Supporting Information

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SI Methods

Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were housed under a 12-h/12-h light/dark cycle and fed for ad libitum consumption. *Nestin*-EGFP mice, a generous gift from M. Yamaguchi, Kochi University, Kochi, Japan (49), were on a C57BL/6 background. Mice were randomly assigned to two groups on E11. The VPA group received an oral administration of 300 mg/kg VPA (Sigma) once a day on E12, E13, and E14. VPA was dissolved in 0.5% (wt/vol) MC (Wako) to a concentration of 30 mg/mL. The control (Ctrl) group received an oral MC administration equal in volume to the VPA group once a day on the same days. Pups from the Ctrl and VPA groups were weaned at P28. Four groups were designated as Ctrl (pups from an MC-treated mother), Ctrl+Running wheel (RW) (pups from an MC-treated mother, with running wheel), VPA (pups from a VPA-treated mother), and VPA+RW (pups from a VPA-treated mother, with RW). Ctrl+RW and VPA+RW mice were housed with a RW and were allowed to run freely in their cage immediately after weaning. All aspects of animal care and treatment were carried out according to the guidelines of the Experimental Animal Care Committee of Kyushu University.

Behavioral Assays. Four- and 12-wk-old mice were used for the examination. To induce seizure, the mice received i.p. injections of 20 mg/kg of KA (Enzo Life Sciences) dissolved in distilled H_2O. Behavior of the mice was observed for 1 h after the injection and a seizure score was recorded, according to previously described criteria (50). Briefly, we used the following seizure scale: no response (0), staring and reduced locomotion (1), activation of extensors and rigidity (2), repetitive head and limb movements (3), sustained rearing with clonus (4), loss of posture (5), and status epilepticus and death (6).

IHC. We performed IHC as described previously (28). Briefly, adult mouse brains were fixed in 4% paraformaldehyde and 40-μm sections were cut with a cryostat. The sections were blocked for 1 h at room temperature with blocking solution (5% FBS and 0.3% Triton X-100) and incubated overnight at 4 °C with primary antibodies. For staining with anti-CR antibody, antigen retrieval was performed by heating sections in target retrieval solution (Dako) at 105 °C for 15 min before blocking. The antibodies used were mouse anti-PV (1:500, MAB1572; Millipore), rat anti-SST (1:500, MAB354; Millipore), rabbit anti-CR (1:500, AB5054; Millipore), goat anti-DCX (1:500, sc8066; Santa Cruz), rabbit anti-Prox1 (1:500, AB5475; Millipore), chick anti-GFP (1:500, GFP-1010; Aves Laboratories), and mouse anti-NeuN (1:500, MAB377; Millipore). Sections were incubated for 2 h with corresponding secondary antibodies, CF-488 donkey anti-mouse IgG (H+L) highly cross-adsorbed (1:500; Biotium), CF-488 donkey anti-chicken IgG (H+L) highly cross-adsorbed (1:500; Biotium), CF-555 donkey anti-rabbit IgG (H+L) highly cross-adsorbed (1:500; Biotium), CF-555 donkey anti-mouse IgG (H+L) highly cross-adsorbed (1:500; Biotium), CF-568 donkey anti-rat IgG (H+L) highly cross-adsorbed (1:500; Biotium), and CF-647 donkey anti-goat IgG (H+L) highly cross-adsorbed (1:500; Biotium). Nuclei were stained using Hoechst 33258 (Nacalai Tesque). Fluorescence images were obtained on a confocal laser microscope (LSM700 and LSM 800; Ziess).

Cell Counts. Cell counting was performed on every 12th coronal section containing DG at the same anatomical level. The number of counted cells was then multiplied by 12 to provide an accurate estimation of the number of cells in the bilateral DG per brain. For calculating the rate of EGCs, the number of NeuN and GFP double-positive cells located in the hilus was divided by the number of NeuN and GFP double-positive cells located in the DG. A cell was determined to be located in the hilus when its soma was clearly located on the side of hilus, relative to a continuous line drawn between SGZ and hilus.

Immunoblot Analysis. Cells were lysed with a buffer containing 0.5% Nonidet P-40, 10 mM Tris·HCl, pH 7.5, 150 mM NaCl, 50 mM NaF, 1 mM Na_3VO_4, and 1% protease inhibitor (Nacalai Tesque). Lysates were sonicated and centrifuged at 20,000 × *g* for 20 min at 4 °C. Total cell lysates were subjected to SDS/PAGE and transferred to a PVDF transfer membrane (GE Healthcare). The blots were blocked with 0.3% skim milk in TBST (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20), and after incubation with primary antibody they were washed with TBST and incubated with peroxidase-conjugated secondary antibody. Immunoreactive bands were detected by enhanced chemiluminescence using ECL Prime Western blotting detection reagent (GE Healthcare). The antibodies used were rabbit anti-CXCR4 (1:100, ab124824; Abcam) and mouse anti-β-actin (1:1,000, 8H10D10; Cell Signaling Technology).

Construction of Vectors and Retrovirus Production. The pMYs-IRES-EGFP retrovirus vector, a generous gift from T. Kitamura, Tokyo University, Tokyo (51), which includes hybrid LTRs containing elements from both Moloney murine leukemia virus and myeloproliferative sarcoma virus/PCC4-cell-passaged myeloproliferative sarcoma virus, was used. For *Cxcr4* overexpression, a retrovirus vector was constructed by cloning murine Cxcr4 cDNA into the pMYs-IRES-EGFP retrovirus vector. The Plat-E packaging cell line (Cosmo Bio) was transiently transfected with retrovirus constructs. Culture supernatants containing retrovirus were collected 24 and 48 h after transfection and centrifuged at 6,000 × *g* overnight at 4 °C. The virus was concentrated using Retro-X Concentrator (TAKARA), and suspended in N2-supplemented DMEM/F-12 medium (N2 medium).

Cell Culture and in Vitro Retroviral Infection. Rat adult hippocampal neural stem cells were plated on a poly-L-ornithine/laminin–coated dish with N2 medium containing 10 ng/mL of basic fibroblast growth factor (PeproTech). The cells were infected with retrovirus 6 h after they were plated. At 24 h after infection, the medium was replaced with fresh N2 medium. The cells were cultured for another 24 h and then collected for immunoblot assay.

In Vivo Retroviral Injection. Four-week-old mice were anesthetized with medetomidine (0.3 mg/kg), midazolam (4 mg/kg), and butorphanol (5 mg/kg). The virus suspension was injected stereotaxically into the bilateral DG using the following coordinates relative to bregma: caudal, −2.0 mm; lateral, ±1.4 mm; ventral, −2.1 mm. Eight weeks after the viral injection, KA was intraperitoneally injected into the mice to evaluate seizure susceptibility as described above. After the 1-h trial, their brains were fixed and IHC was performed. Mice lacking GFP-positive cells in the bilateral DG were excluded from the study.

Isolation of NS/PCs. NS/PCs were isolated as described previously (52, 53). Briefly, after cervical dislocation, the forebrain (E15) or DG (P5 and 12w) was dissected from *Nestin*-EGFP mice and minced with a scalpel. The tissue was transferred into prewarmed PDD enzyme mix (papain, 2.5 U/mL; dispase, 1 U/mL; and DNase,

250 U/mL) and dissociated into single cells by pipetting with a fire-polished Pasteur pipette. Activity of the PDD enzyme mix was stopped by the addition of a solution containing 4% BSA. After sucrose gradient centrifugation, the cells were suspended in Hanks' balanced salt solution. The cells were sorted on a FACS Aria II (BD Biosciences), with 7-AAD (559925; BD Pharmingen) added to exclude nonviable cells from the analysis. Cells dissociated from the forebrain or DG of WT mice were used to draw gates for GFP+ and GFP− cell populations. The GFP+ NS/PCs were sorted into N2 medium, centrifuged at 2,000 × *g* for 10 min at 4 °C, and stored in liquid nitrogen.

mRNA Isolation and cDNA Library Preparation. We isolated ∼150,000 cells from one mouse at E15 and P5 to construct a cDNA library for each sample. However, we used 7–10 mice at 12w to obtain 4,000–5,000 cells for cDNA library preparation because we were able to isolate only ∼500 NS/PCs per mouse. mRNAs were isolated from the NS/PCs with a Dynabeads mRNA DIRECT Micro Kit (Invitrogen), and the purified mRNAs were subjected to cDNA library construction using an NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs) following the manufacturer's protocols. Briefly, polyadenylated mRNA was isolated directly from the cells using Dynabeads Oligo $(dT)_{25}$ (Invitrogen). mRNA was fragmented in NEBNext First Strand Synthesis Reaction Buffer by heating at 94 °C for 15 min. First-strand cDNA was reverse-transcribed from the fragmented mRNA and then used as template to synthesize second-strand cDNA. dTTP was replaced with dUTP during the second-strand cDNA synthesis. The cDNA was end-repaired, dA-tailed, and ligated with NEBNext Adaptor. The second-strand cDNA containing dUTP was digested by USER enzyme, and a sequencing tag and barcode were introduced through a 15-cycle PCR amplification. The cDNA library was purified using AMPure XP beads (Beckman Coulter). Quality of the cDNA library was assessed using an Agilent 2100 BioAnalyzer (Agilent Technologies).

RNA Sequencing and Bioinformatics Analysis. RNA was sequenced by 50-bp single-end sequencing using an Illumina HisEq 2500. Obtained reads were processed with the FASTX tool kit (54) to remove short (<20 bp) and low-quality (quality score <20) reads, followed by trimming of the adaptor sequence. Processed reads were mapped to the mouse mm10 genome using TopHat/Bowtie2 (55). Cuffdiff (56), a program in the Cufflinks software platform, was used for differential gene expression analysis. Hierarchical clustering was performed with R, based on gene expression levels. GO analysis was performed using the Database for Annotation, Visualization and Integrated Discovery. We analyzed GO term enrichment in the Biological Process category. To focus on highly expressed genes, up- and down-regulated genes whose expression was less than 10 fragments per kilobase of transcript per million mapped reads in NS/PCs of VPA mice and Ctrl mice, respectively, were filtered for GO analysis. GSEA was carried out using signal-to-noise as the ranking metric and with the "weighted" scoring scheme. For extracting genes associated with neuronal migration and for GSEA, gene lists were prepared from the Mouse Genome Informatics database.

Fig. S1. The number of GABAergic interneurons in the hippocampus of adult mice is not altered by prenatal VPA exposure. (*A*) Representative images of PV-positive (green) interneurons in the hippocampus. (Scale bar, 200 μm.) (*B* and *C*) Quantification of the number of PV-positive cells in DG (*B*) and CA3/CA1 (*C*) ($n = 3$ animals each). (*D*) Representative images of SST-positive (red) interneurons in the hippocampus. (Scale bar, 200 μm.) (*E* and *F*) Quantification of the number of SST-positive cells in DG (*E*) and CA3/CA1 (*F*) ($n = 3$ animals each). (*G*) Representative images of CR-positive (red) interneurons in the hippocampus. (Scale bar, 200 μm.) (*H* and *I*) Quantification of the number of CR-positive cells in DG (*H*) and CA3/CA1 (*I*) ($n = 6$ animals each). n.s., not significant.

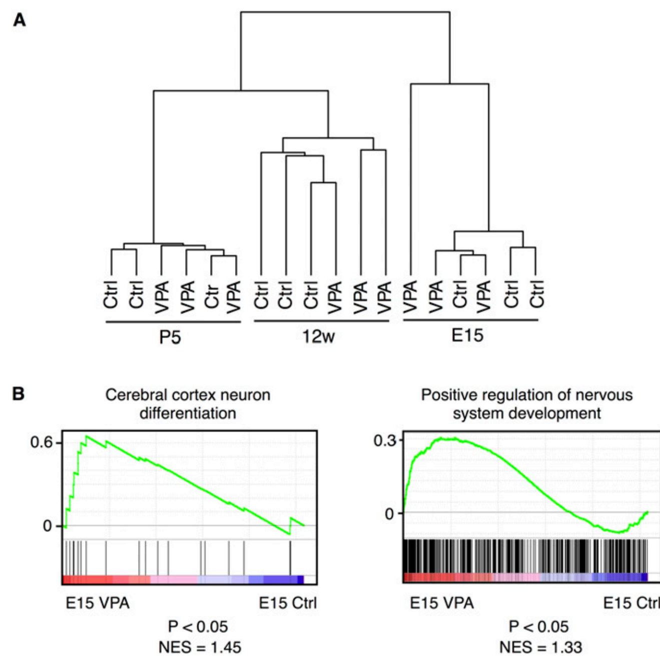

Fig. S2. Transcriptome profiling of NS/PCs in control and VPA mice during development. (*A*) Hierarchical clustering of NS/PCs isolated from Ctrl and VPA mice at the three developmental stages. (*B*) GSEA plots of NS/PC genes expressed in E15 Ctrl and VPA mice for the lists of genes categorized in GO terms “cerebral cortex neuron differentiation” and “positive regulation of nervous system development.” NES, normalized enrichment score.

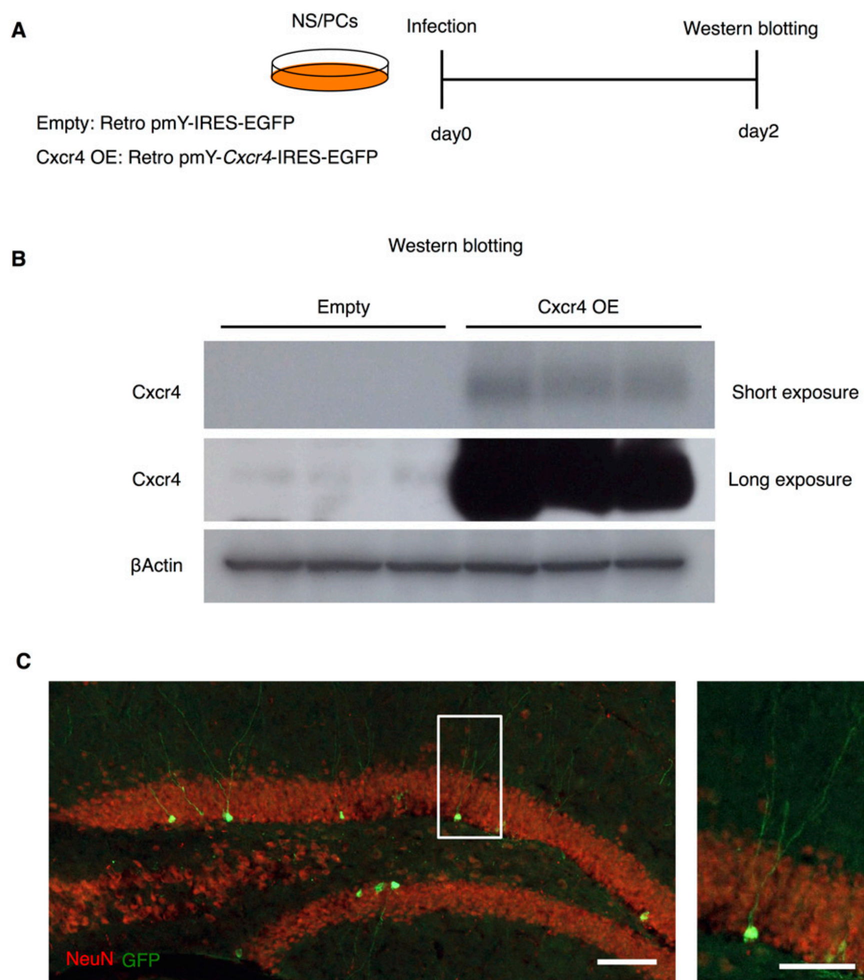

Fig. S3. Retrovirus-mediated expression of Cxcr4 in NS/PCs. (*A*) Schematic representation of retroviral infection in NS/PCs in vitro. (*B*) Western blotting analysis showing the level of Cxcr4 protein, which was increased in NS/PCs infected with *Cxcr4*-containing retrovirus. Expression of endogenous Cxcr4 protein was also detected with a long time exposure. (*C*) Representative image of GFP (green) and NeuN (red) dual-positive newborn neurons in the DG at 8 wk after retrovirus injection. (Scale bar, 100 μm.) The area outlined by the white rectangle is enlarged to the right. (Scale bar, 50 μm.)

Fig. S4. Impairment of hippocampal neurogenesis and seizure susceptibility are not yet explicit in 4w mice. (*A*) Experimental scheme for investigating seizure susceptibility and hippocampal neurogenesis in 4w Ctrl and VPA mice. (*B*) Representative images of DCX-positive (cyan) immature neurons in the DG. (*Insets*) H33258 nuclear staining of each field. (Scale bar, 200 μm.) (*C* and D) Quantification of the number of DCX-positive cells in SGZ/GCL (*C*) and hilus (*D*) (n = 5 animals each). (*E*) Seizure response to KA treatment over time in Ctrl (n = 10 animals) and VPA mice (n = 12 animals). Two-way repeated measures ANOVA was used for statistical analysis (treatment: $F_{1,260} = 3.12$, $P = 0.0785$; time: $F_{12,260} = 2.59$, $P = 0.0029$; treatment × time interaction: $F_{12,260} = 0.2691$, $P = 0.9933$). n.s., not significant.

Fig. S5. Model for prenatal VPA exposure-induced impairment in adult neurogenesis and seizure susceptibility. Prenatal VPA exposure induces ectopic neurogenesis resulting in augmentation of seizure susceptibility, probably due to the altered expression of cell migration-related genes including *Cxcr4*. Voluntary running largely restores these defects.