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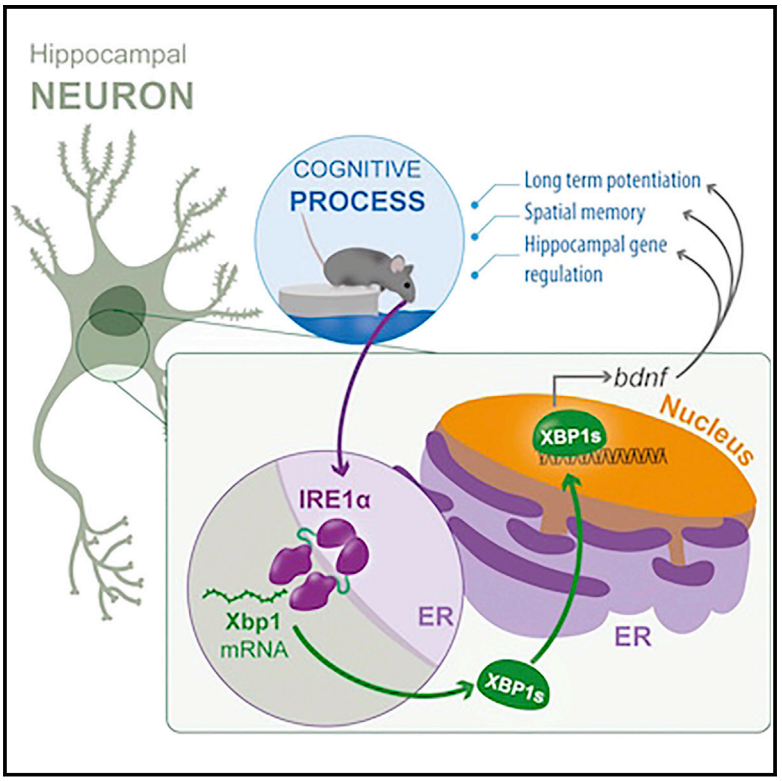
Regulation of Memory Formation by the Transcription Factor XBP1

Graphical Abstract



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In Brief

Using gain- and loss-of-function approaches, Martinez et al. demonstrate that XBP1, a master regulator of the unfolded protein response (UPR), regulates learning and memory-related processes. This function of XBP1 in the nervous system involves the control of BDNF expression in the hippocampus.

Highlights

- Cognitive processes activate the IRE1 branch of the UPR pathway in the hippocampus
- The UPR transcription factor XBP1 controls learning and memory-related processes
- Enforced expression of XBP1s in the hippocampus improves spatial memory
- XBP1 controls synaptic plasticity-related genes, including the expression of BDNF

Martínez et al., 2016, Cell Reports *14*, 1382–1394
February 16, 2016
http://dx.doi.org/10.1016/j.celrep.2016.01.028

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Regulation of Memory Formation by the Transcription Factor XBP1

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http://dx.doi.org/10.1016/j.celrep.2016.01.028

SUMMARY

Contextual memory formation relies on the induction of new genes in the hippocampus. A polymorphism in the promoter of the transcription factor XBP1 was identified as a risk factor for Alzheimer's disease and bipolar disorders. XBP1 is a major regulator of the unfolded protein response (UPR), mediating adaptation to endoplasmic reticulum (ER) stress. Using a phenotypic screen, we uncovered an unexpected function of XBP1 in cognition and behavior. Mice lacking XBP1 in the nervous system showed specific impairment of contextual memory formation and long-term potentiation (LTP), whereas neuronal XBP1s overexpression improved performance in memory tasks. Gene expression analysis revealed that XBP1 regulates a group of memory-related genes, highlighting brain-derived neurotrophic factor (BDNF), a key component in memory consolidation. Overexpression of BDNF in the hippocampus reversed the XBP1-deficient phenotype. Our study revealed an unanticipated function of XBP1 in cognitive processes that is apparently unrelated to its role in ER stress.

INTRODUCTION

The control of protein synthesis and, hence, the induction of gene expression are key to the formation and maintenance of long-term memories (Costa-Mattioli et al., 2009). Mice lacking the α and δ isoforms of the cyclic AMP (cAMP) response element-binding protein (CREB) revealed a key role for this transcription factor in long-term memory storage (Kogan et al., 1997; Bourtchuladze et al., 1994), corroborated by CREB-mediated gene expression in response to stimuli leading to long-term potentiation (LTP) in the hippocampus (Impey et al., 1996). Local inhibition of protein synthesis through phosphorylation of the protein translation initiation factor eIF2α, along with consequent downstream expression of the CREB family member ATF4, represses long-term synaptic plasticity and memory consolidation (Costa-Mattioli et al., 2009). Although eIF2α phosphorylation is a hallmark of the endoplasmic reticulum (ER) stress response through activation of protein kinase RNA-like ER kinase (PERK) (Hetz, 2012), the engagement of this pathway during learning and memory is linked to protein kinases not related to protein-folding stress, including protein kinase R (PKR) and general control nonderepressible 2 (GCN2) (Costa-Mattioli et al., 2009; Zhu et al., 2011; Costa-Mattioli et al., 2005). In contrast, in the context of Alzheimer's disease, activation of PERK due to protein-folding stress impairs memory-related functions (Ma et al., 2013).

The basic region/leucine zipper transcription factor X-box binding protein-1 (XBP1) is a third member of the CREB family that binds to CRE (cAMP-responsive element)-like sequences in target genes containing unfolded protein response elements (UPRE). Although it is best known as a key regulator of the UPR downstream of the ER stress sensor IRE1α (Hetz et al., 2015), XBP1 also drives gene expression programs that are not directly connected with cellular stress (see examples in Martinon et al., 2010; Lee et al., 2008; and Rutkowski and Hegde, 2010). IRE1α is a kinase and endonuclease protein that catalyzes the unconventional splicing of *Xbp1* mRNA, introducing a

frameshift that leads to the production of a transcriptionally active form of XBP1, termed XBP1s (Walter and Ron, 2011). XBP1s expression is essential for maintaining the function of specialized secretory cells and tissues (i.e., B cells, exocrine and endocrine pancreas, and salivary glands) by controlling the expression of a cluster of genes involved in protein folding, secretion, lipid biosynthesis, and ER-associated protein degradation (Lee et al., 2003; Acosta-Alvear et al., 2007; Cornejo et al., 2013).

Alteration to the ER proteostasis network is emerging as a relevant player in most common neurodegenerative diseases involving protein misfolding (Oakes and Papa, 2015). Although extensive reports have described a functional impact of the unfolded protein response (UPR) on neurodegenerative diseases (Hetz and Mollereau, 2014), its potential physiological role in higher functions of the brain remains unexplored. Accumulating studies in models of neurodegeneration support the idea that the contribution of the UPR to the disease process is complex and highly dependent on the specific signaling branch affected and the disease context. For example, we have shown that targeting either XBP1 or ATF4 in spinal cord injury models diminishes locomotor recovery (Valenzuela et al., 2012), whereas XBP1 deficiency has no impact on optic nerve injury (Hu et al., 2012). Unexpectedly, ablating XBP1 expression in models of amyotrophic lateral sclerosis and Huntington's disease has protective effects through the modulation of autophagy (Hetz et al., 2009; Vidal et al., 2012). In contrast, ATF4 deficiency protects against the development of amyotrophic lateral sclerosis by reducing the expression of pro-apoptotic factors such as CHOP (Matus et al., 2013), whereas it has no impact on Huntington's disease progression (Vidal et al., 2012). Conversely, XBP1 expression is irrelevant in prion-related disorders (Hetz et al., 2008), whereas PERK activity has a pro-degenerative effect, possibly through an inhibitory modulation of the expression of synaptic proteins (Moreno et al., 2012). Many other studies also support a role of the UPR in neurodegeneration in different model organisms (Hetz and Mollereau, 2014). Likewise, the UPR is also emerging as a relevant target in other important pathologies, including cancer, autoimmunity, and metabolic disorders (Oakes and Papa, 2015; Hetz et al., 2013).

Although extensive studies associate the UPR and XBP1 with brain diseases, the possible involvement of the pathway in the normal physiology of the nervous system remains mostly unexplored. Interestingly, genomic screens have identified a polymorphism in the XBP1 promoter as a risk factor for Alzheimer's disease (Liu et al., 2013), bipolar disorder, and schizophrenia in certain human populations (Kakiuchi et al., 2003, 2004), suggesting that XBP1 may modulate cognitive processes. In support of this hypothesis, *Xbp1* mRNA appears to be upregulated in animals exposed to enriched environments (Rampon et al., 2000), and *Xbp1* mRNA splicing is activated locally in neurites in response to brain-derived neurotrophic factor (BDNF) treatment, enhancing neurite outgrowth in vitro (Hayashi et al., 2007, 2008). Neuronal function involves different functional aspects of the secretory pathway, including synthesis and trafficking of various plasma membrane receptors and ion channels, engagement of ER calcium signaling, synthesis of membranes, and assembly of protein complexes. Consequently, it is possible to speculate that components of the UPR might play an important role in brain function through classical ER stress outputs.

In this study, we have uncovered an unexpected function of XBP1 in the control of learning and memory that, surprisingly, is ER stress independent. This activity of XBP1 in the brain is linked to the transcriptional regulation of BDNF in the hippocampus. This report identifies a physiological role of this UPR signaling branch in the nervous system, unrelated to its function as an ER stress-responsive factor.

RESULTS

XBP1 Deficiency in the Nervous System Reduces Learning and Memory

To explore the possible participation of XBP1 in cognitive, sensory, or motor functions of the CNS, we performed a behavioral screen on a neural-specific *Xbp1* conditional knockout mouse model ($XBP1^{Nes-/-}$) that we previously described (Hetz et al., 2008) (Figure S1A, upper panel). In earlier studies, we did not observe any obvious spontaneous motor or behavioral abnormalities in these animals, and we did not detect histological alterations in their brains under normal conditions (Hetz et al., 2008, 2009; Valenzuela et al., 2012; Vidal et al., 2012). However, mutant animals displayed a specific impairment in the contextual and cued fear-conditioning tests (Figure 1A; Figures S1A and S1B), which assesses hippocampus- and amygdala-dependent memory (Kim et al., 1993). We also measured the learning capacity of $XBP1^{Nes-/-}$ animals using the memory flexibility paradigm, a hippocampus-dependent test related to episodic memory (Chen et al., 2000). $XBP1^{Nes-/-}$ mice required more trials than control animals to perform the task, indicating significant memory impairment (Figure 1B). This phenotype occurred in the absence of alterations in brain cortex-dependent recognition memory, motor, anxiety, or reflex control, as assessed by novel object recognition, rotarod, startle response, hot plate tests, (Figure S1A) or the swimming velocity in the memory flexibility (Figure S1C).

To further explore the effects of XBP1 on hippocampal synaptic function, we measured glutamatergic transmission evoked by Schaffer collaterals' stimulation and recorded field excitatory postsynaptic potentials (fEPSPs) in the CA1 region. LTP, a long-lasting form of synaptic plasticity whose expression relies on postsynaptic mechanisms (Granger and Nicoll, 2014), was drastically impaired in $XBP1^{Nes-/-}$ animals, as measured after theta-burst stimulation (TBS) (Figure 1C) or high-frequency stimulation (HFS) (Figure S2A). We observed a significant reduction in basal synaptic transmission in $XBP1^{Nes-/-}$ animals, evaluated by input-output curves (Figure 1D). Presynaptic fiber volley (FV) amplitudes—but not fEPSP slopes, a measure of postsynaptic activity—were significantly reduced in $XBP1^{Nes-/-}$ animals (Figures S2B and S2C). These results are indicative of a decreased number of presynaptic neurons firing action potentials. Interestingly, the LTP alterations observed in $XBP1^{Nes-/-}$ mice were maintained for up to 3 hr (Figures S2D and S2E), well into the protein-synthesis-dependent phase of late LTP that is commonly associated with long-term memory. Thus, ablation of XBP1 in the nervous system induces multiple functional deficits in hippocampal synapses. Taken together, these results indicate that

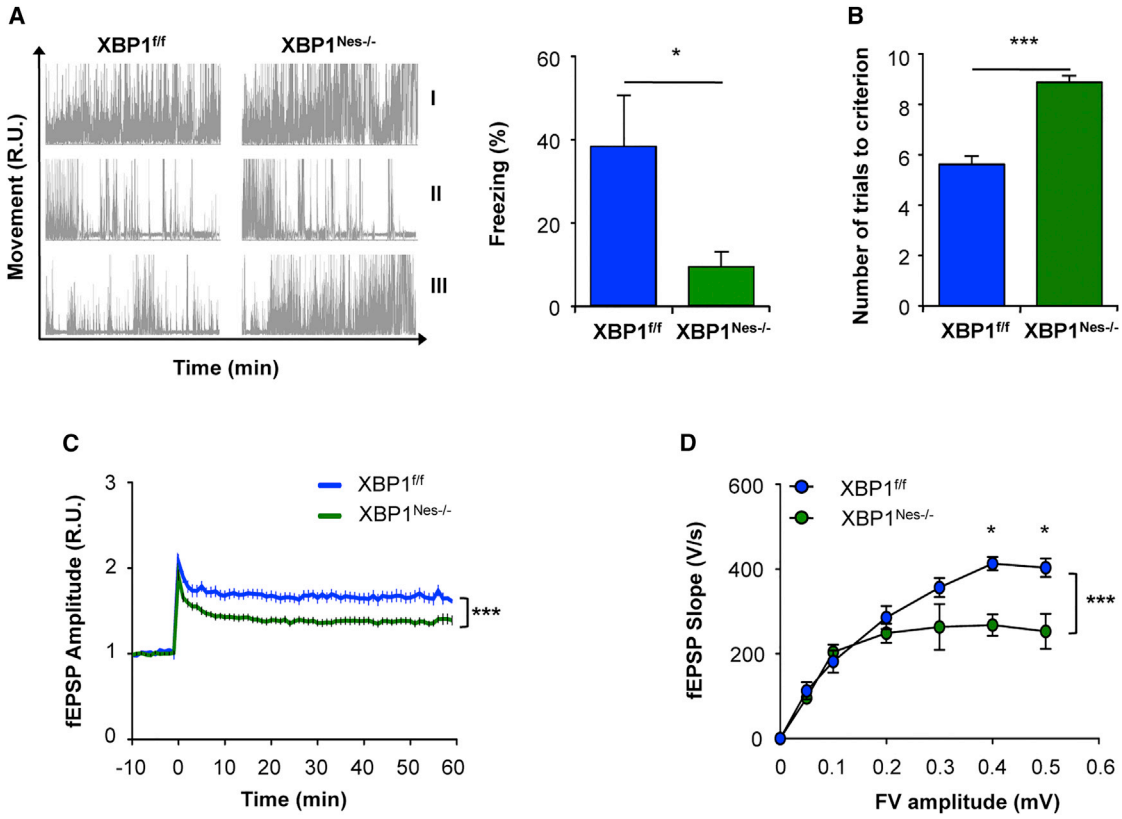

Figure 1. Altered Long-Term Memory and LTP in XBP1 Conditional Knockout Mice

(A) Left panel: representative images of movement traces in time at different stages of the contextual fear-conditioning assay. I, habituation; II, training; and III, test. Right panel: XBP1$^{Nes-/-}$ mice and littermate control (XBP1$^{f/f}$) male mice were tested in the contextual fear-conditioning assay. Percentage of freezing events during the test was calculated (XBP1$^{f/f}$, n = 4; XBP1$^{Nes-/-}$, n = 6), and statistical analysis was performed using Student's t test. R.U., relative units.

(B) Animals were trained and tested using the memory flexibility paradigm. The analysis shows the average number of trials to reach criterion in 4 consecutive days (n = 4 per group). Statistical analysis was performed using Student's t test.

(C) LTP was induced by TBS (n = 20 slices from three XBP1$^{Nes-/-}$ mice; 21 slices from 3 XBP1$^{f/f}$ mice). Statistical analysis was performed using two-way ANOVA.

(D) Basal synaptic transmission recorded in hippocampal CA1 area was assessed by input-output curves plotting FV amplitudes against fEPSP slopes. (n = 26 slices from three XBP1$^{Nes-/-}$ mice; 36 slices from three XBP1$^{f/f}$ mice). Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test).

Data are presented as mean ± SEM. *p < 0.05; ***p < 0.001.

See also Figures S1, S2, and S4.

reducing XBP1 expression in the brain impairs learning and memory-related processes.

Enforced Expression of XBP1s Improves Learning and Memory

To test whether increasing active XBP1s in the nervous system alters the learning and memory capacity of mice, we generated a transgenic mouse model using the prion promoter to drive expression of the active XBP1s form in neurons (Tg^{XBP1s}). These animals were viable, did not show alterations in Mendelian ratios at birth, and did not develop any overt phenotypes. Restricted expression of the transgene to the CNS was confirmed by western blot and real-time PCR analysis (Figure 2A, left panel; Figures S3A and S3B). Immunofluorescence staining for XBP1s in the hippocampus confirmed that the transgene is expressed almost exclusively in neurons (Figure 2A, right panel). Remarkably, sustained neuronal expression of XBP1s improved performance in the memory flexibility (Figure 2B) and contextual fear-conditioning tests (Figure 2C). Memory flexibility improvement in transgenic mice was not related to changes in swimming velocity (Figure S3C), suggesting normal motor performance. Consistent with these results, hippocampal slices derived from Tg^{XBP1s} mice showed enhanced LTP compared with wild-type slices (Figure 2D), which extended to the protein-synthesis-dependent phase of late LTP (Figures S3D and S3E). In addition, overexpression of XBP1s led to increased basal synaptic transmission determined through input-output measurements (Figure 2E). Overall, these results indicate that overexpression of XBP1s in the nervous system improves the basal learning capacity in mice, associated with improved LTP and synaptic transmission in the hippocampus.

To rule out possible compensatory effects of manipulating XBP1 levels during development in the transgenic mice, and to directly investigate XBP1 function in the hippocampus, we

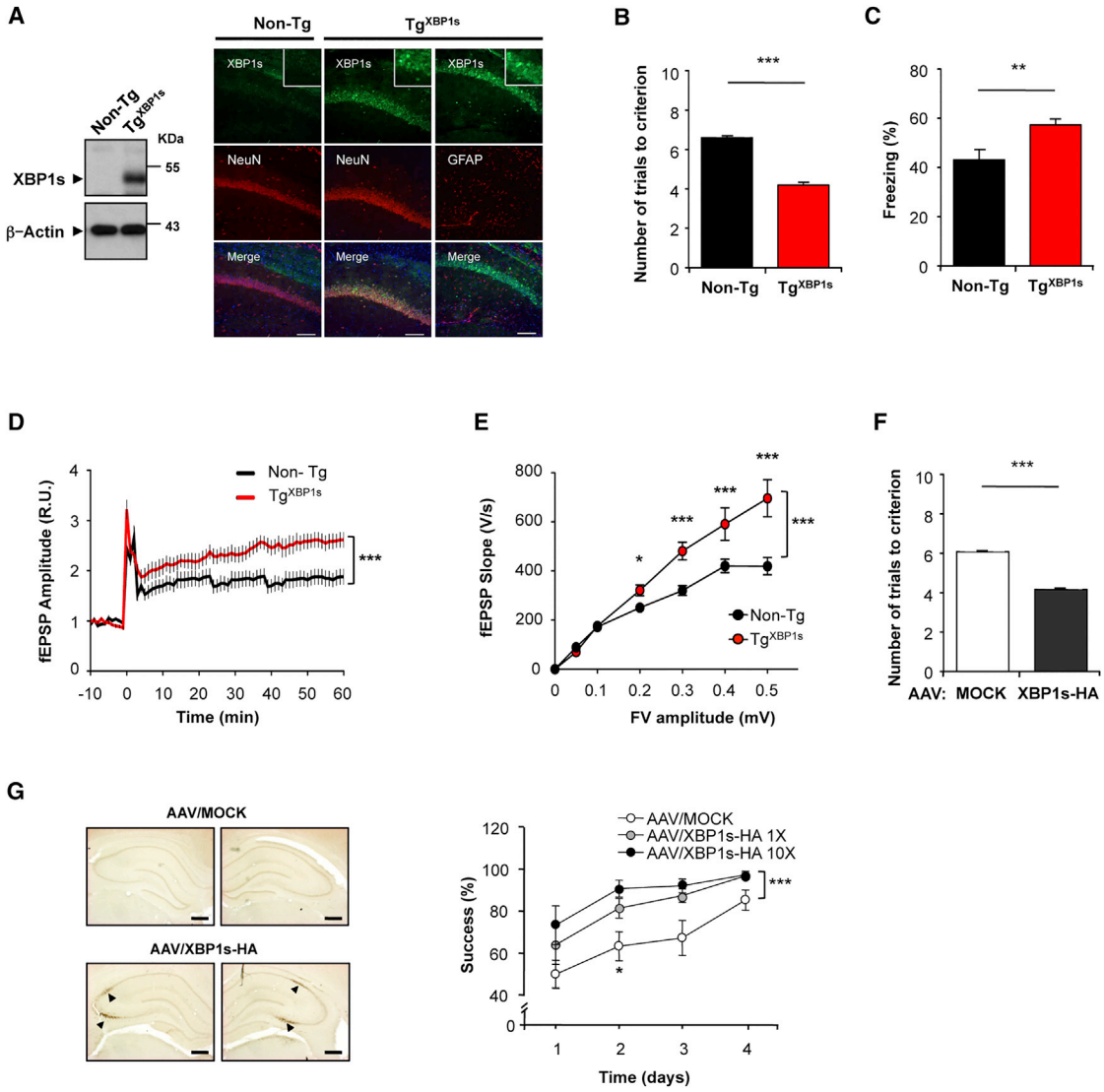

Figure 2. Overexpression of XBP1s in Neurons Enhances Long-Term Memory

(A) An XBP1s neuron-specific transgenic mouse strain was generated using the prion promoter to drive its expression in the nervous system (Tg^{XBP1s}). Left panel: XBP1s levels in the hippocampus were analyzed by western blot using β-actin as loading control. Right panel: Expression of XBP1s, NeuN, and glial fibrillary acidic protein (GFAP) was evaluated in the hippocampus using immunofluorescence analysis. Scale bars, 100 μm.

(B) Learning performance was assessed in Tg^{XBP1s} and littermate control non-transgenic (Non-Tg) animals using the memory flexibility test. The analysis shows the average number of trials to reach criterion in 4 consecutive days (n = 4 per group). Statistical analysis was performed using Student's t test.

(C) Tg^{XBP1s} mice and littermate control (Non-Tg) male mice were tested in the contextual fear-conditioning assay. Percentage of freezing events during the test was calculated (Tg^{XBP1s}, n = 11; and Non-Tg, n = 11), and statistical analysis was performed using Student's t test.

(D) LTP induced by TBS was measured in hippocampal slices from Tg^{XBP1s} and control animals (n = 7 slices from five mice per group). Statistical analysis was performed using two-way ANOVA. R.U., relative units.

(E) Input-output curves showing the relationship between FV amplitude and fEPSP slope (n = 70 slices from five Non-Tg mice; 36 slices from three Tg^{XBP1s} mice). Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test.

(F) Three-month-old mice were injected with serotype 6 AAVs to deliver XBP1s-HA (AAV/XBP1s-HA) or empty vector (AAV/MOCK) into the hippocampus using bilateral stereotaxis. Fourteen days after injection, animals were trained and tested in the memory flexibility test. The analysis shows the average number of trials to reach criterion in 4 consecutive days (n = 4 per group). Statistical analysis was performed using Student's t test.

(G) Rats were injected with AAV particles described in (F) into the hippocampus using two different viral titers (1×, 1×10^6 transducing units [TUs]; 10×, 1×10^7 TUs) through bilateral brain stereotaxis. Left panel: representative immunohistochemistry images of injected brains are shown after staining with an anti-HA antibody. Arrowheads indicate positive HA neurons. Scale bar, 100 μm. Right panel: 14 days post-injection, rats were trained in the oasis maze task and the percentage of successful trials was recorded over time (1X titer: AAV/MOCK n = 9; AAV/XBP1s-HA n = 10; 10X titer: AAV/MOCK n = 5; AAV/XBP1s-HA n = 5). Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test (*$p < 0.05$; ***$p < 0.001$).

Data are presented as mean and SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

See also Figures S3 and S4.

performed bilateral stereotactic injections of adeno-associated viruses (AAVs) to deliver XBP1s (AAV/XBP1s-HA [hemagglutinin]) or a control vector (AAV/MOCK) into the CA1 hippocampal region of adult wild-type mice. Two weeks after AAV injection, mice were tested in the memory flexibility assay. As in Tg^{XBP1s} animals, local expression of XBP1s in the hippocampus resulted in improved memory flexibility without significant changes in swimming velocity (see Figures 2F, S4A, and S4B for a global comparison of all tests).

We further validated these results in a separate rodent model using a cognitive test that assesses hippocampal-dependent memory. We bilaterally injected the CA3 hippocampal region of rats with two different titers of AAV/XBP1s-HA and, 2 weeks after surgery, evaluated their behavior in the Oasis Maze (Clark et al., 2005). Rats expressing XBP1s-HA in the hippocampus (Figure 2G, left panel) showed a significant increase in the percentage of successful attempts to find hidden food in the maze (Figure 2G, right panel). Thus, enforcement of XBP1s expression in the hippocampus of wild-type animals is sufficient to improve higher cognitive functions involved in learning and memory tasks.

XBP1 Regulates BDNF Expression in the Hippocampus

In the ER stress response, XBP1 supports secretory cell function through the transcriptional control of a cluster of genes involved in protein folding, quality control, and the secretory pathway (Lee et al., 2003; Acosta-Alvear et al., 2007); therefore, we measured UPR gene expression (*Wfs1*, *Edem1*, and *Bip*) in the hippocampus of $XBP1^{Nes-/-}$ mice. Surprisingly, no changes were observed in the basal expression of these canonical ER stress-related genes (Figure 3A). Alternatively, the learning and memory deficits observed in $XBP1^{Nes-/-}$ animals could be explained by compensatory overactivation of PERK signaling, resulting in eIF2α phosphorylation and ATF4 induction. Thus, we measured markers of translational control in the hippocampus of $XBP1^{Nes-/-}$ mice but did not detect any alterations in eIF2α phosphorylation or ATF4 expression (Figure 3B).

Based on the cognitive defects identified in $XBP1^{Nes-/-}$ animals, we evaluated the expression of a panel of 26 known genes linked to learning and memory (Table 1). Unexpectedly, XBP1 deficiency led to a marked reduction of *Bdnf* mRNA levels in the brain of $XBP1^{Nes-/-}$ animals (Figure 3C), in addition to a moderate decrease in the expression of genes that participate in neurotransmission and synaptic plasticity, including *Kif17* and *Ampa3* (Figure 3C). Other important memory-related genes were not significantly altered upon deletion of *Xbp1* in the brain, including glutamate receptors, ryanodine receptors, CREB, syntaxin 17, and calcineurin, among others (Table 1). These changes in gene expression were specific to the hippocampus, since they were not detected in dissected amygdala from $XBP1^{Nes-/-}$ mice (Figure 3D). We also confirmed reduced BDNF and KIF17 protein expression in the hippocampus of $XBP1^{Nes-/-}$ animals (Figure 3E). Consistent with these results, Tg^{XBP1s} mice also displayed increased levels of BDNF in the hippocampus compared to littermate control animals (Figure 3F). Finally, we also evaluated the levels of *Bdnf* mRNA levels in the hippocampus of mice injected with AAV/XBP1s-HA and detected a significant up-regulation of *Bdnf* expression (Figure 3G). Taken together, these results demonstrate that XBP1 expression increases the levels of *Bdnf* in the mouse hippocampus.

XBP1 Transactivates the BDNF Promoter, Enhancing Learning and Memory Processes

Through a bioinformatic analysis of the proximal 1-kb 5′ promoter region of *Bdnf*, *Kif17*, and *Ampa3*, we identified a putative consensus binding site for XBP1 in BDNF promoter IV (Figure 4A, upper panel) and in the proximal promoter region of Kif17 (data not shown). Since BDNF is a master regulator of neuronal plasticity and memory-related processes (Park and Poo, 2013), we focused our analysis on the relationship between XBP1 expression and BDNF transcription. The canonical XBP1 binding site with a UPRE B core (Acosta-Alvear et al., 2007) in the mouse BDNF promoter IV is located 108 bp upstream of the transcription start site and is conserved in mouse, rats, and humans. To test whether XBP1s transactivates the BDNF promoter, we generated a luciferase reporter construct including the promoter region from −604 to +53 bp (Figure 4A, upper panel). Transient co-expression of XBP1s led to enhanced activity of the BDNF promoter in HEK293T cells (Figure 4A, lower panel). To functionally validate the identified UPRE B site, we deleted its core ACGT region (ΔBNDF-LUC) and tested XBP1s-mediated transactivation. This mutation fully ablated the ability of XBP1s to activate the BDNF promoter construct (Figure 4A, lower panel). Finally, using chromatin immunoprecipitation (ChIP) in the neuronal cell line Neuro2a transiently transfected with an XBP1s-HA expression vector, we confirmed the physical interaction between XBP1s and the UPRE B site in the BDNF promoter (Figure 4B). Importantly, the binding of XBP1s-HA to the BDNF promoter was comparable to its binding to the *Dgat2* promoter (Figure 4B), a well-known XBP1s-target gene (Lee et al., 2008). We also confirmed a positive binding of XBP1s to the BDNF promoter at basal levels, using ChiP in hippocampus extracts of wild-type mice (Figure 4C). Thus, XBP1s drives changes in hippocampal gene expression that are not associated with a classical UPR response but instead involve transactivation of the BDNF promoter.

To investigate a functional connection between decreased levels of BDNF and the memory deficits observed in $XBP1^{Nes-/-}$ animals, we injected serotype 6 AAVs into the hippocampus of $XBP1^{Nes-/-}$ mice to deliver BDNF-GFP or control vector (Figure 5A, left panel; Figure S4C) and then performed the memory flexibility test. Remarkably, local expression of BDNF-GFP was effective in restoring memory performance of $XBP1^{Nes-/-}$ animals up to a level comparable with that of control animals without significant changes in swimming velocity (Figure 5A, right panel; Figure S4D). This result was confirmed in an independent experimental group of animals pre-trained before AAV/BDNF-GFP injections that were then re-evaluated 2 weeks post-injection (Figure 5B). Importantly, BDNF overexpression did not alter the performance of control animals, suggesting that the expression levels of BDNF obtained in our experimental setting are not sufficient to enhance basal performance but can restore the deficiency observed in $XBP1^{Nes-/-}$ animals. Moreover, exogenous BDNF also restored hippocampal synaptic transmission, measured as LTP in $XBP1^{Nes-/-}$ mice pre-injected with AAV/BDNF-GFP (Figure 5C). The recovery of synaptic connectivity was also confirmed

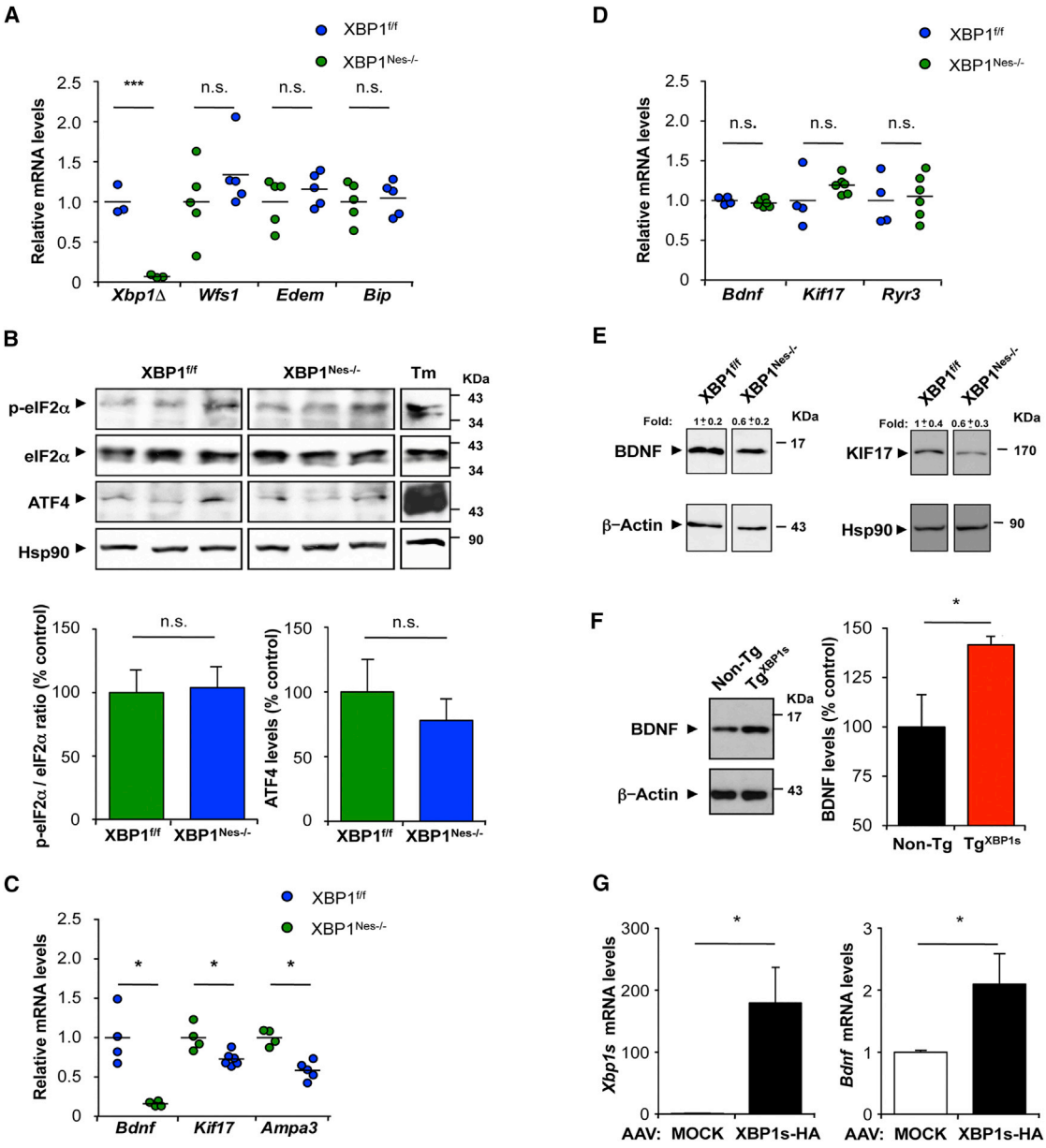

Figure 3. XBP1 Regulates the Expression of BDNF and a Group of Memory-Related Genes in the Hippocampus

(A) The mRNA levels of indicated UPR-target genes were measured in dissected hippocampus from XBP1$^{Nes-/-}$ mice or littermate XBP1$^{f/f}$ control animals using real-time PCR. The analysis was performed at 6 months of age (n = 3 for *Xbp1Δ*—deleted *Xbp1* mRNA—and n = 5 for *Wfs1*, *Edem*, and *Bip*).

(B) Upper panel: phosphorylated eIF2α (peIF2α) and ATF4 protein levels were measured in dissected hippocampus from XBP1$^{Nes-/-}$ mice or littermate XBP1$^{f/f}$ animals using western blot (n = 3), with Hsp90 and total eIF2α as loading controls. A total lysate from mouse embryonic fibroblasts (MEFs) treated with 5 μg/ml tunicamycin (Tm) for 16 hr was included as positive control. Lower panel: quantification of peIF2α/eIF2α ratio and ATF4 expression levels after normalization to Hsp90 expression levels. Statistical analysis was performed using Student's t test.

(C) The expression levels of several memory-related genes were measured in the hippocampus of XBP1$^{f/f}$ (n = 4) and XBP1$^{Nes-/-}$ (n = 5–6) mice using real-time PCR (see also Table 1 for the complete dataset).

(D) The mRNA levels of memory-related genes indicated in (C) were measured in dissected amygdala using real-time PCR. In (A), (C), and (D), averages are shown, and statistical analysis was performed using Mann-Whitney U test. All samples were normalized to β-actin mRNA levels.

(E) BDNF and KIF17 protein levels were analyzed by western blot, using hippocampus extracts obtained from 6-month-old XBP1$^{f/f}$ and XBP1$^{Nes-/-}$ animals. Levels of β-actin or Hsp90 were used as loading control. Mean and SEM are presented as fold change compared to control animals (n = 3). Bands were spliced from the same gel under identical film exposure.

(F) BDNF levels were analyzed in dissected hippocampus from Tg^{XBP1s} and control mice by western blot (left panel); β-actin was used as loading control. Right panel: Quantification of BDNF protein levels normalized to β-actin levels (Non-Tg, n = 5; Tg^{XBP1s}, n = 7 mice). Statistical analysis was performed using Student's t test.

(legend continued on next page)

Table 1. Gene Expression Profile in the Hippocampus of XBP1$^{Nes-/-}$ Animals

Gene	Mean; SEM XBP1$^{f/f}$	Mean; SEM XBP1$^{Nes-/-}$	p Value	Gene Description
Ttr	1; 0.25	0.81; 0.39	0.571	transthyretin
Reln	1; 0.32	0.68; 0.05	0.73	reelin
Gria1	1; 0.16	0.75; 0.11	0.286	glutamate receptor, ionotropic, AMPA1 (alpha1)
Gria2	1; 0.06	0.77; 0.06	0.063	glutamate receptor, ionotropic, AMPA2 (alpha 2)
Gria3[a]	1; 0.05	0.58; 0.05	0.016	glutamate receptor, ionotropic, AMPA3 (alpha3)
Gria4	1; 0.12	0.93; 0.08	0.113	glutamate receptor, ionotropic, AMPA4 (alpha4)
Myo5b	1; 0.27	0.44; 0.08	0.111	myosin VB
Creb	1; 0.05	0.98; 0.22	1	CREB
Bdnf[a,b]	1; 0.17	0.16; 0.18	0.029	BDNF
CamkII	1; 0.27	1.30; 0.26	0.762	calcium/calmodulin-dependent protein kinase II
Ryr1	1; 0.24	0.93; 0.19	0.762	ryanodine receptor 1
Ryr2	1; 0.16	0.68; 0.06	0.114	ryanodine receptor 2
Ryr3	1; 0.15	0.61; 0.04	0.067	ryanodine receptor 3
Nr2a	1; 0.01	1.01; 0.01	0.914	ionotropic glutamate receptor subunit NR2A
Nr2b	1; 0.07	1.21; 0.10	0.257	ionotropic glutamate receptor subunit NR2B
Pp2b/Caln	1; 0.09	1.09; 0.16	0.914	calcineurin
Kif17[a,b]	1; 0.08	0.73; 0.03	0.019	kinesin family 17
Stx17	1; 0.14	0.76; 0.05	0.171	syntaxin 17
Kcnk1	1; 0.17	0.76; 0.09	0.257	potassium channel, subfamily K, member 1
Xpo4	1; 0.18	0.93; 0.14	0.905	exportin 4
Csnk2a	1; 0.14	1.01; 0.07	0.686	casein kinase 2, alpha 1 polypeptide
Adrb1	1; 0.06	1.14; 0.11	0.610	adrenergic receptor, beta 1
Pten	1; 0.12	1.18; 0.16	0.352	phosphatase and tensin homolog
Map2k3	1; 0.07	1.14; 0.09	0.476	mitogen-activated protein kinase kinase 3
Uqcr10	1; 0.45	1.94; 0.49	0.171	ubiquinol-cytochrome c reductase, complex III subunitX
Nipsnap1	1; 0.37	1.35; 0.33	0.610	4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1

The mRNA levels of indicated genes involved in learning and memory were measured in dissected hippocampus from male mice using real-time PCR (XBP1$^{f/f}$: n = 4; XBP1$^{Nes-/-}$: n = 5–6). For comparison, values are shown as fold change of gene expression in XBP1$^{Nes-/-}$ in relation to the average of the value obtained in control XBP1$^{f/f}$. Mean and SEM are presented. p values were obtained with the Mann-Whitney test.

[a]Genes showing statistically significant differences in expression.

[b]Genes that contain putative XBP1s binding sites in their proximal 1-kb promoter region. Promoter sequences were analyzed with the TESS program, using the TRANSFAC and JASPAR matrices.

through input-output measurements (Figure 5D; Figures S5A and S5B). These findings indicate that the cognitive and synaptic defects observed in XBP1$^{Nes-/-}$ animals are highly dependent on BDNF expression, implying that XBP1 modulates learning and memory through the regulation of a distinct group of genes, with a predominant role in the control of BDNF expression.

Xbp1 mRNA Splicing Is Activated by BDNF In Vitro and In Vivo

BDNF is known to drive its own expression, a relevant process in memory consolidation (Minichiello, 2009), and to trigger IRE1α activation in primary neuronal cultures (Hayashi et al., 2007). We confirmed the induction of *Xbp1* mRNA splicing in primary neuronal cultures exposed to recombinant BDNF, in addition to neuronal depolarization with extracellular KCl, suggesting that this UPR branch is engaged by neuronal stimulation (Figures 6A and 6B). We further demonstrated that recombinant BDNF enhances *Xbp1* mRNA splicing in an IRE1α-dependent manner, as determined by treating neuronal cultures with the IRE1α inhibitor STF083010 (Figure 6C). The effect of BDNF on *Xbp1* mRNA splicing occurs in the absence of general UPR activation, since *Bip* and *Chop* were not induced by BDNF treatment (Figure 6D). We validated these results in vivo by monitoring the levels of *Xbp1s* mRNA in the hippocampus of mice injected with

(G) *Xbp1s* (left panel) and *Bdnf* (right panel) mRNA levels were measured by real-time PCR in total cDNA obtained from the hippocampus of wild-type mice injected with AAV/XBP1s-HA or AAV/MOCK particles. Expression values were normalized to β-actin mRNA levels and expressed as fold change with respect to the AAV/MOCK group (n = 6). Statistical analysis was performed using Student's t test.

Data are presented as mean and SEM. *p < 0.05; ***p < 0.001; n.s., non-significant.

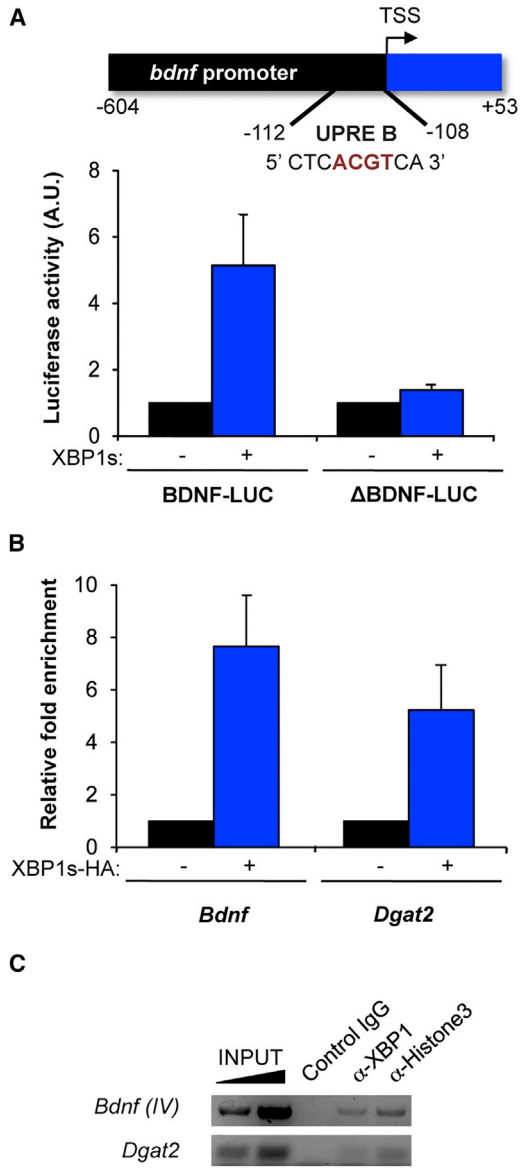

Figure 4. XBP1 Binds to the Promoter IV of *Bdnf*

(A) The indicated region of *Bdnf* promoter IV was cloned from mouse genomic DNA into a luciferase reporter plasmid (BDNF-LUC). A schematic representation of the construct is shown highlighting the presence of a conserved UPRE B sequence in the proximal promoter region (upper panel; TSS, transcription starting site). Bottom panel: HEK293 cells were co-transfected with an expression vector for active XBP1s and the BDNF-LUC reporter construct (n = 5) or a mutated version where the ACTG core sequence in the UPRE B was deleted (ΔBDNF-LUC) (n = 4). After 48 hr, luciferase activity was measured as described in Experimental Procedures. Mean and SEM are shown as fold change compared to control.

(B) ChIP of XBP1 using an anti-XBP1 antibody was performed in N2A cells after transfection with an expression vector encoding an HA-tagged version of XBP1s (XBP1-HA). Binding of XBP1s to the UPRE B region in the *Bdnf* promoter was assessed by real-time PCR. As positive control, the binding of XBP1s to the *Dgat2* promoter region (a well-known XBP1 target) was measured. Values represent relative increase compared to the signal obtained with the control antibody. Mean and SEM of three independent experiments are shown.

(C) Qualitative in vivo ChIP of XBP1 in hippocampal tissue of wild-type mice. Anti-Histone H3 antibody or rabbit IgG (immunoglobulin G) were used as positive and negative IP controls, respectively. Input PCR reactions of *Bdnf* and *Dgat2* promoter regions were performed with 0.1% or 1% of total starting material.

BDNF-GFP AAV particles (Figure 6E). Then, we determined whether the training of animals in the contextual fear-conditioning test engages the IRE1α pathway. Indeed, the mRNA levels of *Xbp1s* were increased in the hippocampus similarly to the immediate early gene *c-Fos* (Figure 6F). Importantly, a direct correlation between *Xbp1* mRNA splicing and *c-Fos* levels was observed in this experiment (Figure S6). Collectively, these results suggest that IRE1α activation is a physiological component of neuronal processes associated with plasticity and learning, which may contribute to modulate BDNF signaling.

DISCUSSION

Memory is defined as the process by which new information is acquired, consolidated, and retrieved by the brain (Alberini, 2009; Kandel, 2001). Long-term memory occurs when new neural synapses are established, allowing the information to be remembered for weeks, months, and even years, in a process that depends on the synthesis of new mRNA and proteins (Costa-Mattioli et al., 2009). BDNF is a neurotrophin that regulates brain development, neuroplasticity, and synaptogenesis in the CNS and peripheral nervous system through binding and activation of neurotrophin receptors TrkB (tropomyosin receptor kinase B) and $p75^{NTR}$, triggering phosphorylation cascades in neurons that lead either to enhanced survival, differentiation, and plasticity or to cell death (Park and Poo, 2013; Bekinschtein et al., 2014). Several studies have established the importance of BDNF signaling in cognitive functions, with a predominant impact on learning and memory-related processes (Lu et al., 2008). BDNF expression levels are dynamically controlled in an activity-dependent fashion during behavioral tasks; however, available studies on its transcriptional regulation are complex, indicating the use of alternative promoters according to the inputs received by the neuron (Hayes et al., 1997; Nakayama et al., 1994). From the nine different functional promoters that generate splicing variants, promoter IV is known to mediate the activity-dependent transcription of *Bdnf* through binding of a number of transcription factors, including the calcium-responsive transcription factor (CaRF) and CREB (Tao et al., 1998, 2002). These transcription factors bind to calcium-responsive elements on promoter IV and are activated by disparate signaling pathways that are triggered by membrane depolarization and calcium entry into cells (Zheng et al., 2012). Despite the relatively thorough molecular characterization of activity-dependent transcription of BDNF, the dynamics and mechanisms that fine-tune BDNF expression during neural development and plasticity are still poorly understood. Here, we provide evidence that XBP1s has a central role in learning and memory-related processes, primarily by modulating BDNF expression in the hippocampus. XBP1s directly binds to and transactivates BDNF promoter IV, which contains an UPRE-like sequence. Our data suggest that the deficits in memory performance caused by *Xbp1* ablation are mostly due

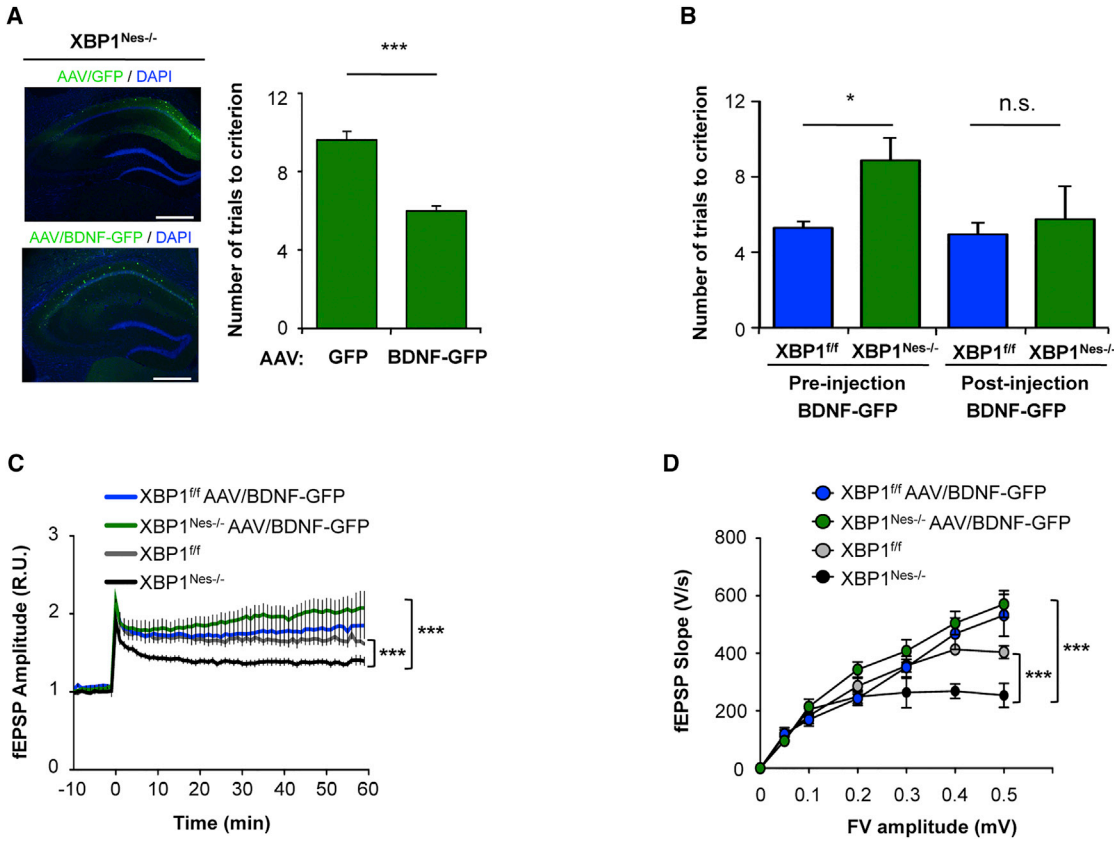

Figure 5. Local Expression of BDNF in the Hippocampus of XBP1-Deficient Animals Improves Long-Term Memory

Three-month-old XBP1$^{Nes-/-}$ animals were bilaterally injected with serotype 6 AAVs to deliver BDNF-GFP (AAV/BDNF-GFP) or control (AAV/GFP) particles into the hippocampi.

(A) Left panel: expression of BDNF-GFP or GFP was confirmed using histological analysis with DAPI co-staining. Scale bars, 100 μm. Right panel: results of the memory flexibility test performed 2 weeks after viral injections are shown as average number of trials to reach criterion in 4 consecutive days (n = 4 per group). Data were analyzed using Student's t test.

(B) Learning performance was assessed in XBP1$^{Nes-/-}$ mice and littermate control (XBP1$^{f/f}$) animals using the memory flexibility test (pre-injection). The average number of trials to reach criterion is presented. After training, the same animals were injected with AAV/BDNF-GFP into the hippocampus using bilateral stereotaxis. Fourteen days after injection, animals were re-trained and tested using the memory flexibility test (XBP1$^{f/f}$, n = 4; and XBP1$^{Nes-/-}$, n = 3). Statistical analysis was performed using Student's t test.

(C) Electrophysiological recordings of LTP were performed in hippocampal slices from the same groups of mice shown in (B) (n = 20 slices from three XBP1$^{Nes-/-}$ mice; 21 slices from three XBP1$^{f/f}$ mice; 22 slices from three XBP1$^{Nes-/-}$ AAV/BDNF-GFP mice; 28 slices from four XBP1$^{f/f}$ mice). Statistical analysis was performed using two-way ANOVA. R.U., relative units.

(D) Basal synaptic transmission recorded in hippocampal slices from animals injected with AAV/BDNF-GFP (n = 26 slices from three XBP1$^{Nes-/-}$ mice; 36 slices from three XBP1$^{f/f}$ mice; 31 slices from 3 XBP1$^{Nes-/-}$ AAV/BDNF-GFP mice; 39 slices from four XBP1$^{f/f}$ AAV/BDNF-GFP mice). Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test.

Data are presented as mean and SEM. *$p < 0.05$; ***$p < 0.001$; n.s., non-significant.

See also Figures S4 and S5.

to BDNF deficiency; however, we cannot rule out additional consequences of targeting XBP1 in the nervous system.

Using gain- and loss-of-function approaches, we demonstrated that XBP1 is required for the optimal establishment of long-term hippocampus-dependent memories. This unexpected function may have an evolutionary origin, since XBP1 is a member of the ATF/CREB superfamily of transcription factors. Indeed, early studies from our group revealed that XBP1 preferentially binds to and transactivates CRE-like sequences containing an ACGT core (Clauss et al., 1996). CREB is a key transcription factor required for long-lasting modulation of synaptic plasticity and memory (Alberini, 2009). XBP1s may act in concert with CREB to induce memory formation and may oppose the repressor activity of ATF4 (Costa-Mattioli et al., 2005, 2007), another closely related transcription factor implicated in the adaptive response to ER stress (Walter and Ron, 2011). The eIF2α and ATF4 pathway has been associated with negative control of long-term memory formation due to its inhibitory activity on protein synthesis and CREB expression, respectively (Costa-Mattioli et al., 2007, 2009). Even though we did not observe significant activation of eIF2α phosphorylation or upregulation of ATF4 levels in the hippocampus of

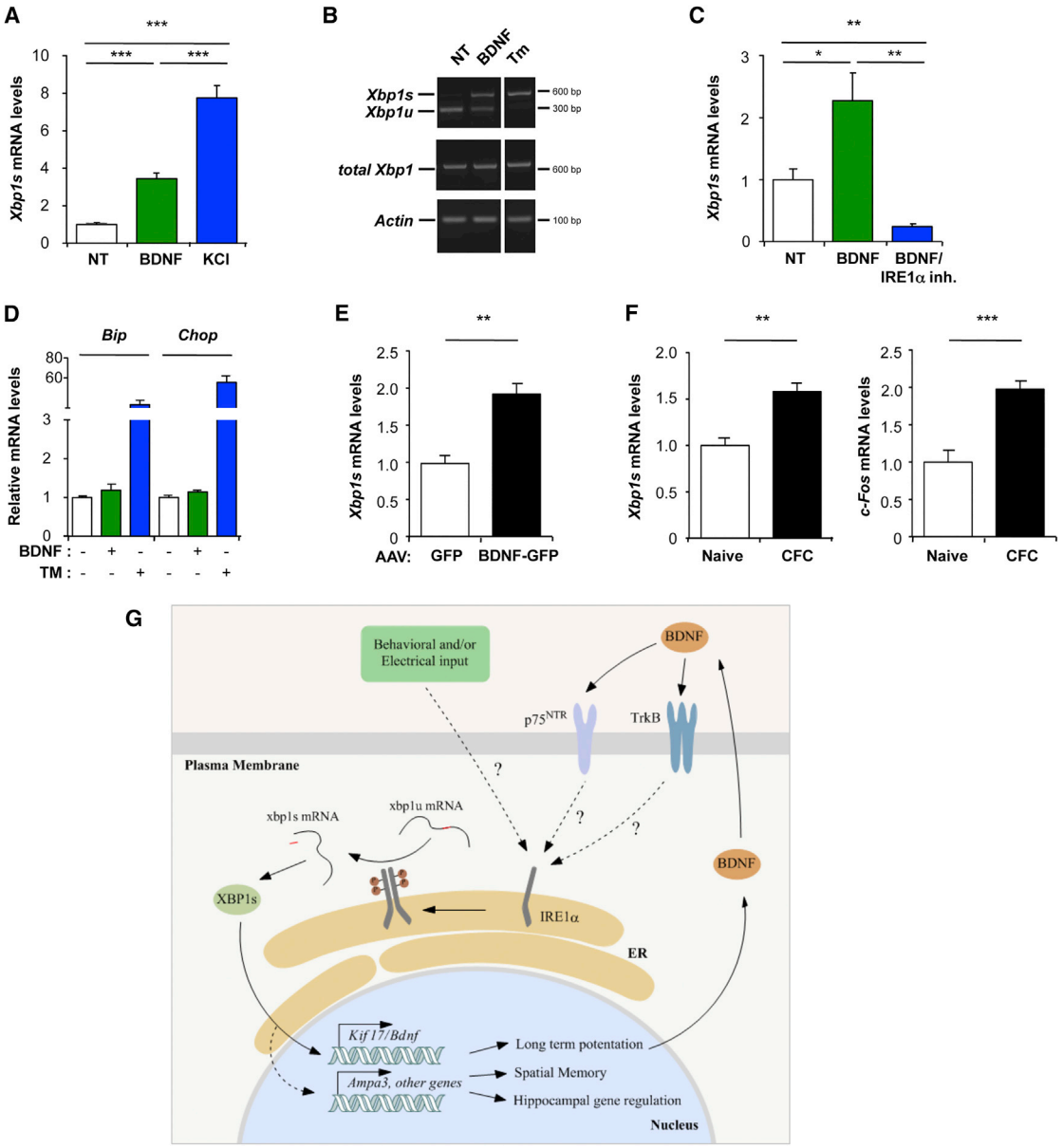

Figure 6. Regulation of XBP1 mRNA Splicing by BDNF

(A) *Xbp1s* mRNA levels were measured by real-time PCR in cortical neuron primary cultures treated with recombinant BDNF (100 ng/ml) or KCl (15 mM) for 16 hr (n = 3). Statistical analysis was performed using Student's t test (***p < 0.001). NT, not treated.

(B) XBP1 mRNA splicing was determined by RT-PCR using a PstI-based assay in mouse hippocampal primary cultures treated with recombinant BDNF (100 ng/ml) for 16 hr. PCR fragments corresponding to the spliced *Xbp1 (Xbp1s*) and unspliced *Xbp1* (*Xbp1u*) mRNA are indicated. As control, cultures were also treated with 10 μg/ml of tunicamycin (Tm). Bands were spliced from the same gel under identical film exposure.

(C) *Xbp1s* mRNA levels were measured by real-time PCR in cortical neuron primary cultures treated with recombinant BDNF in the presence or absence of 50 μM of an IRE1α pharmacological inhibitor (inh.) (n = 3). Statistical analysis was performed using Student's t test.

(D) mRNA levels of the ER stress-target genes *Bip* and *Chop* were determined in samples shown in (B) using real-time PCR. TM, tunicamycin.

(E) Wild-type mice were injected with AAV/GFP or AAV/GFP-BDNF particles into the hippocampus using brain stereotaxis. Two weeks after injection, *Xbp1s* mRNA levels were measured by real-time PCR in total cDNA obtained from dissected hippocampus (n = 3 per group). Expression values were normalized to β-actin levels. Statistical analysis was performed using Student's t test.

(F) *Xbp1s* (left panel) and *c-Fos* (right panel) mRNA levels were measured by real-time PCR in total cDNA obtained from the hippocampus of wild-type mice after 3 hr of testing in the contextual fear-conditioning assay (CFC). Expression values were normalized to β-actin mRNA levels and expressed as fold change with respect to naive mice (naive, n = 5; post-test, n = 10). Statistical analysis was performed using Mann-Whitney U test.

(G) Working model: expression of XBP1 in hippocampal neurons directly or indirectly (dotted lines) controls the expression of different genes involved in the establishment of memory and other cognitive processes. Direct regulation of BDNF expression occurs through the binding of XBP1 to an UPRE B binding site

(legend continued on next page)

XBP1-deficient animals, the opposing interplay of both pathways during neural plasticity needs further elucidation.

XBP1 deficiency in the nervous system did not result in significant alterations in the basal levels of canonical UPR-target gene expression. Instead, our results suggest that XBP1 has a unique role in the brain under physiological conditions that is unrelated to its identity as a key ER stress protein. This result is consistent with recent evidence that XBP1-mediated transcription is context dependent (Cornejo et al., 2013; Hetz et al., 2015). XBP1s physically interacts with different protein partners, establishing distinct transcriptional programs. For example, XBP1s forms heterodimers with other transcription factors of the same superfamily, such as ATF6f (activating transcription factor 6 fragment), to regulate specific gene expression patterns (Shoulders et al., 2013), whereas in the liver, XBP1s specifically controls genes involved in lipid and cholesterol synthesis (Lee et al., 2008). We also reported that, in the context of cancer, XBP1s physically interacts with HIF1, driving the expression of genes involved in angiogenesis (Chen et al., 2014). Additionally, XBP1 represses the expression of the transcription factor FOXO1, regulating glucose metabolism (Zhou et al., 2011) and autophagy in a model of Huntington's disease (Vidal et al., 2012). Based on this evidence, we speculate that XBP1s may control the expression of memory-related genes in the hippocampus through the interaction with specific transcription factors and activity-dependent signaling pathways, a hypothesis that we are currently investigating through interactome studies.

BDNF signaling has at least two modalities: one leading to transient phosphorylation of TrkB and short-lived downstream messaging and a second sustained mode that chronically activates TrkB phosphorylation and leads to enhanced neurite branching and structural changes associated with long-term plasticity (Park and Poo, 2013). One explanation for this dual dynamics is the self-amplification of BDNF signaling through an autocrine-positive feedback (Cheng et al., 2011). We speculate that the activation of the IRE1α/XBP1 signaling module in neurons may operate as an amplification loop reinforcing BDNF signaling to fine-tune synaptic plasticity and learning and memory-related processes (see model in Figure 6G). We are currently investigating the downstream signaling events that engage IRE1α activation by BDNF or electrical stimulation in neurons, which may occur through posttranslational modifications of IRE1α as reported in other systems (Hetz et al., 2015). Our data, together with the discovery of a polymorphism in the XBP1 promoter associated with bipolar disorder (Kakiuchi et al., 2003), schizophrenia (Kakiuchi et al., 2004), and Alzheimer's disease (Liu et al., 2013), suggest that alterations in XBP1 function may lead to the development of serious cognitive disorders in humans. Strategies to enhance XBP1s activity in specific areas of the brain, such as gene therapy, may thus translate into beneficial effects in the context of cognitive impairment associated with prevalent diseases such as Alzheimer's disease.

EXPERIMENTAL PROCEDURES

Behavioral Tests

Animal care and experimental protocols were performed according to procedures approved by the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996), the Institutional Review Board's Animal Care and Use Committee of the Harvard School of Public Health (approved animal protocols 04137 and 04567), the Bioethical Committee of the Faculty of Medicine, University of Chile (protocol number CBA0503 FMUCH), and the Bioethical Committee of the Pontifical Catholic University of Chile.

Behavioral experiments were performed in a blinded fashion, using groups of age-matched male mice. For contextual and cued fear conditioning: on the first day, mice were allowed to habituate in the contextual fear-conditioning chamber (Med Associates) for 5 min. After 24 hr, animals were trained for 2 min and then exposed to 80 db of white noise (conditioned stimulus; CS) for 30 s. After 2 s, mice were exposed to a 0.5-mA electric shock (unconditioned stimulus) that was then repeated five times. On the next day, freezing events were scored for 5 min without CS in the same chamber (contextual) or with CS in a different context (cued) using an automated system. For memory flexibility: mice were trained in a standard Morris water maze for up to 15 trials per day until they learned the location of the hidden platform. After training, the position of the platform in the maze was changed daily. The number of trials to criterion represents the number of trials necessary to learn the new location of the platform each day. The criterion is defined as three consecutive successful trials in an average of 20 s, without any trial over 30 s.

Electrophysiological Measurements

Transverse slices (350 μm) from the dorsal hippocampus and fEPSPs were evoked in CA1 with a bipolar cathodic stimulation to Schaffer collateral fibers. To generate LTP, we used TBS or HFS. TBS consists of four theta epochs with ten trains of four 100-Hz pulses delivered at 5 Hz. HFS consists of three trains of 100-Hz pulses, 500 ms each, with an inter-train interval of 20 s. Recordings were filtered at 2.0–3.0 kHz, sampled at 4.0 kHz using an A/D converter, and analyzed with pClamp 10 (Molecular Devices).

Biochemical Analysis

Western blot, real-time PCR, and molecular biology assays to address the impact of XBP1 on gene regulation and BDNF expression were performed using standard methods.

Statistical Analysis

All data are shown as mean ± SEM. Significance was calculated as indicated in the figure legends using Student's t test, Mann-Whitney test, or two-way ANOVA followed by Bonferroni correction.

Detailed methods are provided in the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.01.028.

AUTHOR CONTRIBUTIONS

Conceptualization, G.M., R.L.V., and C.H.; Methodology, G.M., R.L.V., and C.H.; Investigation, G.M., R.L.V., P.M., F.G.S., A.O.A., P.V., C.W., and P.T.; Resources, B.K., G.M., R.V., P.V., and B.L.S.; Supervision, L.H.G., A.G.P., J.L.V., and N.C.I.; Writing – Original Draft, G.M., P.M., A.O.A., and C.H.; Writing – Review & Editing, G.M., P.M., A.O.A., L.H.G., and C.H.; Project Administration, G.M. and C.H.; Funding Acquisition, L.H.G., A.G.P., J.L.V., N.C.I., and C.H.

located in the proximal *Bdnf* promoter region. Behavioral and/or electrical stimulus may control IRE1α activation. In addition, BDNF could induce IRE1α activation and XBP1s expression, in part, through TrkB or $p75^{NTR}$ signaling.
Data are presented as mean and SEM. *p < 0.05; **p < 0.01; ***p < 0.001; n.s., non-significant.
See also Figure S6.

ACKNOWLEDGMENTS

We thank Andrés Couve, Paola Haeger, Cecilia Hidalgo, Jorge Parodi, and Cristián Sánchez for helpful discussions. We also thank Silke Escobar, Javiera Ponce, Jean Cosme Dodart, and Gemma Casadesus for technical support in animal studies. This work was primarily funded by Millennium Institute no. P09-015-F and Office of Naval Research-Global (ONR-G) N62909-16-1-2003 (C.H.). We also thank FONDAP 15150012, the Frick Foundation, ALS Therapy Alliance 2014-F-059, Muscular Dystrophy Association 382453, CONICYT-USA2013-0003, Michael J Fox Foundation for Parkinson's Research, COPEC-UC Foundation, Ecos-Conicyt C13S02, FONDECYT no. 1140549, and ALSRP Therapeutic Idea Award AL150111 (C.H.), FONDECYT no. 3150637 (G.M.), and FONDECYT no. 3140388 (P.M.). We also received funding from FONDECYT no. 1150608 (R.L.V.); FONDECYT no. 3130759 (A.O.A.); and FONDECYT no. 1140162 (B.K.). The Centro de Estudios Científicos is funded by the Centers of Excellence Basal Financing Program of CONICYT (B.K.), a CONICYT doctoral scholarship (P.V. and F.G.S.), the Swiss National Science Foundation, Sinergia grant 147660 (B.L.S.), Iniciativa Cientifica Milenio ICM-P10-001-F (J.L.V.), and Iniciativa Cientifica Milenio ICM-P09-022-F (A.O.A. and A.G.P.). N.C.I. was supported by Basal Center of Excellence in Aging and Regeneration grant CONICYT-PFB12/2007 and FONDECYT 1120156. L.H.G. was supported by the NIH and a gift from an anonymous foundation.

Received: July 20, 2015
Revised: November 2, 2015
Accepted: January 5, 2016
Published: February 4, 2016

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