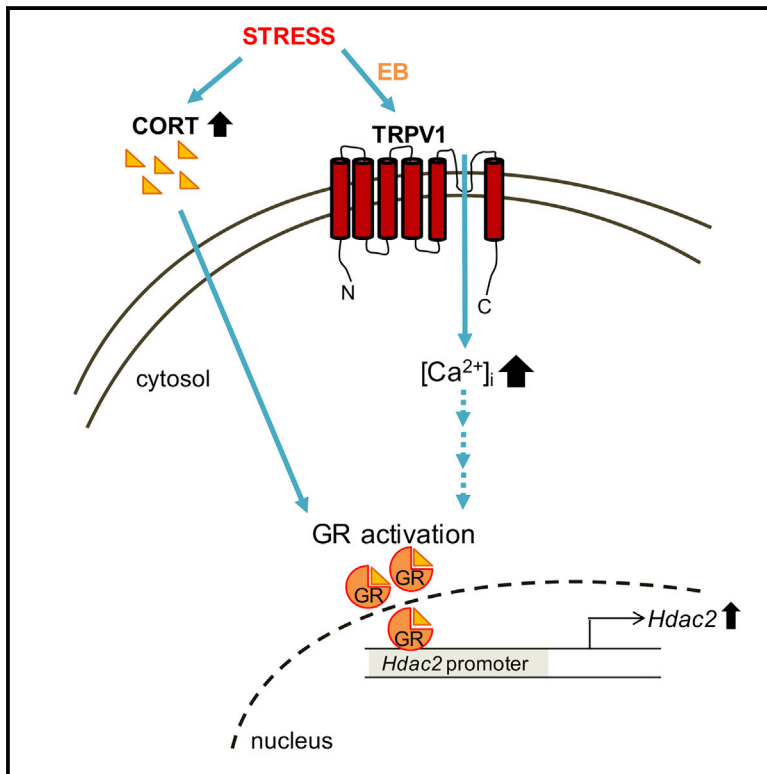


TRPV1 Regulates Stress Responses through HDAC2

Graphical Abstract



Authors

Sung Eun Wang, Seung Yeon Ko, Sungsin Jo, ..., Yong-Seok Kim, Sung Jun Jung, Hyeon Son

Correspondence

hyeonson@hanyang.ac.kr

In Brief

Wang et al. show that *Trpv1*^{-/-} mice are more stress resilient than control mice following chronic unpredictable stress and these are associated with reduced histone deacetylase (HDAC) 2 expression and activity. As a consequence, HDAC2-regulated, cell-cycle- and neuroplasticity-related molecules are altered in *Trpv1*^{-/-} mice. Their findings indicate that HDAC2 is a molecular link between TRPV1 activity and stress responses.

Highlights

- TRPV1 deficiency increases resilience to acute and chronic stress
- *Trpv1*^{-/-} mice exhibit reduced recruitment of GR to the *Hdac2* promoter
- TRPV1 deficiency stimulates hippocampal neurogenesis
- Blocking TRPV1 during stress provides a beneficial effect via chromatin regulation



TRPV1 Regulates Stress Responses through HDAC2

Sung Eun Wang,¹ Seung Yeon Ko,¹ Sungsin Jo,² Miyeon Choi,¹ Seung Hoon Lee,¹ Hye-Ryeong Jo,¹ Jee Young Seo,¹ Sang Hoon Lee,¹ Yong-Seok Kim,^{1,3} Sung Jun Jung,^{1,4} and Hyeon Son^{1,3,5,*}

¹Graduate School of Biomedical Science and Engineering, Hanyang Biomedical Research Institute

²Hanyang University Hospital for Rheumatic Diseases

³Department of Biochemistry and Molecular Biology, College of Medicine

⁴Department of Physiology, College of Medicine

Hanyang University, 222 Wangsimni-ro, Seongdong-gu, Seoul 04763, Republic of Korea

⁵Lead Contact

*Correspondence: hyeonson@hanyang.ac.kr

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SUMMARY

Stress causes changes in neurotransmission in the brain, thereby influencing stress-induced behaviors. However, it is unclear how neurotransmission systems orchestrate stress responses at the molecular and cellular levels. Transient receptor potential vanilloid 1 (TRPV1), a non-selective cation channel involved mainly in pain sensation, affects mood and neuroplasticity in the brain, where its role is poorly understood. Here, we show that *Trpv1*-deficient (*Trpv1*^{-/-}) mice are more stress resilient than control mice after chronic unpredictable stress. We also found that glucocorticoid receptor (GR)-mediated histone deacetylase 2 (HDAC) 2 expression and activity are reduced in the *Trpv1*^{-/-} mice and that HDAC2-regulated, cell-cycle- and neuroplasticity-related molecules are altered. Hippocampal knock-down of TRPV1 had similar effects, and its behavioral effects were blocked by HDAC2 overexpression. Collectively, our findings indicate that HDAC2 is a molecular link between TRPV1 activity and stress responses.

INTRODUCTION

Chronic stress causes pathophysiological and psychological alterations associated with brain function, leading to maladaptive responses including clinical depression and anxiety (Koob, 2008; Krishnan and Nestler, 2008). Mounting evidence suggests that acute and chronic stress, especially the stress-induced release of glucocorticoids, alters neurotransmission and decreases adult hippocampal neurogenesis in the mammalian brain, thereby influencing some aspects of stress-induced neuropsychiatric disorders (Popoli et al., 2011). However, it remains unclear how neurotransmission systems orchestrate stress responses at the molecular and cellular levels in the brain.

There is accumulating evidence that glucocorticoids are closely associated with the endocannabinoid (EB) system in the brain. Glucocorticoids rapidly stimulate EB production in the brain, whereupon EBs bind to cannabinoid receptor 1

(CB1) and transient receptor potential vanilloid 1 (TRPV1), resulting in inhibition of neurotransmitter release (Popoli et al., 2011). The TRPV1 channel, which is mainly expressed in primary sensory neurons of the dorsal root and trigeminal ganglia of the peripheral nervous system (Mezey et al., 2000), is thought to extend to many regions of the brain, including hippocampus, amygdala, and hypothalamus (Chávez et al., 2010; Mezey et al., 2000; Cristino et al., 2006; Huang et al., 2014). TRPV1 is expressed in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG), a neurogenic region that contributes to neuroplasticity, in line with its suggested role in psychiatric disorders (Chahl, 2011; Stock et al., 2014). Acute restraint stress increases expression of TRPV1 in the hippocampus, and TRPV1 antagonism reverses the behavioral deficits induced by stress in rats (Socała and Wlaź, 2016). TRPV1 deficiency also has behavioral anxiolytic and antidepressant-like effects (Yu et al., 2009; Marsch et al., 2007). Ca²⁺ influx through TRPV1 activates protein kinase C (PKC) in neurons (Gunthorpe and Chizh, 2009), and calcium-mediated PKC activation in fibroblast cells activates glucocorticoid receptors (GRs) (Basta-Kaim et al., 2002). Although these studies point to a role of TRPV1 in the CNS response to stress, it is unclear how it acts.

Epigenetic regulation is closely involved in the pathophysiology of stress (Tsankova et al., 2007). Class I histone deacetylase (HDAC) 2 controls behavioral adaptations to chronic emotional stress via chromatin modulation (Covington et al., 2009). We focus here on HDAC2 because its expression is tightly regulated by neuronal activity, Ca²⁺, and the GR, and the latter is responsive to glucocorticoids produced under stress (Gräff and Tsai, 2013).

Based on the idea that stress is generally associated with EBs (Morena et al., 2016), we used *Trpv1*^{-/-} mice to investigate whether TRPV1 is involved in stress-induced behaviors. We show that *Trpv1*^{-/-} mice have reduced HDAC2 activity and altered neuronal gene repressor activity, and we demonstrate that this is associated with the presence of HDAC2 in the promoter regions of these genes. Furthermore, the absence of TRPV1 enhances basal hippocampal neurogenesis and has an antidepressant-like effect. Our results imply that TRPV1 loss of function increases histone acetylation and neuronal gene expression and leads to a stress-resilient phenotype, thus implying that TRPV1 regulates stress responses in the brain through epigenetic regulation via HDAC2.



RESULTS

Trpv1^{-/-} Mice Exhibit Enhanced Basal Hippocampal Neurogenesis and Are Less Susceptible to Stress

We obtained *Trpv1*^{-/-} mice and confirmed the absence of TRPV1 protein in their brains (Figures S1A and S1B) at 8 weeks, a peak time of TRPV1 expression in the murine brain (Huang et al., 2014). In addition, activation of TRPV1 with the specific agonist capsaicin (CAP) induced Ca²⁺ influx in the DG granule cells of hippocampal slices obtained from wild-type (WT) mice, but not from *Trpv1*^{-/-} mice (Figures S1C–S1E).

Mice subjected to chronic unpredictable stress (CUS) suffer subsequent neuronal and behavioral deficits, including atrophy of pyramidal neurons and inhibited DG neurogenesis (Russo et al., 2012). We examined the effects of stress on hippocampal neurogenesis in *Trpv1*^{-/-} mice and their WT littermates after exposure to CUS using bromodeoxyuridine (BrdU) to label proliferation in the SGZ, a neuroproliferative region, and scored the viability of neural progenitor cells (NPCs) after 24 hr and a 3-week chase (Figures 1A–1C). We first found that TRPV1 deficiency increased the number of BrdU(+) cells in the SGZ and granular cell layer (GCL) (Figures 1A–1C and S2B) in non-stressed mice, indicating that basal levels of cell proliferation and survival were increased in *Trpv1*^{-/-} mice. CUS reduced the survival of NPCs in the GCL of WT and *Trpv1*^{-/-} mice essentially to the same extent; therefore, remaining survival levels were still higher in *Trpv1*^{-/-} mice than in WT mice (Figures 1D–1F). These results suggest that TRPV1 deficiency enhances the basal hippocampal neurogenesis, consistent with the evidence that loss of TRPV1 expression promotes neurogenesis (Stock et al., 2014) but does not affect CUS-induced reduction of hippocampal neurogenesis.

Stress induces increased activity of the hypothalamus-pituitary gland-adrenal cortex (HPA) axis, which is related to reduced feedback inhibition by endogenous glucocorticoids and can lead to stress-induced disorders (Russo et al., 2012). To explore potential changes in the HPA axis in the *Trpv1*^{-/-} mice, we characterized plasma corticosterone (CORT) concentrations before testing (no behavioral) and 24 hr after exposure to behavioral tests (post-behavioral) in mice experiencing CUS (Figure 1G). Exposure to behavioral tests for 3–4 consecutive days increased basal CORT in the WT mice, but not the *Trpv1*^{-/-} mice, indicating that only the former had developed reduced feedback inhibition of the HPA axis (i.e., HPA-axis deregulation). Furthermore, CUS increased post-behavioral CORT about 2-fold in the WT mice, but not in the *Trpv1*^{-/-} mice. This suggests that *Trpv1*^{-/-} mice are less likely to develop HPA-axis deregulation than WT mice, which is consistent with the finding that inhibiting TRPV1 lowers plasma CORT in stressed mice (Navarria et al., 2014).

We then evaluated whether TRPV1 deficiency affects stress-induced behaviors, as indicated by responsiveness in the forced swim test (FST) and the learned helplessness test (LHT), two widely used models of behavioral despair, and the novelty-suppressed feeding test (NSFT), an animal model of anxiety. The FST measures immobility time after being placed in a tank filled with water from which animals cannot escape. Many groups have used the forced swim stress as an acute stressor (Porsolt et al., 1977). We found that immobility time was halved in the *Trpv1*^{-/-} mice, a typical antidepressant response (Figure 1H).

The *Trpv1*^{-/-} mice also displayed reduced latency to feeding in the NSFT (Figure 1I), consistent with a previous report of anxiolytic behavior in *Trpv1*^{-/-} mice (Marsch et al., 2007). In the LHT, the latency to escape and the number of escape failures showed a tendency to be lower in the *Trpv1*^{-/-} mice than the WT mice (Figures 1J, S1I, and S1J), which is also an indication of antidepressant-like behavior. Using the CUS approach, we also assessed the role of TRPV1 in chronic stress-induced behaviors. In WT mice, CUS caused the expected increase in immobility in the FST and in latency to escape in the LHT, whereas these effects were absent from the *Trpv1*^{-/-} mice (Figures 1H and 1J). The latency to feed in the NSFT was also lower in *Trpv1*^{-/-} mice that experienced CUS than in WT mice (Figure 1I). Altogether, these results demonstrate that *Trpv1*^{-/-} mice are less susceptible to stress states. As in a previous report (Porsolt et al., 1977), there was no effect on spontaneous locomotor activity, including average speed, total distance traveled, and distance traveled from the center or margin, indicating that the effects in the FST were not due to general ambulatory differences (Figures S1F–S1H). There was also no difference between *Trpv1*^{-/-} and WT mice in contextual fear memory (Figure S1K), indicating that the stress-resilient effects of TRPV1 ablation are unlikely to result from a difference in perception of the environment.

HDAC2 Expression and Activity Are Reduced in the Hippocampal DG of *Trpv1*^{-/-} Mice

Because hippocampal neurogenesis, stress-induced behaviors, and gene expression are tightly linked to chromatin remodeling (Franklin et al., 2012), we tested the effect of deletion of TRPV1 on the expression of HDACs in the hippocampal DG and CA1 subregions, in which TRPV1 receptors are highly expressed (Chávez et al., 2010; Mezey et al., 2000; Cristino et al., 2006). The protein levels of HDAC2 and HDAC5 were reduced in the DG, but not in the CA1, of *Trpv1*^{-/-} mice, whereas other HDACs were not affected (Figures 2A, 2B, and S3A). The reduced levels of HDAC2 and HDAC5 seemed to be restricted to the DG: western blots of the prefrontal cortex and whole hippocampal tissue punches contained similar levels of HDAC2 and HDAC5 in *Trpv1*^{-/-} and WT mice (Figures S3B–S3D). It has been previously shown that TRPV1 is involved in GR signaling via EBs (Popoli et al., 2011) and GR activation promotes HDAC2 expression in the brain (Gräff and Tsai, 2013), whereas HDAC5 is not known to be involved in GR signaling. We therefore examined whether the GR was involved in the relationship between TRPV1 deletion and HDAC2 expression. Chromatin immunoprecipitation (ChIP) assays showed that the GR was more enriched in the *Hdac2* promoter region of the WT mice than in that of the *Trpv1*^{-/-} mice (Figure 2C). In agreement with this, the level of HDAC2 mRNA was also higher in the WT mice (Figure 2D).

We next investigated the activities of other class II HDACs. Paralleling the lower HDAC2 and HDAC5 expression found in the TRPV1-deficient DG, we observed reduced activities of these HDACs in the DG of *Trpv1*^{-/-} mice (Figures 2E and 2F). Overall, hippocampal levels of acetylated histone 3 (acH3), acetylated histone 4 (acH4), and histone 4 on lysine (Lys) 8 (acH4K8) were also elevated (Figures 2G and 2H). In particular, we detected increased acetylation on Lys14 and serine (Ser) 10

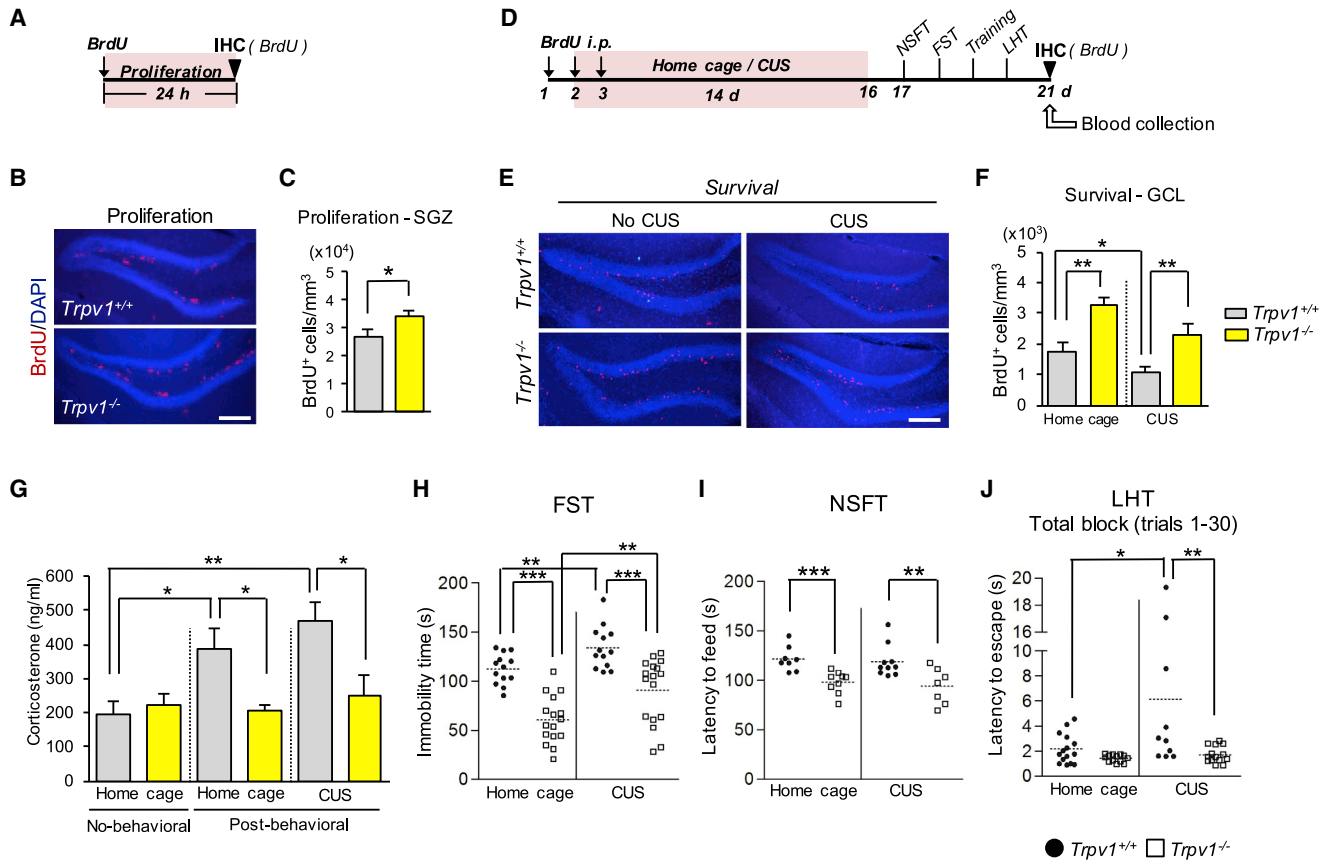


Figure 1. *Trpv1*^{-/-} Mice Display Enhanced Hippocampal Neurogenesis and a Reduced Overall Response to Stress

(A) Experimental design of cell proliferation studies.
 (B) TRPV1 deficiency increased the number of BrdU(+) cells in the SGZ.
 (C) Quantification of BrdU(+) cells (n = 5 per genotype).
 (D) Experimental design of the cell survival studies following CUS for 14 days starting on day 2. BrdU (50 mg/kg) was given three times every 24 hr during an initial period of 3 days. The behavioral performance was measured for 3–4 consecutive days starting on day 17.
 (E) Representative images of DG.
 (F) CUS reduced the survival of NPCs in the GCL of WT and *Trpv1*^{-/-} mice to the same extent. Two-way ANOVA showed the main effects of stress ($F_{1,17} = 7.664$, $p = 0.0132$) and genotype ($F_{1,17} = 22.82$, $p = 0.0002$) but no interaction ($F_{1,17} = 0.2041$, $p = 0.6571$). Significance was determined by unpaired two-tailed t test (n = 5 per group).
 (G) *Trpv1*^{-/-} prevented an increase in plasma CORT after behavioral tests (home-caged WT, n = 6; home-caged *Trpv1*^{-/-}, n = 5; post-behavior tests WT, n = 8; post-behavior tests *Trpv1*^{-/-}, n = 11; CUS WT, n = 7; CUS *Trpv1*^{-/-}, n = 8). Two-way ANOVA (main effects of interaction, $F_{2,39} = 4.306$, $p = 0.0204$; behavior test and stress, $F_{2,39} = 5.365$, $p = 0.0087$; genotype, $F_{1,39} = 11.88$, $p = 0.0014$) was followed by Bonferroni's multiple comparison test.
 (H) FST. *Trpv1*^{-/-} mice had a shorter immobility time than WT mice in the home cage and the stressful condition (n = 13, 16, 13, 17).
 (I) NSFT. *Trpv1*^{-/-} mice had a shorter latency than WT mice in the home cage or under CUS (n = 9, 8, 10, 7).
 (J) LHT. There was no difference between WT and *Trpv1*^{-/-} mice in the home cage, whereas the latency to escape was lower in *Trpv1*^{-/-} mice under CUS (n = 15, 13, 10, 14). Two-way ANOVA (main effects of interaction, $F_{1,48} = 4.874$, $p = 0.0321$; stress, $F_{1,48} = 6.502$, $p = 0.0140$; genotype, $F_{1,48} = 9.438$, $p = 0.0035$) was followed by Bonferroni's multiple comparison test. BrdU (red) and DAPI (blue).
 Data are mean ± SEM. (H and I) Unpaired two-tailed t test. Scale bar, 200 μm. *p < 0.05, **p < 0.01, ***p < 0.001.

phosphorylation of H3 (pSer10-acK14H3), a mitotic marker that has a key role in activating neuronal gene expression (Reul et al., 2009) (Figures 2G and 2H), indicating that mitotic activity is elevated in *Trpv1*^{-/-} mice.

HDAC2 is of particular interest given its reported role in transcriptional repression of cell-cycle-related genes such as p21 in neurogenesis and neuronal differentiation (Jawerka et al., 2010; Marqués-Torrejón et al., 2013; Guan et al., 2009). It is recruited to specific loci by mSin3A and co-repressor element-1

silencing transcription factor (CoREST), two epigenetic regulators of neural cell fates (Qureshi et al., 2010). We therefore examined the association of HDAC2 with these corepressors in *Trpv1*^{-/-} mice and found reduced interaction of HDAC2 with CoREST, but not with mSin3A (Figures 2I and 2J). Because HDAC2 represses gene expression (Guan et al., 2009), this suggests that gene repression in the hippocampus of *Trpv1*^{-/-} mice is further relieved by reduced interaction between HDAC2 and CoREST; this would facilitate the transcription of genes required

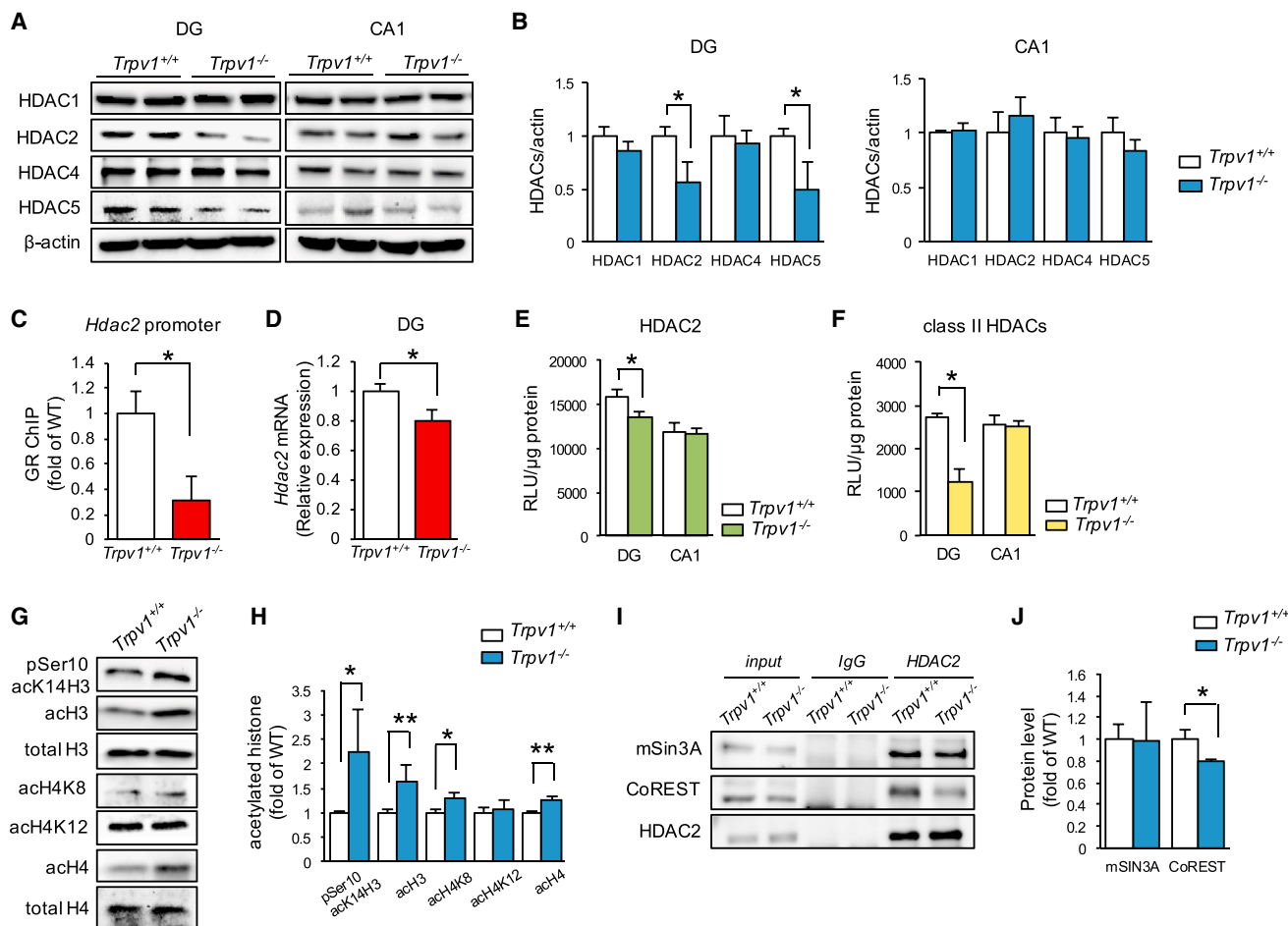


Figure 2. Mice Lacking TRPV1 Express Lower Levels of HDAC2

(A) Representative blots of HDACs in DG and CA1 extracts.

(B) Quantification of (A) showing specific reductions of HDAC2 and HDAC5 in the DG of *Trpv1*^{-/-} mice (WT, n = 4; *Trpv1*^{-/-}, n = 3).

(C) ChIP assays. Decreased binding of GR to the *Hdac2* promoter in the hippocampus of *Trpv1*^{-/-} mice. (WT, n = 3; *Trpv1*^{-/-}, n = 4).

(D) *Hdac2* mRNA levels were decreased in the DG of *Trpv1*^{-/-} mice (n = 3 per genotype).

(E and F) Reduced relative luciferase units (RLUs) of HDAC2 activity (E) and decreased RLU of class II HDAC activity (F) in DG lysates of *Trpv1*^{-/-} mice. Luciferase activities were normalized to protein content (n = 4 independent experiments performed in triplicate).

(G) Representative blots showing histone acetylation in hippocampal histone extracts.

(H) Quantification of (G) showing increased histone modification in *Trpv1*^{-/-} mice. Loading was normalized to total H3 or H4 (n = 3 per genotype).

(I) Representative blots of CoREST and mSin3A following immunoprecipitation of hippocampal extracts with Ab against HDAC2.

(J) Quantification of (I). Protein levels were normalized to inputs. There was less HDAC2 bound to co-repressor complexes in *Trpv1*^{-/-} mice (n = 3 per genotype). Values are means ± SEM (C, D, E, and F) or means ± SD (B, H, and J). Unpaired t test. *p < 0.05, **p < 0.01, ***p < 0.001.

for neuronal functions. Altogether, these results indicate that TRPV1 deficiency lowers GR-mediated HDAC2 expression, which may contribute to stress resilience.

Expression of HDAC2-Regulated Genes Affecting Neurogenesis and Neuroplasticity Is Altered in *Trpv1*^{-/-} Mice

HDAC2 suppresses the expression of genes implicated in neuronal activity and synaptic remodeling by binding to their promoters (Guan et al., 2009). To examine the action of HDAC2 in *Trpv1*^{-/-} mice, we examined the transcription of neurogenesis- and neuroplasticity-related genes (Guan et al., 2009)

by surveying the association of HDAC2 with the promoters of ten genes, including *Fos*, *Creb1*, *Synaptophysin*, *Synapsin2*, *p16*, *p21*, and *p57*, by chromatin immunoprecipitation (ChIP). Cyclin-dependent kinase inhibitors (CDKis) involved in adult neurogenesis (Schmetsdorf et al., 2005)—p16, a member of the Ink4 family, and p21, a member of the Cip/Kip family—were studied. HDAC2 was more highly enriched in the promoters of *p16* and *p21* in hippocampal preparations from *Trpv1*^{-/-} mice than in those from WT mice (Figure 3A). Because HDAC2 is linked to repression of the activities of specific promoters, these results are consistent with the reduction of p16 and p21 mRNAs and proteins in the DG (Figures 3D, 3E, and S3E). Furthermore, levels

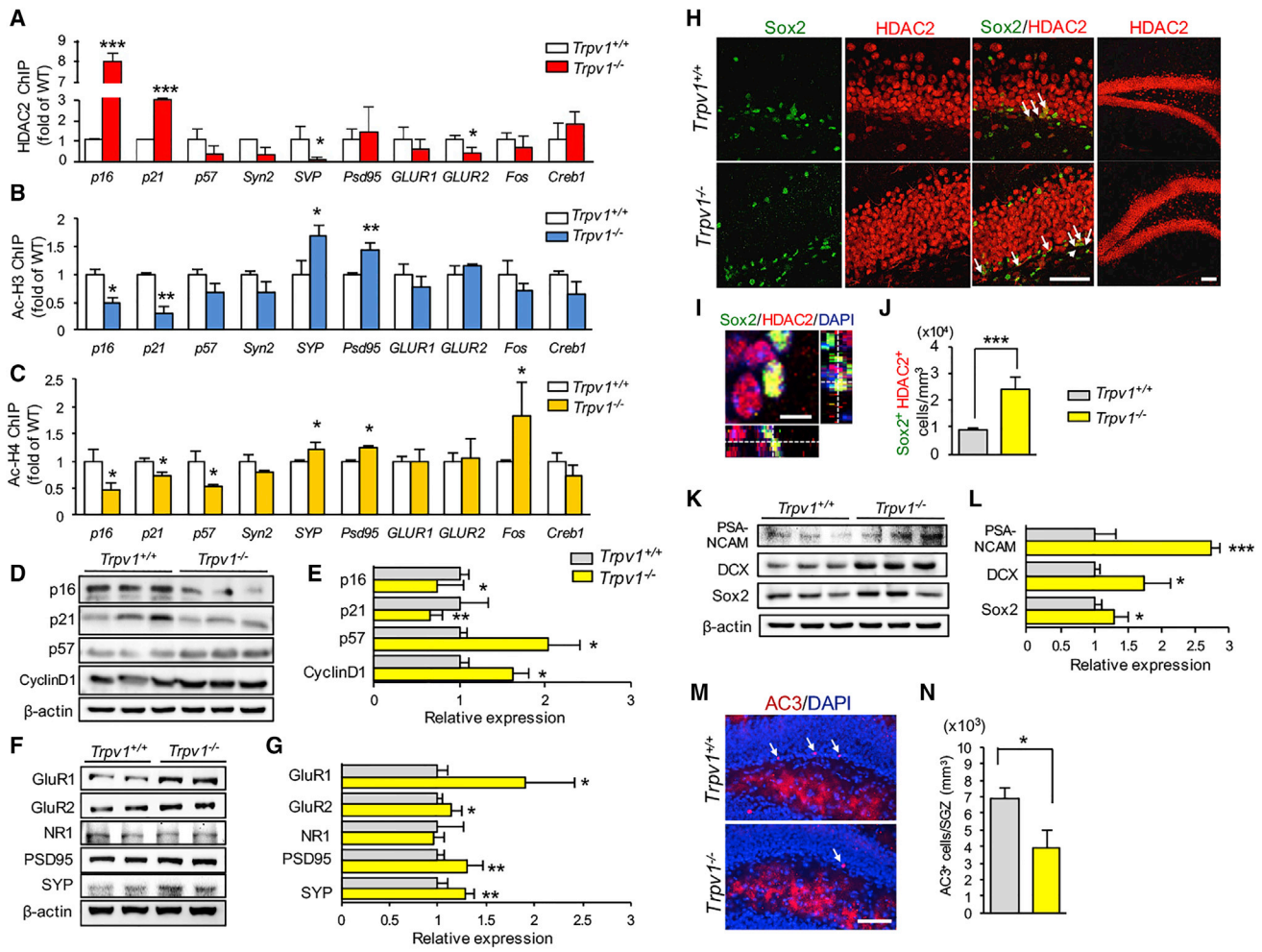


Figure 3. TRPV1 Reduces Expression of Neurogenesis- and Plasticity-Related Genes via HDAC2 in the Hippocampus

(A) ChIP assays. Effects of TRPV1 deficiency on association of HDAC2 with gene promoters. Increased binding of HDAC2 to the *p16* and *p21* promoters and decreased binding to the *Synaptophysin* (*SYP*) and *GLUR2* promoters in the hippocampus of *Trpv1*^{-/-} mice (n = 3 per genotype). (B) ChIP assays. Effect of TRPV1 deficiency on association of acH3 with gene promoters (n = 3 per genotype). (C) ChIP assays. Effect of TRPV1 deficiency on association of acH4 with gene promoters (n = 3 per genotype). (D) Representative blots in DG extracts. (E) Quantification of the experiments in (D), showing decreased levels of CDKis in the DG of *Trpv1*^{-/-} mice (n = 4 per genotype). (F) Representative blots of synaptic molecules. (G) Quantification of the results in (F). *GluR1*, *GluR2*, *PSD95*, and *SYP* protein levels are elevated in the DG of *Trpv1*^{-/-} mice (n = 5 per genotype). (H) Representative confocal images of NPCs in the DG, immunostained for Sox2 (green) and HDAC2 (red). (I) High-power confocal image of a cell (arrowhead in H) co-expressing Sox2 (green) and HDAC2 (red) and stained for DAPI (blue). The bottom and right panels show images merged across the x and z axes. (J) The number of Sox2(+) and HDAC2(+) cells (n = 3 per genotype). (K) Representative western blot of neurogenesis-related proteins in DG. (L) Quantification of experiments in (K) showing increased levels of NPC markers in the DG of *Trpv1*^{-/-} mice (n = 4 per genotype). (M) Immunoreactivity of AC3. (N) AC3(+) cell counts in the SGZ (n = 3 per genotype).

Values represent means \pm SEM of three or four independent experiments performed in triplicate (A, B, C, J, and N) and means \pm SD (E, G, and L). Scale bars, 50 μ m (H and K) and 15 μ m (I). Unpaired t tests. *p < 0.05, **p < 0.01, ***p < 0.001.

of acH3 and acH4 were reduced in the *p16* and *p21* promoters of the *Trpv1*^{-/-} mice (Figures 3B and 3C). The CDKi p57, which promotes cell-cycle exit and neuronal differentiation of NPCs in the CNS (Tury et al., 2012), was elevated in *Trpv1*^{-/-} mice (Figures 3D and 3E). These results suggest that although HDAC2

is downregulated in its expression and activity, its increased binding to the promoters of cell-cycle inhibition genes accounts for the enhanced neurogenesis observed in *Trpv1*^{-/-} mice.

Next, we examined the association of chromatin factors with the promoters of neuroplasticity-related genes. We observed

less enrichment of HDAC2 at the promoters of *GLUR2* and *Synaptophysin* in *Trpv1*^{-/-} mice than in WT mice (Figure 3A), with a concomitant increase in GluR2 and Synaptophysin mRNAs and proteins in the hippocampus (Figures 3F, 3G, S3E, and S3F). Increased acH3 and acH4 were observed in the promoter regions of *Synaptophysin* and *Psd95* (Figures 3B and 3C), consistent with the upregulated mRNA and protein levels (Figures 3F, 3G, S3E, and S3F). HDAC2 was similarly enriched in the promoter of *GLUR1*, a post-synaptic ion channel involved in depolarization (Chávez et al., 2010), in the WT compared with the *Trpv1*^{-/-} mice (Figure 3A), as were *GluR1* mRNA levels (Figure S3F). However, GluR1 protein level was elevated in the hippocampi of *Trpv1*^{-/-} mice (Figures 3F and 3G), possibly pointing to stabilization of the GluR1 receptors (Chávez et al., 2010). Altogether, these results indicate that loss of TRPV1 function affects the expression of cell-cycle proteins and synaptic molecules.

The preceding results demonstrate that selective deletion of the gene coding for the TRPV1 channel is associated with increased HDAC2-mediated expression of cell-cycle- and differentiation-related molecules expected to enhance neurogenesis in response to stress. We further analyzed changes in adult neurogenesis thought to involve HDAC2 (Jawerka et al., 2010). It has been reported that p21 binds directly to a Sox2 enhancer and negatively regulates Sox2 expression in neural stem cells (NSCs) (Marqués-Torrejón et al., 2013). We investigated whether the decreased expression of p21 due to HDAC2 elevates expression of Sox2 in the DG. The number of those Sox2(+) cells in *Trpv1*^{-/-} mice was almost 2.5-fold higher than in WT mice, confirming that TRPV1 deficiency results in the expansion of NPCs (Figures 3H–3J).

Polysialylated-neural cell adhesion molecule (PSA-NCAM) and doublecortin (DCX), both progenitor cell markers (Hanson et al., 2011), were more highly expressed in the hippocampal DG of *Trpv1*^{-/-} mice than that of WT mice (Figures 3K, 3L, S2F, and S2G), confirming that newly generated neurons increased in the *Trpv1*^{-/-} mice. Because TRPV1 activation induces apoptosis in the adult hippocampus (Kong et al., 2010), we investigated whether apoptosis is altered in *Trpv1*^{-/-} mice by examining the expression of activated caspase-3 (AC3) (Walker et al., 2015). The DG of *Trpv1*^{-/-} mice contained less AC3 (–43%) than did that of WT mice (Figures 3M and 3N), implying a neuroprotective effect of TRPV1 deletion. This effect and the increased multiplication of NPCs may combine to enhance hippocampal neurogenesis in *Trpv1*^{-/-} mice.

These results suggest that HDAC2-mediated neuroplasticity and neurogenic molecules are elevated in the adult hippocampus of *Trpv1*^{-/-} mice and are consistent with the notion that *Trpv1*^{-/-} mice have greater DG long-term potentiation (LTP) (Chávez et al., 2010) and learning ability (Stock et al., 2014).

Knockdown of TRPV1 in the DG Mimics the Phenotype of *Trpv1*^{-/-} Mice

To see whether knockdown of TRPV1 has similar effects to deletion of TRPV1, we infused lenti-shTRPV1 into the hippocampal DG of WT mice to reduce TRPV1 expression (Figure 4A). TRPV1 expression was markedly attenuated at both mRNA and protein levels (Figure 4B). There was an obvious increase

in the number of BrdU(+) cells in the DG of these mice (Figures 4C and 4D), pointing to enhanced survival of newly generated cells. If TRPV1 knockdown in neurons is associated with reduced HDAC2, lenti-shTRPV1 should lower the expression of HDAC2. HDAC2, p16, and p21 expression were clearly reduced, while expression of Sox2, DCX, CyclinD1, and GluR1 was elevated (Figures 4E, 4F, and S4A). Consistent with the involvement of TRPV1 in neuroplasticity and the upregulated expressions in *Trpv1*^{-/-} mice, the protein and mRNA levels of GluR2, PSD95, and Synaptophysin and the number of synaptic puncta were elevated in the cells expressing lenti-shTRPV1 (Figures 4G and 4H). At the behavior level, in the non-stress condition, lenti-shTRPV1-infused mice had a reduced immobility time in the FST (Figure 4I) and a shorter latency to escape during the initial block of LHT (Figure 4J). This short-term attenuation of TRPV1 was not sufficient to produce behavioral changes in the NSFT, and total locomotor activity and anxiety were unchanged (Figures S4B–S4E). Animals infused with lenti-shTRPV1 and then receiving CUS displayed antidepressant-like behaviors in the FST and during the initial block of LHT, but not in the NSFT (Figures 4I, 4J, and S4E). These findings are consistent with antidepressant-like phenotype of *Trpv1*^{-/-} mice; they indicate that the antidepressant-like effects seen in *Trpv1*^{-/-} mice are not due to a developmental compensation mechanism and suggest that TRPV1 deficiency in the hippocampus has a critical role in protection against stress-induced behavioral responses.

Capsaicin, a TRPV1 Agonist, Upregulates HDAC2 and Enhances Susceptibility to Stress

Our results indicate that multiple facets of HDAC2 function are altered in *Trpv1*^{-/-} mice. If TRPV1 deficiency affected HDAC2 downregulation, pharmacological activation of TRPV1 with the specific agonist CAP would likely upregulate HDAC2 levels and enhance susceptibility to stress in animal models of behavioral despair. We found that CAP alone increased HDAC2 levels in a control group infused with control virus (Figures 5A–5C). CAP also reduced expression of GluR2 and PSD95, as well as spine density in the DG, and this reduction was prevented by knockdown of HDAC2 (Figures 5D–5G). Consistent with this, CAP increased immobility time and the latency to escape in the FST and LHT, respectively, both of which were blocked by HDAC2 knockdown (Figures 5H and 5I). Lenti-shHDAC2 did not influence locomotor activity in WT mice (Figures S5A–S5C). Resiniferatoxin (RTX), a synthetic TRPV1 agonist, also increased HDAC2 expression (Figure S5I) and produced depression-like behaviors in the FST, which were blocked by lenti-shHDAC2 (Figures S5D–S5H). The protein level of PSD95 was decreased in the DG of mice injected with RTX, and this was prevented by lenti-shHDAC2 (Figures S5I and S5J). These results are consistent with the view that reduced TRPV1 activity is associated with antidepressant-like effects and show that at least part of the TRPV1 activity-dependent behavior is mediated through HDAC2.

To further demonstrate the involvement of HDAC2 in the behavioral effects of TRPV1 deletion, we compared the behaviors of *Trpv1*^{-/-} and WT mice overexpressing HDAC2 in the FST. We subcloned mouse *HDAC2* cDNA into a lenti-mCherry vector (lenti-HDAC2-mCherry) (Figure 5J) and confirmed that

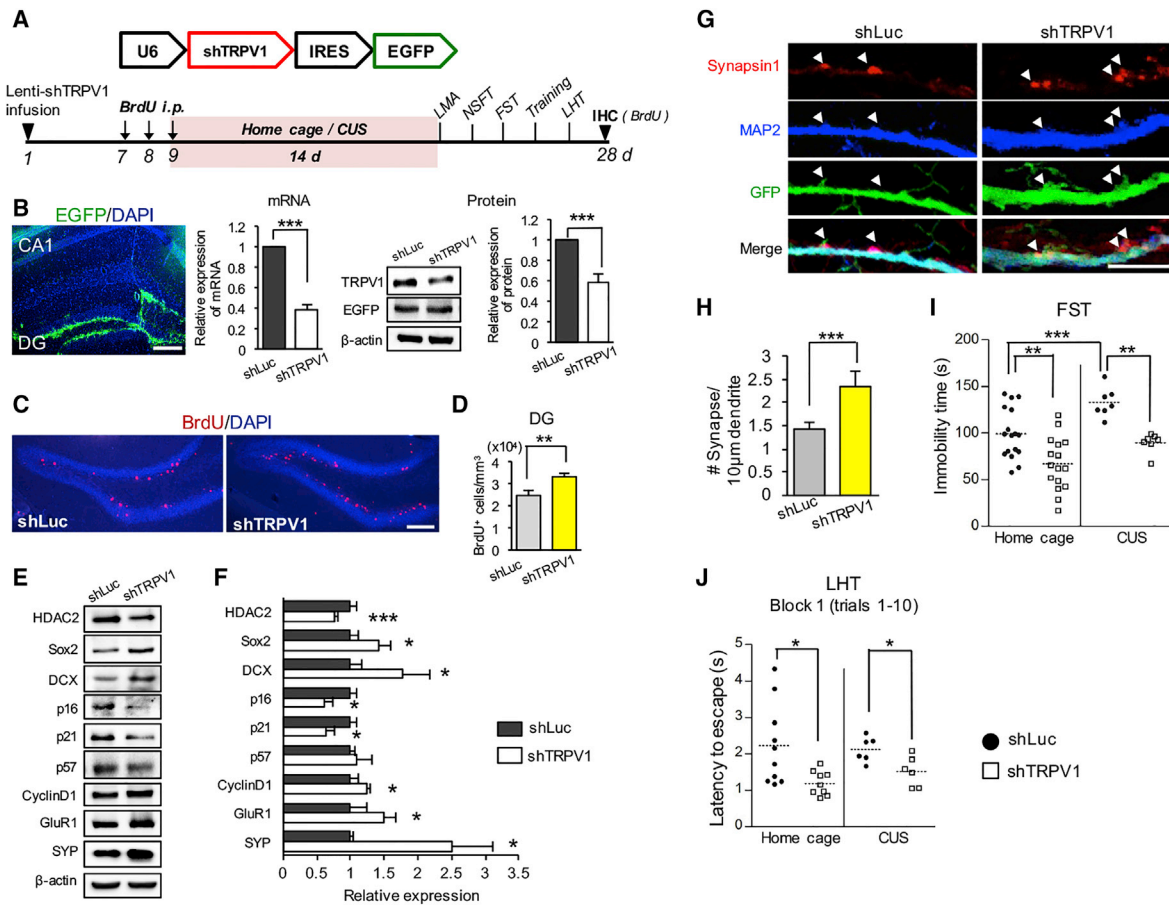


Figure 4. Knockdown of TRPV1 in the DG Produces Stress Resilience and Enhances Hippocampal Neurogenesis

(A) Targeting strategy for deleting TRPV1 mRNA. The mouse U6 promoter was used to drive the expression of TRPV1-shRNA (upper). Experimental design (lower).

(B) Localization of the lentivirus infection in the DG by GFP staining (left). Mouse TRPV1 and β -actin mRNA levels were quantified by qPCR (n = 6 per group) (middle), and TRPV1 protein was quantified by western blotting (n = 5 per group) (right).

(C) Representative images of DG. BrdU (red) and DAPI (blue).

(D) Quantification of BrdU(+) cells in (C) (n = 6 per group).

(E) Representative blots of DG lysates.

(F) Quantification of the results in (E) (n = 3 per group).

(G) Triple-immunostaining of lenti-shLuc (shRNA luciferase gene) or lenti-shTRPV1 infected neurons with anti-synapsin1, anti-MAP2 (microtubule-associated protein 2), and anti-GFP antibodies.

(H) Quantification of the number of synapsin1(+) MAP2(+) synaptic puncta per 10 μ m of dendrite in lenti-shRNA-infected neurons (n = 15).

(I) FST. Animals infused with lenti-shTRPV1 displayed antidepressant-like behaviors in the FST in both non-stress and CUS conditions compared with animals infused with control virus (n = 17, 16, 7, 7 per group).

(J) LHT. Lenti-shTRPV1-infused mice had a shorter latency to escape than animals infused with control virus in non-stress and CUS conditions during trials 1–10 (n = 10, 9, 6, 6).

(B, D, F, and H) Unpaired one-tailed t test. Data are mean \pm SEM. (I and J) Unpaired two-tailed t test. Scale bars, 100 μ m (B), 200 μ m (C), and 10 μ m (G). *p < 0.05, **p < 0.01, ***p < 0.001.

HDAC2 protein was overexpressed (Figure S6A). Then we infected the DG bilaterally with the viral vector (Figure S6B). HDAC2 activity was increased in DG extracts of lenti-HDAC2-mCherry-infused mice (Figure 5K), and lenti-HDAC2-mCherry blocked the decrease in the immobility time in the FST in *Trpv1*^{-/-} mice, indicating that HDAC2 overexpression overcomes the antidepressant-like effects of TRPV1 deletion (Figure 5L). In the last one-third block (trials 21–30) of LHT, lenti-HDAC2-mCherry attenuated the stress-resilient phenotype

of *Trpv1*^{-/-} mice, but it did not influence locomotor activity in the WT and *Trpv1*^{-/-} mice (Figures S6C–S6F). These behavioral results are consistent with the finding that lenti-HDAC2-mCherry induced a decrease in the protein levels of GluR2 and PSD95 in *Trpv1*^{-/-} mice (Figures 5M and 5N). However, protein levels of p16 and CyclinD1, cell-cycle proteins, were not altered by lenti-HDAC2-mCherry in *Trpv1*^{-/-} mice. Collectively, these results indicate that at least part of the stress resilience resulting from TRPV1 deletion or reduction is due to

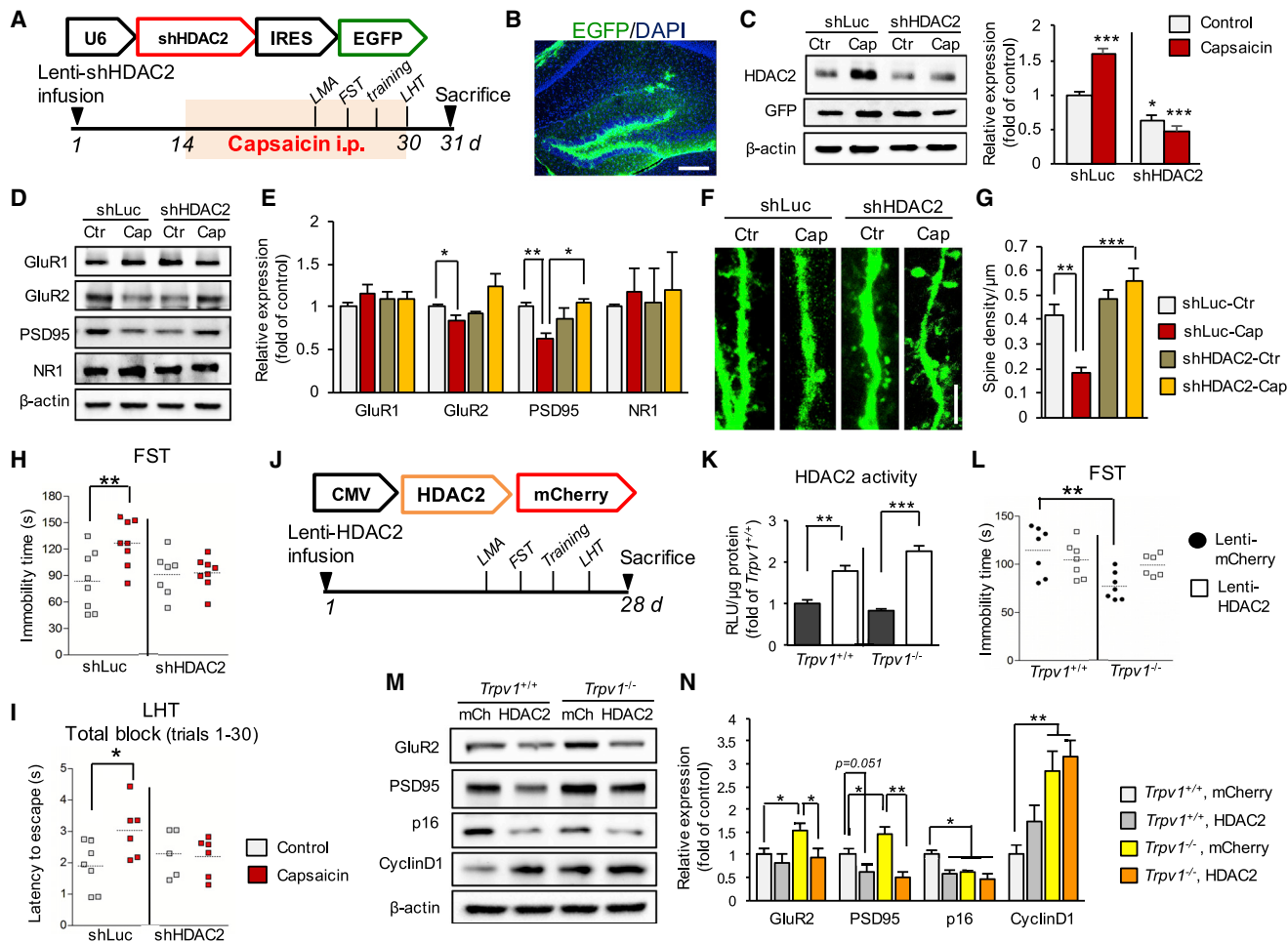


Figure 5. Capsaicin, a TRPV1 Agonist, Upregulates HDAC2 and Enhances Susceptibility to Stress

(A) Targeting strategy for blocking HDAC2 mRNA synthesis. The mouse U6 promoter was used to drive the expression of HDAC2-shRNA (upper). Experimental design (lower).

(B) GFP staining demonstrates the localization of the lentivirus infection of the DG.

(C) To assess the efficiency of lenti-shHDAC2, HDAC2 and β -actin protein levels were analyzed in lysates of the lenti-shHDAC2-infused DG by western blotting (n = 4 per group). Two-way ANOVA (main effect of interaction, $F_{1,12} = 28.4$, $p = 0.0002$; drug, $F_{1,12} = 9.999$, $p = 0.0082$; virus, $F_{1,12} = 117.3$, $p < 0.0001$) followed by Bonferroni's multiple comparison test compared lenti-shLuc-infused with WT mice.

(D) Representative blots of synaptic molecules in the DG.

(E) Quantification of the results in (D). GluR2 and PSD95 protein levels were decreased in the DG of WT mice treated with CAP compared to control (n = 2–4 per group).

(F) Representative images of GFP(+) dendrites of granular cell layers (GCs).

(G) Quantification of dendritic spine density of the GCs shown in (F) (16–20 neurons from three or four animals per group). Two-way ANOVA (main effect of interaction, $F_{1,67} = 14.31$, $p < 0.0003$; drug, $F_{1,67} = 4.281$, $p = 0.0424$; virus, $F_{1,67} = 29.34$, $p < 0.0001$) followed by Bonferroni's multiple comparison test.

(H) FST. WT mice treated with CAP had a longer immobility time than mice treated with vehicle (n = 8, 8, 7, 8 per group). Two-way ANOVA (main effect of interaction, $F_{1,27} = 4.73$, $p = 0.038$; drug, $F_{1,27} = 5.552$, $p = 0.026$) was followed by Bonferroni's multiple comparison test.

(I) Lenti-shHDAC2-infused WT mice had a shorter latency to escape than WT mice exposed to CAP in trials 1–10 (n = 7, 6, 5, 6 per group).

(J) Targeting strategy for overexpressing HDAC2 protein. Lentiviral vector containing the cytomegalovirus (CMV) promoter was used to drive the expression of HDAC2 mRNA (upper). Experimental design (lower).

(K) RLU of HDAC2 activity were elevated in DG lysates of lenti-HDAC2-mCherry-infused mice. Luciferase activities were normalized to protein content. (n = 5 per group).

(L) Lenti-HDAC2-mCherry-infused $Trpv1^{-/-}$ mice had a longer immobility time than lenti-mCherry-infused $Trpv1^{-/-}$ mice (n = 7, 7, 7, 6 per group). Two-way ANOVA (main effect of interaction, $F_{1,23} = 4.947$, $p = 0.0362$; genotype, $F_{1,23} = 8.957$, $p = 0.0065$) was followed by Bonferroni's multiple comparison test.

(M) Representative blots of GluR2, PSD95, p16, and CyclinD1 proteins in DG extracts.

(N) Quantification of the results in (M) (n = 3–4 per group).

(E, I, K, and N) Unpaired two-tailed t test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Scale bars, 100 μm (B) and 10 μm (F). Values represent mean \pm SEM.

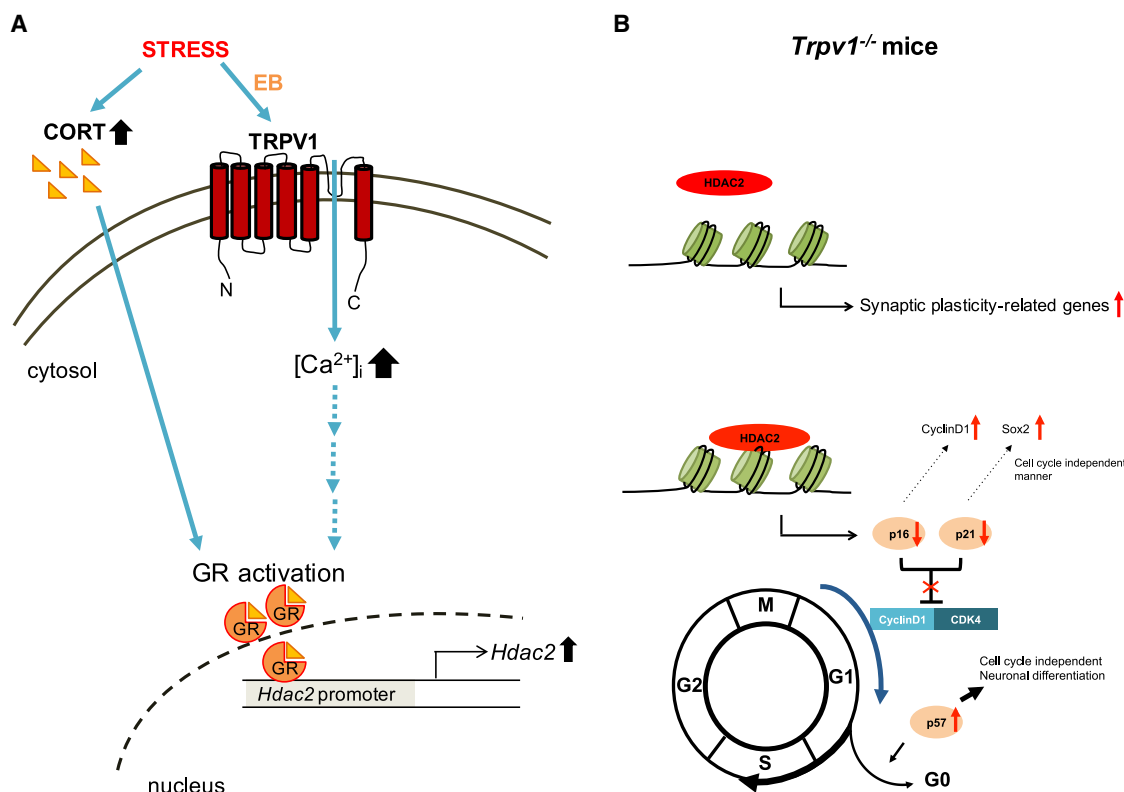


Figure 6. Schematic Diagram Illustrating the Involvement of TRPV1 in Stress Resilience via HDAC2

(A) Exposure to stress alters EBs, which are endogenous agonists of the TRPV1 receptor. Calcium influx through TRPV1 activation activates GR-induced gene transcription by Ca^{2+} -dependent kinases (e.g., PKC).

(B) GR, as a transcription factor, contributes to elevated levels of HDAC2, which leads to repression of genes related to synaptic plasticity. In the nuclei of *Trpv1*^{-/-} DG, repression of synaptic plasticity-related genes is reduced. HDAC2 binds to the CDKi promoters and negatively regulates expression. Reduction of p16 and p21 expression in TRPV1-deficient NPCs may lead to release of inhibition of cyclinD1/CDK4 complexes and in turn accelerate neuronal precursor proliferation. Reduction of p16 and p21 levels induces expression of CyclinD1 and Sox2, respectively, at the transcriptional level in a cell-cycle-independent manner. Increased p57 expression in the TRPV1-deficient DG may interact with basic-helix-loop-helix (bHLH) factors to regulate differentiation in a time- and context-dependent manner. This process may be related to p57 function in cell-cycle exit.

downregulation of HDAC2 and is associated with enhanced neuroplasticity.

DISCUSSION

We have shown that TRPV1 deficiency increases resilience to acute and chronic stress and that the behavioral effects of TRPV1 deficiency are accompanied by reduced recruitment of GR to the *Hdac2* promoter. Because previous studies reported that Ca^{2+} influx through TRPV1 induces PKC activation (Guntorpe and Chizh, 2009), which is involved in GR activation (Budziszewska et al., 2000), our results suggest that TRPV1 deficiency contributes to the downregulation of HDAC2 expression and activity. We further suggest that the reduced susceptibility to stress is due, at least partly, to this downregulation of HDAC2 and a resulting increase in global acetylation of histones H3 and H4, which stimulates the expression of neuroplasticity-related genes (Figure 6A). Furthermore, TRPV1 deficiency led to increased basal hippocampal neurogenesis, as did knock-down of TRPV1 in the hippocampal DG. Our results extend pre-

vious reports of the ability of TRPV1 to modify gene expression and point to a beneficial effect of blocking TRPV1 action at the level of chromatin regulation on stress behavior.

HDAC2 expression patterns in the hippocampus likely reflect the intrinsic characteristics of both the circuitry and the function of subregions (Hou et al., 2014). The DG subregion is a novelty detector for incoming sensory stimuli (Hou et al., 2014; Chandramohan et al., 2007), whereas CA1 is involved in spatial memory formation (Tsien et al., 1996). In this regard, the effects of *Trpv1* knockout on HDAC2 levels and activity in the DG may indicate that HDAC2 expression is, at least partly, regulated by information flows occurring via TRPV1. The observations that a postsynaptic Ca^{2+} rise via TRPV1 mediates long-term depression (LTD) of excitatory synaptic transmission in the mouse DG (Chávez et al., 2010) and that TRPV1 deficiency stimulates the induction of LTP in the hippocampal DG (Chávez et al., 2010) support the involvement of TRPV1 in neuronal activity-induced processes in the DG. These results are also in agreement with our finding that neuroplasticity-related genes are upregulated in the DG of *Trpv1*^{-/-} mice. Altogether, these observations suggest that

TRPV1 plays an important role in the recognition of signals generated by neuronal activity, at least partly in a manner dependent on epigenetic regulation.

We observed downregulation of p21 and upregulation of p57 in the hippocampal DG of *Trpv1*^{-/-} mice. This is in line with previous findings that p21 is decreased (increasing cell proliferation) (Marqués-Torrejón et al., 2013) and p57 is increased (Anacker et al., 2011) in hippocampal neurons in response to antidepressant treatment. p21 directly binds the *Sox2* enhancer and negatively regulates *Sox2* expression in NSCs in a cell-cycle-independent manner (Marqués-Torrejón et al., 2013), which is consistent with the elevated *Sox2* protein level in *Trpv1*^{-/-} mice. Both pharmacological activation of TRPV1 (Kong et al., 2010) and TRPV1 activity (Stock et al., 2014) impair hippocampal neurogenesis. Our results thus suggest that the ability of TRPV1 deletion to influence hippocampal neurogenesis is related to coordinated regulation by p16, p21, and p57 (Figure 6B).

The present data show that TRPV1 ablation produces antidepressant-like behaviors, and this appears to be accompanied by an increase in basal hippocampal neurogenesis. The antidepressant-like phenotype displayed by *Trpv1*^{-/-} mice is associated with the normalization of HPA-axis deregulation. These results demonstrate that TRPV1 activity, together with downregulation of HDAC2, is able to regulate neuroplasticity and behavioral responses. Although the beneficial effects of TRPV1 deficiency on behaviors were equally evident under non-stress and CUS conditions, TRPV1 activity in the brain under CUS may occur in response to a new chemical balance due to the possible effects of EB signaling, in combination with stress. One possible mechanism by which TRPV1 activity might play a role under CUS is if TRPV1 located in the CNS were activated to a greater extent in CUS than under non-stress basal conditions. In support of this, previous work demonstrated that activation of TRPV1 receptors located in the CNS was responsible for the enhanced depression-like behaviors; it also showed that these sites could be activated by the endogenous ligand during forced swim stress imposed by immersion in cold water (25°C–26°C) (Abdelhamid et al., 2014). Because our CUS paradigm involved cold stress, and the FST took place at low temperature (25°C–26°C), CUS may have induced the release of sufficient endogenous ligand during the CUS to activate the centrally located TRPV1 (Abdelhamid et al., 2014), thus leading to depression-like behavior in WT mice, but not in *Trpv1*^{-/-} mice. However, hippocampal neurogenesis may not be directly associated with behavioral improvement in the *Trpv1*^{-/-} mice in the CUS condition, because CUS-induced reduction of hippocampal neurogenesis appears to be largely unaffected by TRPV1 deficiency. The biochemical mechanisms underlying the stress-resilience effects of TRPV1 removal in CUS require future study; these may include complex signaling processes different from those in the absence of CUS.

Our findings provide evidence that HDAC2 mediates TRPV1 signaling, which implies that epigenetic mechanisms play an important role in TRPV1-mediated emotional responses. TRPV1 receptors have been reported in DG granule cells (Chávez et al., 2010), and our results provide evidence for functional TRPV1 receptors in these cells. The presence of TRPV1 in the brain may permit the development of new approaches to the

treatment of stress-induced disorders. One should remember, though, that EBs bind to CB1, as well as TRPV1 (Di Marzo et al., 2004). Thus, blockade of EB signaling through TRPV1 may potentiate EB signaling via CB1, and preventing TRPV1 signaling may decrease stress and anxiety but at the same time alter behavior via CB1. Therefore, the relative importance of EB signaling via TRPV1 versus CB1 for the function of the DG in relation to psychological states such as stress warrants further investigation. In addition, studies of TRPV1 reporter mice have shown that TRPV1 brain expression is low and limited to a few discrete brain regions in the mouse, rat, and human (Cavanaugh et al., 2011). Furthermore, TRPV1 stimulation can induce tumor cell death (Stock et al., 2012). These effects need to be understood if TRPV1 is to be exploited as a therapeutic target in the treatment of stress-induced affective disorders.

EXPERIMENTAL PROCEDURES

Mice

Trpv1^{-/-} mice (Caterina et al., 2000) were obtained from The Jackson Laboratory. They were backcrossed onto the C57BL/6J (Charles River Korea) background, and mice of the N7 or higher generation were used. Heterozygous breeders were crossed to generate WT, heterozygous, and knockout littermates, and genotypes were determined by PCR analysis. All experiments were conducted with 8- to 10-week-old male *Trpv1*^{-/-} mice. Age-matched male WT littermates were randomly assigned to each experiment. All experiments were performed and analyzed blind to genotyping or group membership except the western blot experiments. Mice were housed two to four per cage in a temperature- and humidity-controlled environment (lights on 07:00–19:00) with access to food and water ad libitum. All animal experiments were approved by the Institutional Animal Care and Use Committee of Hanyang University and were performed in accordance with relevant guidelines and regulations.

Chromatin Immunoprecipitation Assays

ChIP assays were performed as previously described (Guan et al., 2009). The antibodies used are given in Table S1. Immunoprecipitated DNA samples were resuspended in distilled H₂O and used for real-time PCR (CFX96 Touch Real-Time PCR Detection System, Bio-Rad). Primer sequences are given in Table S2 and were previously reported (Guan et al., 2009). Input and immunoprecipitated DNAs were PCR amplified in triplicate in the presence of SYBR Green (Bio-Rad).

Chronic Unpredictable Stress Procedure

CUS experiments were performed as described previously (Schmidt and Duman, 2010). Mice were exposed to a sequence of mild and unpredictable stressors for 14 days. The stressors used are described in Supplemental Experimental Procedures.

Novelty Suppressed Feeding Test

Mice deprived of food for 24 hr were presented with food pellets placed in the center of a bright plastic box (50 × 50 × 20 cm). Latency before beginning eating was measured as previously described (Lee et al., 2009).

Forced Swim Test

Mice were placed individually in a chamber (height, 30 cm; diameter, 15 cm) filled with 12 cm of water (temperature 24 ± 1°C) for 6 min. The lengths of periods of immobility or struggling in the last 4 min of the 6-min test period were measured as previously described (Lee et al., 2009).

Learned Helplessness Test

The learned helplessness procedure was based on previously described procedures (Duman et al., 2007). Mice were placed on a commercial shuttle box divided into two equal compartments by a central barrier (Gemini Avoidance

System, San Diego Instruments). The mean escape latency and total number of escape failures over 30 successive escape trials were measured.

Statistical Analyses

Differences between groups were analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test, for multiple comparisons between groups when assessing the effect of genotype on stress, and unpaired two-tailed Student's *t* tests, when assessing the effect of genotype on gene expression and the effect of lentivirus (lenti)-short hairpin RNA (shRNA) infusion on behaviors. GraphPad Prism v.5.0 (GraphPad Software) was used for statistical analysis. $p < 0.05$ was considered statistically significant. Biochemical data are presented as mean \pm SEM of multiple independent experiments performed in triplicates. For experiments with low sample sizes, mean \pm SD was used.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2017.03.050>.

AUTHOR CONTRIBUTIONS

H.S. defined the research theme and designed the experiments. S.E.W. designed the methods and experiments. S.E.W., S.Y.K., S.J., M.C., Seung Hoon Lee, H.R.J., J.Y.S., Sang Hoon Lee, and Y.S.K. carried out the laboratory experiments. S.E.W. and H.S. analyzed the data, interpreted the results, and wrote the paper. S.J.J. provided critical review and discussion and contributed reagents and materials. All authors have contributed to the manuscript and have seen and approved it.

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