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Reduced Adult Hippocampal Neurogenesis and Cognitive Impairments following Prenatal Treatment of the Antiepileptic Drug Valproic Acid

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SUMMARY

Prenatal exposure to valproic acid (VPA), an established antiepileptic drug, has been reported to impair postnatal cognitive function in children born to VPA-treated epileptic mothers. However, how these defects arise and how they can be overcome remain unknown. Using mice, we found that comparable postnatal cognitive functional impairment is very likely correlated to the untimely enhancement of embryonic neurogenesis, which led to depletion of the neural precursor cell pool and consequently a decreased level of adult neurogenesis in the hippocampus. Moreover, hippocampal neurons in the offspring of VPA-treated mice showed abnormal morphology and activity. Surprisingly, these impairments could be ameliorated by voluntary running. Our study suggests that although prenatal exposure to antiepileptic drugs such as VPA may have detrimental effects that persist until adulthood, these effects may be offset by a simple physical activity such as running.

INTRODUCTION

Epilepsy is one of the most common neurological disorders in the world and is characterized by uncontrollable seizures (Chang and Lowenstein, 2003). Epilepsy can affect anyone, at any age, and there are an estimated 50 million afflicted people worldwide (Meinardi et al., 2001; Ngugi et al., 2010; Joint Epilepsy Council, 2011). The incidence of epilepsy is estimated to be higher than 0.5 cases per 1,000 of population per year (Sander, 2003). Around 30% of sufferers are women of childbearing age (Joint Epilepsy Council, 2011). During pregnancy, epileptic patients must balance the maternal and fetal risks associated with seizures against the potential teratogenicity of antiepileptic drugs (AEDs) (Battino and Tomson, 2007). Although it was recently reported that several commonly used AEDs could produce postnatal impairment of cognitive function if taken during pregnancy (Meador et al., 2009, 2011, 2012, 2013), the precise pathology underlying such impairment remains unknown, and effective treatments for affected children of epileptic mothers who took AEDs during their pregnancy are therefore currently unavailable.

Valproic acid (VPA [2-propylpentanoic acid]) is an established drug in the long-term treatment of epilepsy (Blaheta and Cinatl, 2002). Several studies have revealed that VPA can directly inhibit histone deacetylase (HDAC) activity and cause hyperacetylation of histones, thereby activating gene transcription (Göttlicher et al., 2001; Phiel et al., 2001). VPA significantly impairs postnatal cognitive function (Meador et al., 2009, 2011, 2012, 2013) and can lead to severe developmental defects if taken in early gestational stages (DiLiberti et al., 1984; Nau et al., 1991). We have previously shown, in several culture systems, that VPA enhances neurogenesis and drives neural precursor cells (NPCs) into the neuronal lineage over the glial lineage by a process involving HDAC inhibition (Hsieh et al., 2004; Abematsu et al., 2010; Juliandi et al., 2012). Here, we show that comparable postnatal cognitive functional impairment after prenatal VPA exposure in mice is caused by the untimely enhancement of embryonic neurogenesis, which leads to depletion of the NPCs pool and consequently a decreased level of adult neurogenesis in the hippocampus. We further show that hippocampal neurons in the offspring of VPA-treated mice have an abnormal morphology and activity. Nevertheless, these impairments can be alleviated by voluntary running.

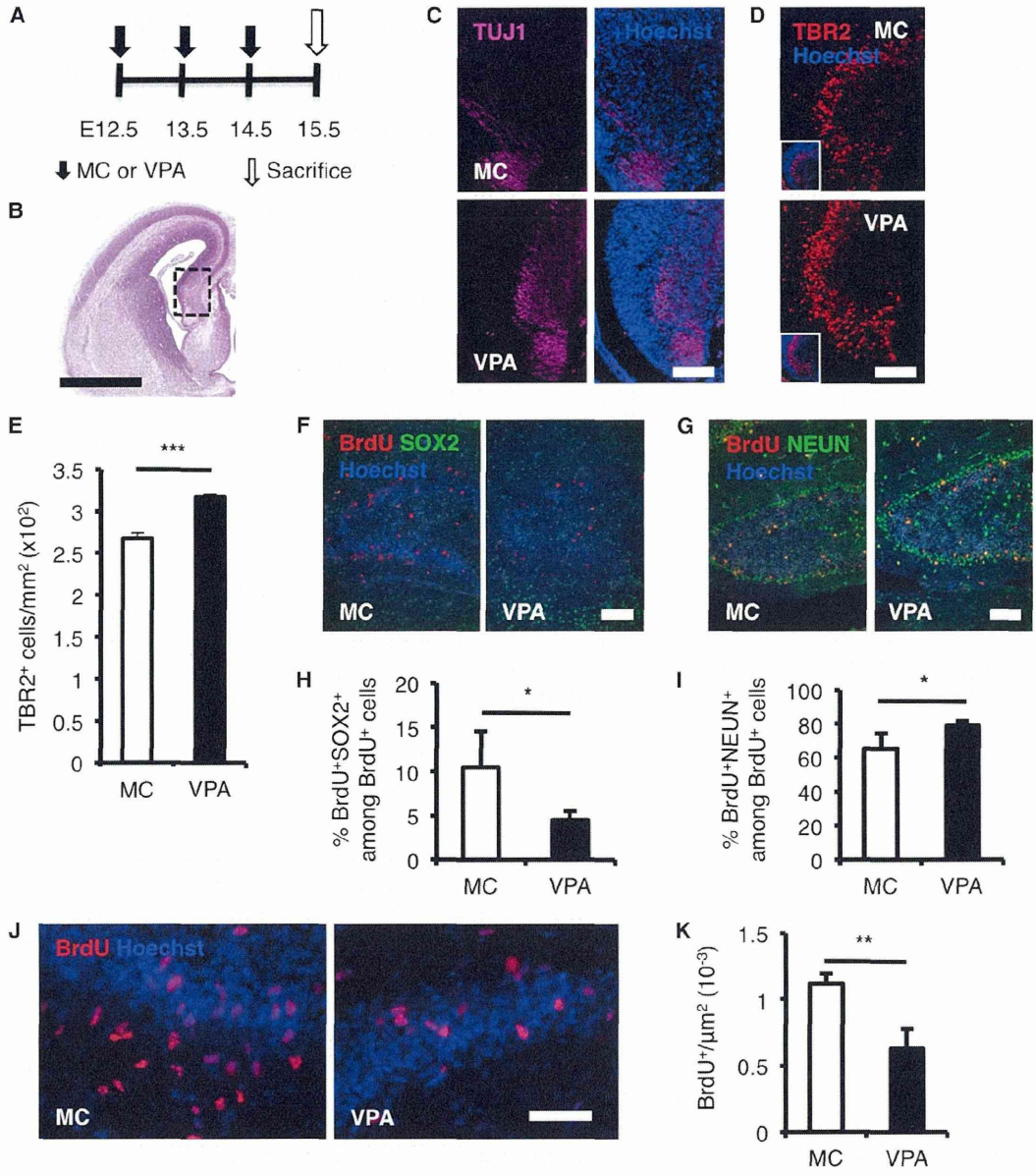

Figure 1. Prenatal VPA Treatment Enhances Untimely Embryonic Neurogenesis

(A) Experimental timeline of prenatal VPA treatment. E, embryonic day.

(B) The region highlighted by the dashed black rectangle in E15.5 forebrain sections was used for analysis in (C) and (D). The image is modified from the Electronic Prenatal Mouse Brain Atlas. Scale bar, 1 mm.

(C) The thickness of the region stained by immature neuron marker β-tubulin isotype III (TUJ1; magenta) is increased in the cortical hem of VPA-treated mice. Scale bar, 100 μm.

(D) VPA treatment increases the production of $TBR2^+$ intermediate neuronal progenitors (red) born in the dentate neuroepithelium. Scale bar, 100 μm.

(E) Quantification of the density of $TBR2^+$ in (D).

(F–I) The proportion of E14.5 BrdU-labeled NPCs (red) expressing NPC marker SOX2 (green; F and H) is reduced, while the ones that had differentiated into $NEUN^+$ neurons are increased at P7 hippocampus after embryonic VPA treatment (green; G and I). Scale bars, 100 μm.

(legend continued on next page)

RESULTS

VPA Enhances Embryonic Neurogenesis and Alters Global Gene Expression through HDAC Inhibition

We orally administered VPA or vehicle (methylcellulose [MC]) to pregnant mice on embryonic day (E)12.5 to E14.5, a mid-gestational period when neurogenesis is prominent (Figure 1A). We found that VPA increased global histone acetylation (Figures S1B–S1D) and enhanced neurogenesis as shown by the increased thickness of TUJ1 stained region in the cortical hem of VPA-treated mice (Figure 1C) and increased production of TBR2-positive intermediate neuronal progenitors born in the dentate neuroepithelium (Figures 1D and 1E) in the developing mouse brain (Figures 1B and S1A; see Figures S1E–S1G for embryonic cortex). We also found that VPA depleted the NPC pool (Figures 1F and 1H, hippocampus; Figures S1E and S1F, cortex) and reduced the number of proliferating NPCs (Figures 1J and 1K, hippocampus; Figures S1H and S1I, cortex). These results were compatible with our previous in vitro observations (Hsieh et al., 2004; Abematsu et al., 2010; Juliandi et al., 2012). Moreover, we found that the fraction of cells that had exited the cell cycle (i.e., differentiated) was higher in the E15.5 forebrain of VPA-treated mice, as shown by an increased number of BrdU-retaining cells that were negative for the proliferation marker KI-67 compared to BrdU-retaining cells that were still KI-67-positive, after a single injection of BrdU on E14.5 to label proliferating cells (Figures S1J and S1K). These results show that VPA treatment enhances embryonic neurogenesis and reduces the pool of NPCs.

Phenotypic changes should be generally induced by gene expression changes, and we wanted to examine whether VPA-induced gene expression change is due to its HDAC inhibiting property. To this end, we administered VPA or valpromide (VPM), an analog AED without HDAC-inhibiting activity, and performed transcriptome analyses at three time points after the final administration. We found that 3 hr after the last administration, global gene expression in the E14.5 telencephalon was changed substantially by VPA, but not by VPM (Figure S1L; GEO: GSE 42904). This global change had almost completely disappeared in both cortex and hippocampus at E18.5 and in hippocampus at P84, although several genes still displayed differential expression levels (Figure S1L). We have found previously that VPM treatment increased neither global histone acetylation nor neurogenesis (Abematsu et al., 2010). Although VPA and VPM might have another pharmacological activity differences beside HDAC-inhibiting activity, taken together our results strongly suggest that VPA alters global gene expression mainly through its HDAC-inhibiting property, and this alteration is short-lived, being restricted mainly to the period when VPA was being given to the mice.

Prenatal VPA Treatment Has the Long-Term Effect of Impairing Adult Neurogenesis, Learning, and Memory

We next investigated whether the enhancement of neurogenesis and changes in gene expression caused by prenatal VPA exposure during the period of prominent neurogenesis could lead to postnatal impairment of cognitive function, as reported in humans (Meador et al., 2009, 2011, 2012, 2013). We conducted several behavioral tests on 12- to 13-week-old male mice (Figure 2A). Although the locomotor activity of VPA-treated mice declined, the decline rate was very slight (Tables S1 and S2) so that we could measure the emotional or cognitive behaviors. We found indeed that VPA-treated mice performed poorly mainly in tests that assessed learning and memory, such as Y-maze (Figure 2B) and contextual and cued fear associative tests (Figures 2C–2E, S4A, S4C, and S4E; Table S1), but not in the other tests (Table S1). VPA-treated mice have a lower correct-arm alternation in Y-maze. VPA-treated mice also have a lower freezing response in contextual and cued fear associative tests than MC-treated mice (control), despite the fact that both groups have similar fear response to foot shock during conditioning (Table S1), indicating that hippocampal-dependent learning and memory (Sarnyai et al., 2000; Van der Borght et al., 2007) are impaired in the VPA-treated mice.

In light of these results, we decided to focus on adult NPCs in the hippocampal dentate gyrus (DG), as they have been shown to play a functional role in learning and memory processes by undergoing neurogenesis to generate adult-born neural cells (Zhao et al., 2008). We injected BrdU once a day for 7 days into 12-week-old mice to label proliferating NPCs in the DG and then sacrificed the mice 1 day (to assess cell proliferation) or 4 weeks (to assess cell survival and fate) after the last BrdU injection (Figure 3A). We found that the number of BrdU-retaining cells in VPA-treated mice was lower at both time points than that in MC-treated control mice (Figures 3B and 3C). We also found fewer proliferating KI-67-positive cells in VPA-treated mice (Figures S2A and S2B). When we traced the fate of BrdU-retaining cells 4 weeks after the last BrdU injection (Figure 3A), we found that a lower proportion

(J and K) Embryonic VPA treatment reduces the number of highly proliferating NPCs (red) labeled by 30 min single-pulse BrdU injection in the P7 DG. See also Figure S1 for other immunostaining data in the cortex of embryonic forebrain.

MC, prenatal methylcellulose (vehicle); VPA, prenatal valproic acid. Data are represented as means. n = 3 for each group. Error bars indicate the SD. *p < 0.05, **p < 0.01, ***p < 0.001, two-tailed t test. Scale bar, 50 μm. See also Figure S1.

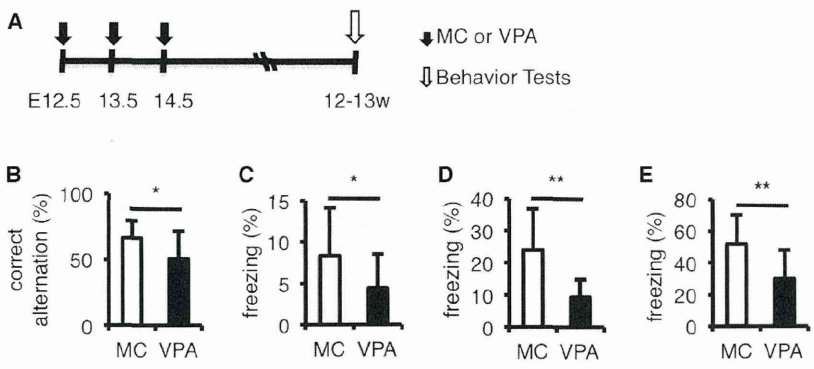

Figure 2. VPA-Treated Mice Perform Poorly in Learning and Memory Tests

(A) Experimental timeline of prenatal VPA treatment and postnatal behavior tests. E, embryonic day; w, weeks old.

(B) VPA-treated mice have a lower correct-arm alternation than MC-treated mice (control).

(C–E) VPA-treated mice have a lower freezing response than MC-treated mice (control) in conditioning (C; day 1), contextual (D; day 2), and cued fear associative tests (E; day 3). See also Figures S4A, S4C, and S4E for time course of freezing response and Table S1 for a summary of behavior data.

MC, prenatal methylcellulose (vehicle); VPA, prenatal valproic acid. Data are represented as means. n = 12 for each group. Error bars indicate the SD. *$p < 0.05$, **$p < 0.01$, two-tailed t test. See also Figure S4 and Table S1.

of BrdU-retaining cells had differentiated into NEUN-positive neurons and S100β-positive astrocytes in VPA-treated mice than in MC-treated mice (Figure 3D). The possibility that more BrdU-retaining cells had died in VPA-treated mice during the 4-week period can be ruled out, because the survival rate of BrdU-retaining cells in these mice was similar to that in MC-treated mice (Figure 3E). These results imply that BrdU-retaining cells in VPA-treated mice either differentiated to another cell type(s) or differentiated more slowly than those in MC-treated mice. The latter explanation is more plausible, because we found that a higher proportion of BrdU-retaining cells in VPA-treated mice still expressed SOX2 (an NPC marker) and KI-67 (a proliferation marker) even 4 weeks after the last BrdU injection (Figures S2C–S2E). We also found that 1 day after the last BrdU injection, almost all BrdU-positive cells were still KI-67-positive in VPA-treated mice, whereas several BrdU-positive but KI-67-negative cells already existed in MC-treated mice (Figure S2F). We also examined amygdala and cortex, other brain regions that were suggested to play important roles in the regulation of memory and fear associative responses (LeDoux, 2003; van Strien et al., 2009). It has been shown previously that amygdala volume associates with differences in fear associative responses (Yang et al., 2008). However, we found that VPA-treated mice have a similar amygdala size to that of MC-treated mice, both at P7 and P84 (Figures S3A–S3D). We also found no significant difference in the expression level of cortical layer-specific genes such as *Cux1*, *Satb2*, and *Ctip2* (Molyneaux et al., 2007) in the cortex of P84 VPA-treated mice (Figures S3E–S3G). Taken together, these results suggest that prenatal VPA treatment has the long-term effect of impairing adult neurogenesis and contribute to the poor performance of VPA-exposed mice in learning and memory tests.

Voluntary Running Restores Learning and Memory Deficiencies in VPA-Treated Mice, Probably through Increased Neurogenesis that Yields Neurons with Normal Morphology

Voluntary running can increase adult neurogenesis in the hippocampal DG (van Praag et al., 1999a, 1999b). We next provided the mice with a running wheel (RW) after they had weaned and repeated the same BrdU-injection experiment when they reached 12 weeks (P84; Figure 3A). We observed an increased number of BrdU-retaining cells in MC- and VPA-treated mice with the RW, both 1 day and 4 weeks after the last BrdU injection (Figures 4A and 4B). We also found an increased proportion of BrdU-retaining cells that had differentiated into NEUN-positive neurons and a reduced proportion of BrdU-retaining cells that still SOX2-positive in MC- and VPA-treated mice with the RW, 4 weeks after the last BrdU injection (Figures 3A, 4C, and 4D). Voluntary running also enabled VPA-treated mice to perform better in hippocampus-dependent learning and memory test, the correct alternation in Y-maze ($F(1, 13) = 22.74$, $p < 0.001$, one-way ANOVA), and this performance was not significantly different in comparison to MC-treated mice (Figure 4E; Table S2). Moreover, we found that voluntary running also led VPA-treated mice performing better in conditioning ($F(1, 13) = 3.06$, $p = 0.10$, one-way ANOVA) and cued fear tests ($F(1, 13) = 6.80$, $p < 0.05$, one-way ANOVA), although not in contextual fear test ($F(1, 13) = 0.47$, $p = 0.50$, one-way ANOVA). As each mouse showed similar normal fear response, the performance of VPA-treated mice after voluntary running in conditioning and cued fear tests, but not in contextual fear test, recovered to levels that were not significantly different from that of MC-treated mice (Figures 4F–4H, S4B, S4D, and S4F; Table S2). However, when we analyzed all experimental groups by two-way ANOVA with both

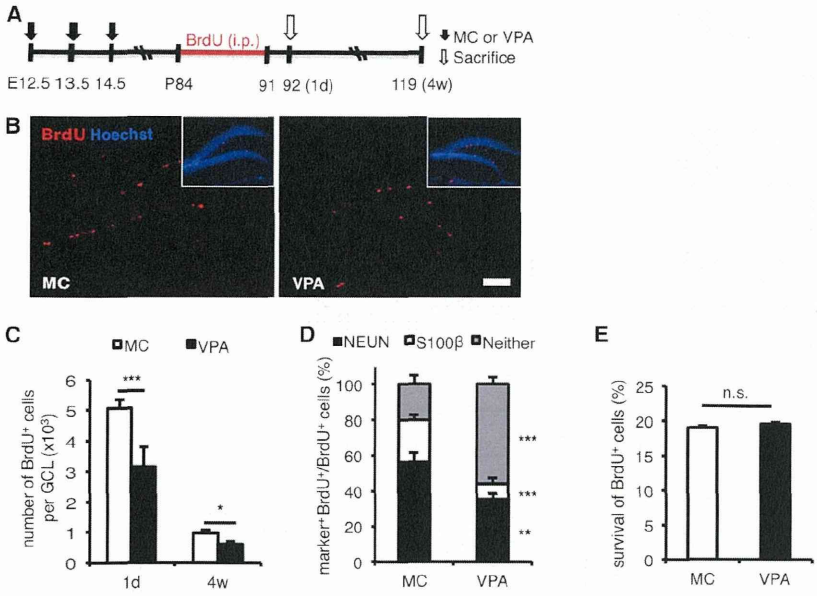

Figure 3. Prenatal VPA Treatment Has the Long-Term Effect on the Adult Neurogenesis

(A) Experimental timeline of prenatal VPA treatment and adult neurogenesis analysis. E, embryonic day; P, postnatal day; 1d, 1 day after the last intraperitoneal (i.p.) BrdU injection; 4w, 4 weeks after the last i.p. BrdU injection.

(B) Representative images of brain sections including the hippocampal DG stained for BrdU (red) and with Hoechst 33258 (blue),1 day after the last BrdU injection. See also Figure S2A for KI-67 staining.

(C) Quantification of $BrdU^+$ cells in the granule cell layer (GCL), 1 day (1d; n = 8 for each group) and 4 weeks (4w; n = 8 for each group) after the last BrdU injection, shows a reduction of $BrdU^+$ cells in the hippocampus. See also Figure S2B for quantification of $KI\text{-}67^+$ cells in the GCL.

(D) NPC differentiation into $NEUN^+$ neurons and $S100\beta^+$ astrocytes is impaired in VPA-treated mice, as shown by a reduced proportion of marker-positive and $BrdU^+$ cells among total $BrdU^+$ cells at 4 weeks after the last BrdU injection (n = 4 for each group).

(E) $BrdU^+$ cell survival is similar in VPA- and MC-treated mice. Quantification of $BrdU^+$ cell survival in each group as a percentage of $BrdU^+$ cells at 4w relative to $BrdU^+$ cells at 1d (n = 8 for each group).

MC, prenatal methylcellulose (vehicle); VPA, prenatal valproic acid. Data are represented as means. Error bars indicate the SD. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s., not significantly different, two-tailed t test. Scale bar, 100 μm. See also Figures S2 and S3.

prenatal treatment and postnatal activity as factors, the results did not show a significant effect of postnatal activity on the performance of mice in hippocampus-dependent learning and memory tests (Table S2). This is mainly because voluntary running in MC-treated mice made no significant contribution to the performance in hippocampus-dependent learning and memory tests (conditioning: $F(1, 13) = 0.13$, $p = 0.72$; contextual: $F(1, 13) = 0.26$, $p = 0.62$; cued: $F(1, 13) = 0.01$, $p = 0.93$; Y-maze: $F(1, 13) = 1.17$, $p = 0.30$; one-way ANOVA). In addition, we found no significant interaction between prenatal drug treatment and postnatal activity in relation to the behavior of mice, except for the correct alternation in Y-maze ($F(1, 26) = 5.59$, $p < 0.05$, two-way ANOVA; Table S2). Therefore, it seems likely that only a certain level of adult neurogenesis is required for mice to perform normally in these hippocampus-dependent learning and memory tests, so that MC-treated mice with or without a RW showed no significant differences in these tests even though MC-treated mice with a RW displayed higher levels of neurogenesis than those without one. However, because the level of adult neurogenesis in the VPA-treated mice was below this threshold, the mice performed poorly in these tests; voluntary running improved their performance, probably by increasing adult neurogenesis in the hippocampus to above the threshold level.

We next sought to determine whether adult neurogenesis in the DG of VPA-treated mice generated normal or abnormal neurons. We found that DCX-positive immature neurons (Figures 5A and S5A) and Golgi-stained mature neurons (Figures 5B and S5B) in the DG of VPA-treated mice displayed an abnormal morphology with shorter total dendritic length (Figures 5D and 5E) and fewer dendrite processes toward the molecular layer, as shown by a wider maximum dendritic span compared to MC-treated mice (Figure 5G). The dendritic complexity of Golgi-stained neurons in VPA-treated mice, however, was similar to that of MC-treated mice (Figures 5F and S5B). Surprisingly, this abnormal morphology could also be overcome by voluntary running (Figures 5A, 5B, 5D, 5E, 5G, S5A, and S5B). Taken together, these observations suggest that the restoration of learning and memory deficiencies in VPA-treated mice through running is involved in increased neurogenesis, which gives rise to neurons with normal morphology.

Several mechanisms have been proposed to be responsible for the voluntary running-induced increase in adult hippocampal neurogenesis. Voluntary running can induce expression of neurotrophic factors such as *brain-derived neurotrophic factor* (*Bdnf*) in the hippocampus (van Praag,

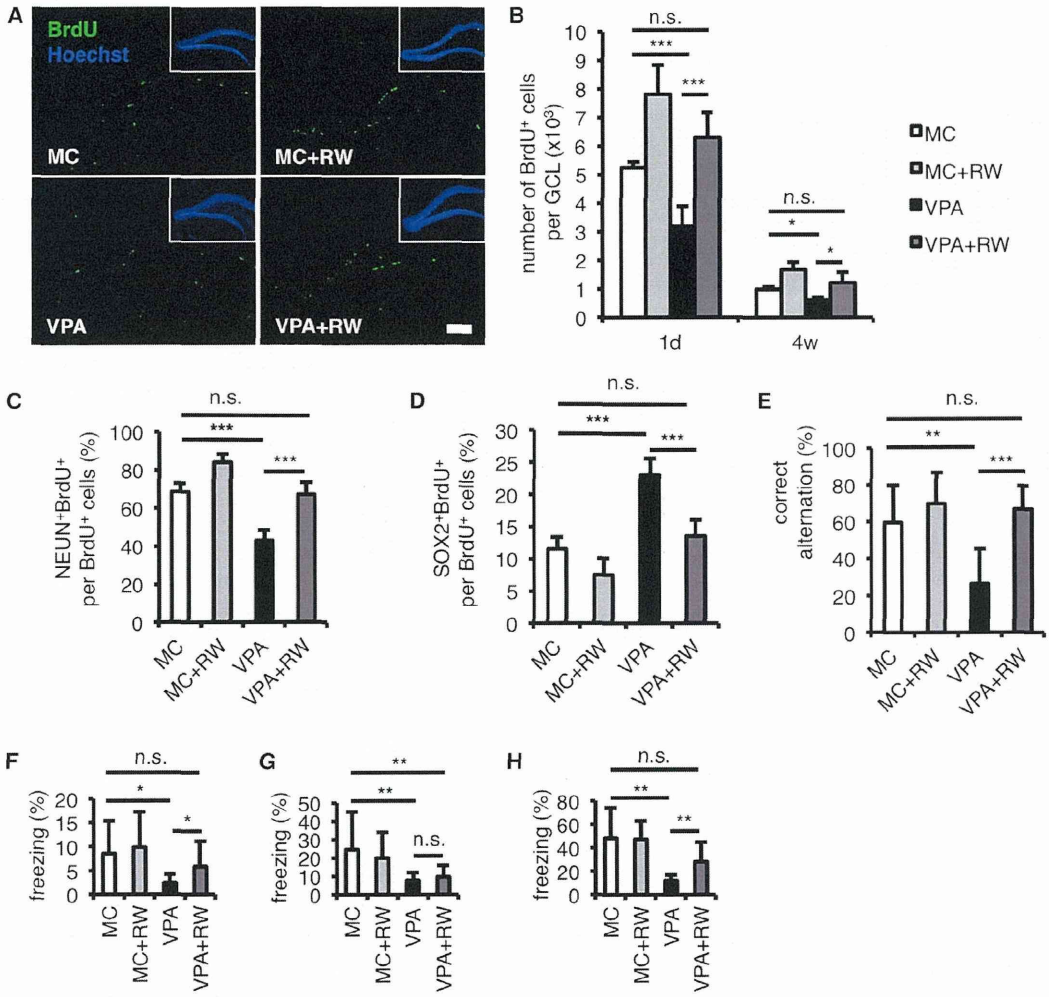

Figure 4. Voluntary Running Restores Adult Neurogenesis and Cognitive Deficiencies of VPA-Treated Mice

(A) Representative images of brain sections including the hippocampal DG stained for BrdU (green) and with Hoechst 33258 (blue), 1 day after the last BrdU injection. See Figure 2A for the experimental timeline.

(B) Quantification of $BrdU^+$ in the granule cell layer (GCL), 1 day (1d; n = 8 for each group) and 4 weeks (4w; n = 8 for each group) after the last BrdU injection, shows an increased number of $BrdU^+$ cells in the hippocampus after voluntary running.

(C and D) Voluntary running recovers the proportion of $BrdU^+$ cells that had differentiated into $NEUN^+$ neurons (C) and the ones that still expressed SOX2 (D) among total $BrdU^+$ cells at 4 weeks after the last BrdU injection (n = 4 for each group).

(E) Reduction of correct-arm alternation in Y-maze tests of VPA-treated mice is recovered by voluntary running (n = 7 for MC and VPA + RW; n = 8 for MC + RW and VPA).

(F–H) Voluntary running recovers the freezing response in conditioning (F; day 1) and in cued fear associative tests (H; day 3), but not in contextual fear associative tests (G; day 2; n = 7 for MC and VPA + RW; n = 8 for MC + RW and VPA). See also Figures S4B, S4D, and S4F for the time course of the freezing response and Table S2 for a summary of behavior data.

MC, prenatal methylcellulose (vehicle); MC + RW, prenatal methylcellulose and postnatal running; VPA, prenatal valproic acid; VPA + RW, prenatal valproic acid and postnatal running. Data are represented as means. Error bars indicate the SD. *p < 0.05, **p < 0.01, ***p < 0.001, n.s., not significantly different, two-tailed t test. Scale bar, 100 μm. See also Figure S4 and Table S2.

2009; Farmer et al., 2004), and *Bdnf* has been suggested to have an important role in adult neurogenesis, neuronal maturation, and dendrite arborization (Tolwani et al., 2002; Bekinschtein et al., 2011; Stranahan, 2011). Indeed, we found an increased level of *Bdnf* expression after running in both MC- and VPA-treated mice hippocampus

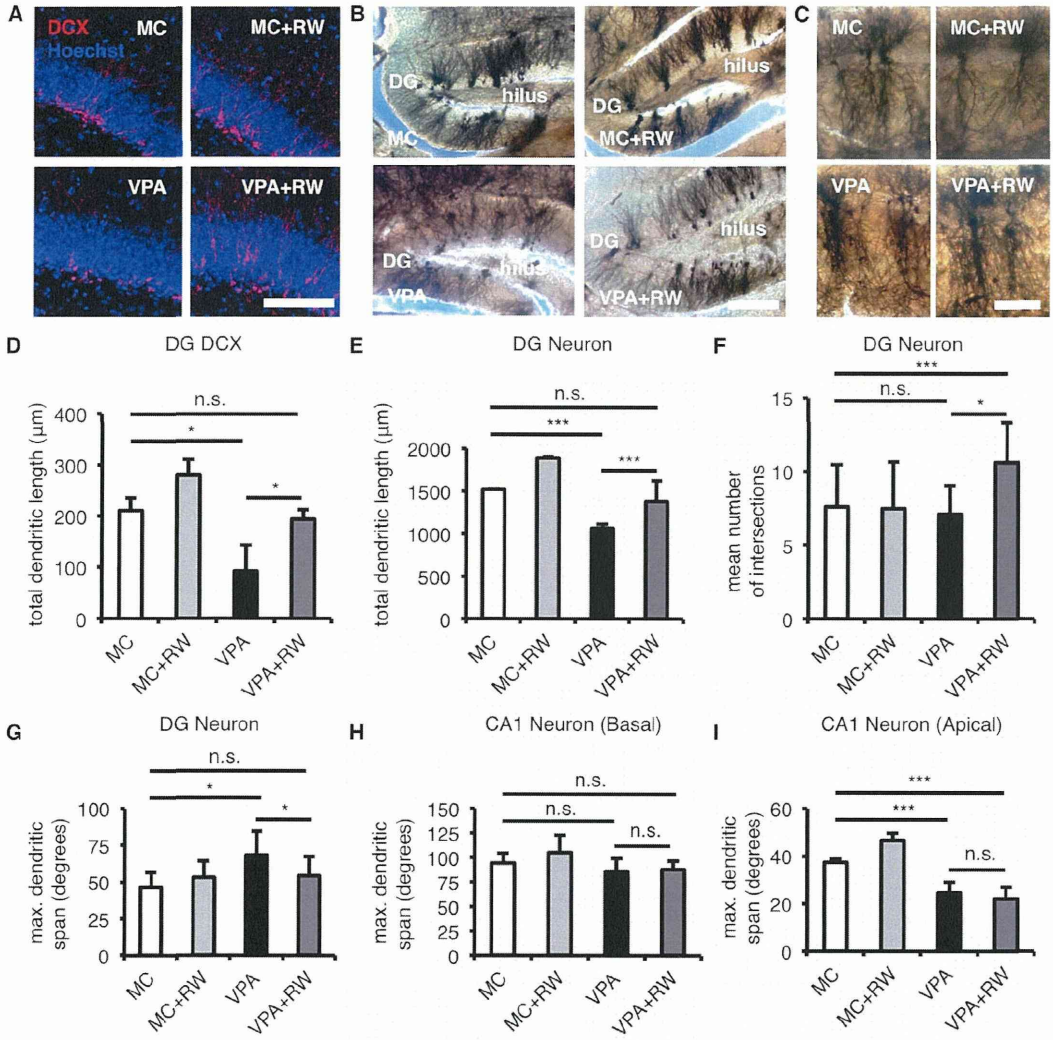

Figure 5. Voluntary Running Restores Neuronal Morphology in VPA-Treated Mice

(A) Impaired morphology of DCX$^+$ young neurons in the DG of VPA-treated mice is recovered by voluntary running (n = 4 for each group). Scale bar, 100 μm.

(B) Impaired morphology of Golgi-Cox stained neurons in the DG of VPA-treated mice is recovered by voluntary running (n = 3 for each group). Note that voluntary running recovered non-molecular layer-oriented dendrites in VPA-treated mice to molecular layer-oriented ones. Scale bar, 100 μm.

(C) Impaired morphology of Golgi-Cox stained neurons in the CA1 of VPA-treated mice is not recovered by voluntary running (n = 3 for each group). Note that the less-ramified and straighter apical dendrites in VPA-treated mice could not be recovered by voluntary running. Scale bar, 50 μm.

(D–F) Voluntary running recovers total dendritic length of DCX$^+$ young neurons (D) and Golgi-Cox stained neurons (E) and increases dendritic complexity of Golgi-Cox stained neurons (F) in the DG of VPA-treated mice. See also Figures S5A and S5B for Sholl analysis.

(G–I) Abnormal dendritic span of DG neurons (G), but not of apical dendritic span of CA1 neurons (I), is recovered by voluntary running in VPA-treated mice, while basal dendrites of CA1 neurons show similar dendritic span across groups (H).

MC, prenatal methylcellulose (vehicle); MC + RW, prenatal methylcellulose and postnatal running; VPA, prenatal valproic acid; VPA + RW, prenatal valproic acid and postnatal running. Data are represented as means. Error bars indicate the SD. *$p < 0.05$, ***$p < 0.001$, n.s., not significantly different, two-tailed t test. See also Figure S5.

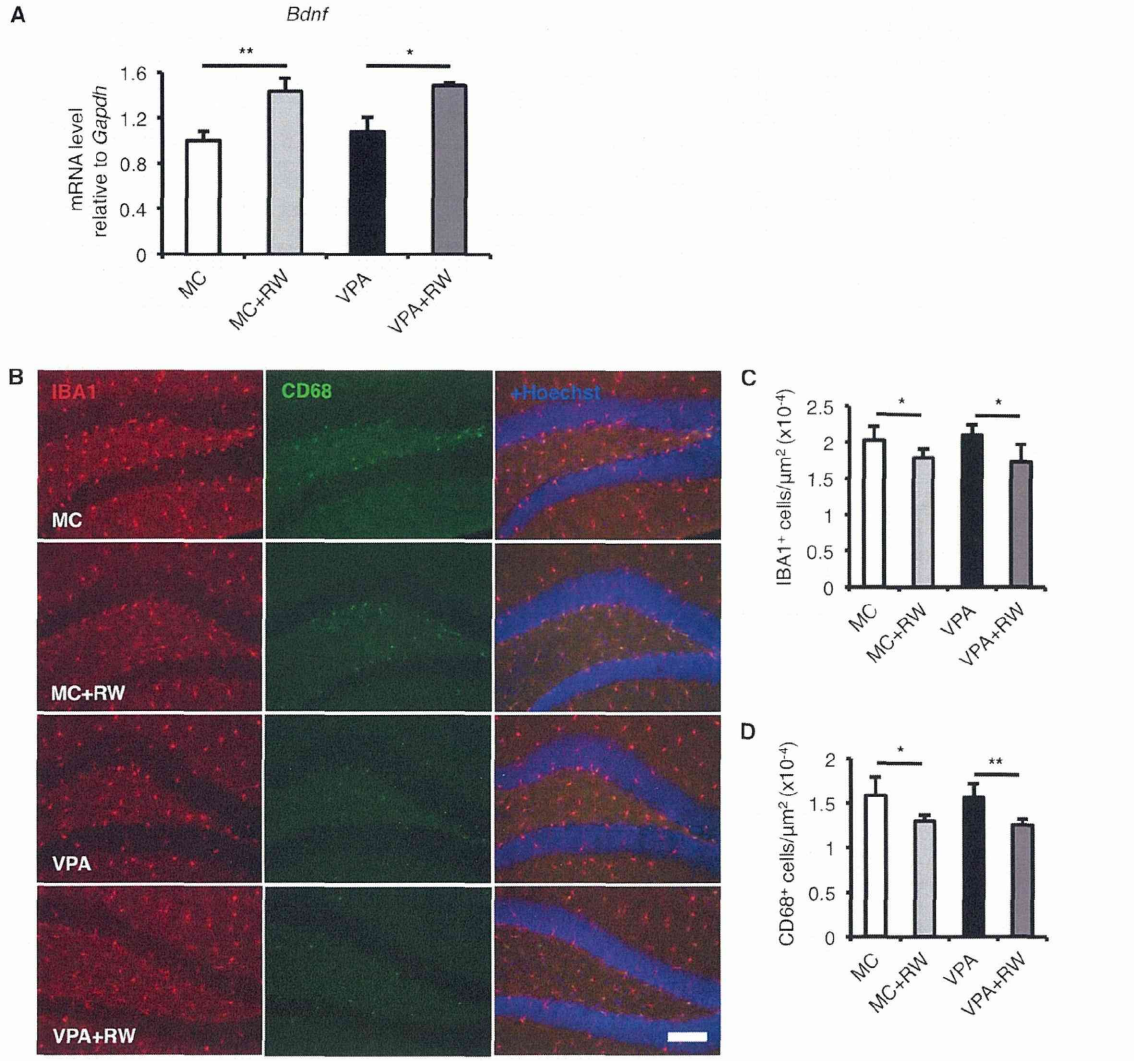

Figure 6. Voluntary Running Increases the *Bdnf* Expression Level and Reduces Microglia and Activated Microglia in the Hippocampus

(A) The expression level of *brain-derived neurotrophic factor* (*Bdnf*) was increased by voluntary running in both MC- and VPA-treated mice. (B–D) Voluntary running reduced the number of IBA1$^+$ microglia (red; B and C) and CD68$^+$-activated microglia (green; B and D) in both MC- and VPA-treated mice.

MC, prenatal methylcellulose (vehicle); MC + RW, prenatal methylcellulose and postnatal running; VPA, prenatal valproic acid; VPA + RW, prenatal valproic acid and postnatal running; *Gapdh*, *glyceraldehyde 3-phosphate dehydrogenase*. Data are represented as means. n = 3 for each group. Error bars indicate the SD. *p < 0.05, **p < 0.01, two-tailed t test. Scale bar, 100 μm.

(Figure 6A). Voluntary running has also been shown to reduce the number of microglia and its activation (Gebara et al., 2013; Kohman et al., 2013), and previous reports showed that microglia can suppress the adult hippocampal neurogenesis (Sierra et al., 2010; Vukovic et al., 2012; Matsuda et al., 2015). Interestingly, we found that the number of IBA1-positive microglia and CD68-positive-activated microglia was also decreased by voluntary running in both MC- and VPA-treated mice (Figures 6B–6D). Therefore, it is plausible that voluntary running helped VPA-treated mice through the increased adult hippocampal neurogenesis that was caused by the increase level of *Bdnf* expression and the reduction of microglia and its activated form in the hippocampus.

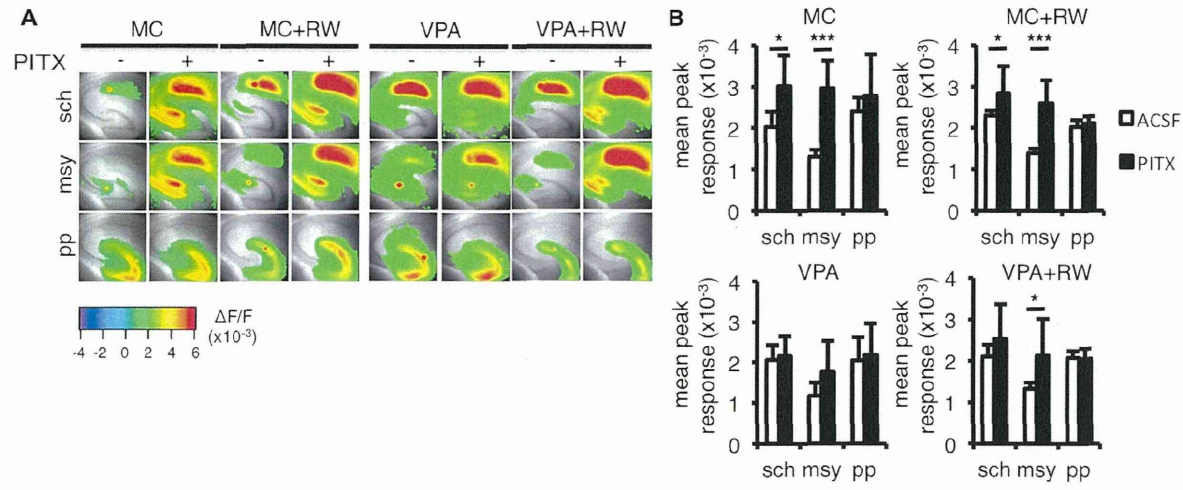

Figure 7. Voluntary Running Restores Neuronal Activity in VPA-Treated Mice

(A) Representative pseudocolor activity map images of brain slices including the hippocampus show that voluntary running can only recover the impairment of $GABA_A$ receptor-mediated inhibition in the mossy fiber pathway (msy) of VPA-treated mice, after treatment with the $GABA_A$ receptor channel antagonist picrotoxin (PITX) (n = 6 for MC, n = 9 for MC + RW, n = 7 for VPA, n = 8 for VPA + RW). Electrical stimulation was applied to Schaffer collateral afferents at the CA3/CA1 border of CA1 (sch); to the granule cell layer to stimulate the mossy fiber pathway (msy); and to the molecular layer of the upper blade in the DG (pp).

(B) Quantification of the neural response in artificial cerebrospinal fluid (ACSF), with (black bars) or without PITX (white bars; n = 6 for MC, n = 9 for MC + RW, n = 7 for VPA, n = 8 for VPA + RW). Note that although the augmentation of the neural response caused by $GABA_A$ receptor-mediated inhibition with PITX application seen in sch and msy was abolished in VPA-treated mice, voluntary running could restore the augmentation only in the msy.

MC, prenatal methylcellulose (vehicle); MC + RW, prenatal methylcellulose and postnatal running; VPA, prenatal valproic acid; VPA + RW, prenatal valproic acid and postnatal running. Data are represented as means. Error bars indicate the SD. $^*p < 0.05$, $^{***}p < 0.001$, two-tailed t test.

Voluntary Running Cannot Mitigate Abnormal Neuronal Morphology or Function of the Hippocampal CA1 Region in VPA-Treated Mice

The freezing response in the contextual fear test after voluntary running by VPA-treated mice did not recover to the level displayed by MC-treated mice (Figures 4G and S4D; Table S2). It has been proposed that recall of contextual memories relies more on CA1 than other regions in the hippocampus (Hall et al., 2001). Indeed, we found that apical dendrite morphology was abnormal in CA1 neurons of VPA-treated mice, and this defect was not repaired by voluntary running (Figures 5C, 5H, and 5I). Moreover, when we examined the region-specific restoration by voluntary running of neuronal activity in the hippocampus, the basal neuronal responses upon electrical stimulation were not affected by VPA nor voluntary running in the three major synaptic connections in the hippocampus (CA3-CA1, Schaffer collateral afferent [sch]; DG-CA3, mossy fiber [msy]; EC-DG, perforant pathway [pp]) possibly due to some homeostatic balancing mechanism (Turrigiano and Nelson, 2004; Turrigiano, 2011). However, when the excitatory activity was measured, with the increase in activity caused by an inhibitor for $GABA_A$ receptor picrotoxin (PITX) application, the effect of VPA became apparent. That is, the PITX induced an increase in sch and msy in MC-treated mice, which was not seen in VPA-treated mice, suggesting the impairment of inhibitory action in VPA-mice. The voluntary running could restore the characteristics only in msy (Figure 7). These results may explain why the recovery of neurogenesis and neuronal morphology of the DG in VPA-treated mice could not ameliorate their poor performance in the contextual associative test. Previous studies have indicated that ablation of adult neurogenesis in the DG leads to defective performance in a contextual associative test (Saxe et al., 2006; Wojtowicz et al., 2008) (but see Shors et al., 2002), but enhanced neurogenesis or running was weakly related to the performance in the test (Wojtowicz et al., 2008).

DISCUSSION

The precise pathology underlying postnatal impairment of cognitive function in children of epileptic expectant mothers treated with VPA, commonly used AED, is