze. J. Neurosci. *19*, 6157–6168.

Man, H.Y., Lin, J.W., Ju, W.H., Ahmadian, G., Liu, L., Becker, L.E., Sheng, M., and Wang, Y.T. (2000). Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. Neuron *25*, 649–662.

Manahan-Vaughan, D., and Braunewell, K.H. (1999). Novelty acquisition is associated with induction of hippocampal long-term depression. Proc. Natl. Acad. Sci. USA *96*, 8739–8744.

Martin, S.J., and Morris, R.G. (2002). New life in an old idea: the synaptic plasticity and memory hypothesis revisited. Hippocampus *12*, 609–636.

Mayford, M., Bach, M.E., Huang, Y.Y., Wang, L., Hawkins, R.D., and Kandel, E.R. (1996). Control of memory formation through regulated expression of a CaMKII transgene. Science *274*, 1678–1683.

McCutchen, M.E. (2004). Interaction of muscarinic LTD and LFS-LTD at hippocampal CA3–CA1 synapses. Paper presented at Society for Neuroscience (San Diego, CA).

Moody, T.D., Thomas, M.J., Makhinson, M., and O'Dell, T.J. (1998). 5-Hz stimulation of CA3 pyramidal cell axons induces a beta-adrenergic modulated potentiation at synapses on CA1, but not CA3, pyramidal cells. Brain Res. *794*, 75–79.

Morishita, W., Connor, J.H., Xia, H., Quinlan, E.M., Shenolikar, S., and Malenka, R.C. (2001). Regulation of synaptic strength by protein phosphatase 1. Neuron *32*, 1133–1148.

Morishita, W., Marie, H., and Malenka, R.C. (2005). Distinct triggering and expression mechanisms underlie LTD of AMPA and NMDA synaptic responses. Nat. Neurosci. *8*, 1043–1050.

Morris, R.G., Garrud, P., Rawlins, J.N., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. Nature *297*, 681–683.

Mulkey, R.M., Herron, C.E., and Malenka, R.C. (1993). An essential role for protein phosphatases in hippocampal long-term depression. Science *261*, 1051–1055.

Nakano, M., Yamada, S., Udagawa, R., and Kato, N. (2004). Frequency-dependent requirement for calcium store-operated mechanisms in induction of homosynaptic long-term depression at hippocampus CA1 synapses. Eur. J. Neurosci. *19*, 2881–2887.

Nguyen, P.V., Abel, T., and Kandel, E.R. (1994). Requirement of a critical period of transcription for induction of a late phase of LTP. Science *265*, 1104–1107.

Nicoll, R.A., Oliet, S.H., and Malenka, R.C. (1998). NMDA receptor-dependent and metabotropic glutamate receptor-dependent forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neurobiol. Learn. Mem. *70*, 62–72.

Nishiyama, M., Hong, K., Mikoshiba, K., Poo, M.M., and Kato, K. (2000). Calcium stores regulate the polarity and input specificity of synaptic modification. Nature *408*, 584–588.

Ohshima, T., Ogura, H., Tomizawa, K., Hayashi, K., Suzuki, H., Saito, T., Kamei, H., Nishi, A., Bibb, J.A., Hisanaga, S., et al. (2005). Impairment of hippocampal long-term depression and defective spatial learning and memory in p35 mice. J. Neurochem. *94*, 917–925.

Oliet, S.H., Malenka, R.C., and Nicoll, R.A. (1997). Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neuron *18*, 969–982.

Patterson, S.L., Pittenger, C., Morozov, A., Martin, K.C., Scanlin, H., Drake, C., and Kandel, E.R. (2001). Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. Neuron *32*, 123–140.

Pavlidis, P., and Noble, W.S. (2001). Analysis of strain and regional variation in gene expression in mouse brain. Genome Biol. *2*. RESEARCH0042. Published online September 27, 2001. 10.1186/gb-2001-2-10-research0042.

Pavlidis, P., Weston, J., Cai, J., and Noble, W.S. (2002). Learning gene functional classifications from multiple data types. J. Comput. Biol. *9*, 401–411.

Petrulis, A., and Eichenbaum, H. (2003). The perirhinal-entorhinal cortex, but not the hippocampus, is critical for expression of individual recognition in the context of the Coolidge effect. Neuroscience *122*, 599–607.

Pittenger, C., Huang, Y.Y., Paletzki, R.F., Bourtchouladze, 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.

Ramanan, N., Shen, Y., Sarsfield, S., Lemberger, T., Schutz, G., Linden, D.J., and Ginty, D.D. (2005). SRF mediates activity-induced gene expression and synaptic plasticity but not neuronal viability. Nat. Neurosci. *8*, 759–767.

Reyes, M., and Stanton, P.K. (1996). Induction of hippocampal long-term depression requires release of Ca2+ from separate presynaptic and postsynaptic intracellular stores. J. Neurosci. *16*, 5951–5960.

Roberts, W.W., Dember, W.N., and Brodwick, M. (1962). Alternation and exploration in rats with hippocampal lesions. J. Comp. Physiol. Psychol. *55*, 695–700.

Rolls, E.T., Cahusac, P.M., Feigenbaum, J.D., and Miyashita, Y. (1993). Responses of single neurons in the hippocampus of the macaque related to recognition memory. Exp. Brain Res. *93*, 299–306.

Staubli, U., and Otaky, N. (1994). Serotonin controls the magnitude of LTP induced by theta bursts via an action on NMDA-receptor-mediated responses. Brain Res. *643*, 10–16.

Stringer, J.L., Belaguli, N.S., Iyer, D., Schwartz, R.J., and Balasubramanyam, A. (2002). Developmental expression of serum response factor in the rat central nervous system. Brain Res. Dev. Brain Res. *138*, 81–86.

Swonger, A.K., and Rech, R.H. (1972). Serotonergic and cholinergic involvement in habituation of activity and spontaneous alternation of rats in a Y maze. J. Comp. Physiol. Psychol. *81*, 509–522.

Thinus-Blanc, C., Bouzouba, L., Chaix, K., Chapuis, N., Durup, M., and Poucet, B. (1987). A study of the spatial parameters encoded during exploration in hamsters. J. Exp. Psychol. *13*, 418–427.

Thompson, R.F., and Spencer, W.A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol. Rev. *73*, 16–43.

Touzani, K., Taghzouti, K., and Velley, L. (1997). Increase of the aversive value of taste stimuli following ibotenic acid lesion of the central amygdaloid nucleus in the rat. Behav. Brain Res. *88*, 133–142.

Vianna, M.R., Alonso, M., Viola, H., Quevedo, J., de Paris, F., Furman, M., de Stein, M.L., Medina, J.H., and Izquierdo, I. (2000). Role of hippocampal signaling pathways in long-term memory formation of a nonassociative learning task in the rat. Learn. Mem. *7*, 333–340.

Vinogradova, O.S. (1995). Expression, control, and probable functional significance of the neuronal theta-rhythm. Prog. Neurobiol. *45*, 523–583.

Wiebel, F.F., Rennekampff, V., Vintersten, K., and Nordheim, A. (2002). Generation of mice carrying conditional knockout alleles for the transcription factor SRF. Genesis *32*, 124–126.

Winson, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science *201*, 160–163.

Yamaguchi, S., Hale, L.A., D'Esposito, M., and Knight, R.T. (2004). Rapid prefrontal-hippocampal habituation to novel events. J. Neurosci. *24*, 5356–5363.

Zakharenko, S.S., Patterson, S.L., Dragatsis, I., Zeitlin, S.O., Siegelbaum, S.A., Kandel, E.R., and Morozov, A. (2003). Presynaptic BDNF required for a presynaptic but not postsynaptic component of LTP at hippocampal CA1–CA3 synapses. Neuron *39*, 975–990.

Zucker, R.S. (1989). Short-term synaptic plasticity. Annu. Rev. Neurosci. *12*, 13–31.