Supporting Information

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SI Materials and Methods

Animals. Male and timed-pregnant female 7-wk-old C57BL/6N mice were obtained from Charles River Japan. All experimental procedures were performed according to the Guidelines for Animal Care and Use at the Yamaguchi University School of Medicine. The experimental protocols were approved by the Committee on the Ethics of Animal Experiments at the Yamaguchi University School of Medicine.

Quantitative PCR and RT-PCR. Quantitative PCR (qPCR) was performed as reported previously (1). The total RNA from dissected tissues was extracted using TRIzol Reagent (Invitrogen) and treated with DNase (DNA-free kit; Ambion). The quality of the RNA (A_{260}/A_{280}) was 1.8–2.0 for all RNA preparations. One microgram of total RNA was used for cDNA synthesis using the QuantiTect Reverse Transcription kit (Qiagen). The cDNA was stored at −80 °C until use. Real-time PCR was performed using the Applied Biosystems 7300 Fast Real-Time PCR System with SYBR green PCR master mix (Applied Biosystems) according to the manufacturer's protocol. The PCR conditions were 15 min at 95 °C followed by 45 cycles of 15 s at 95 °C and 30 s at 60 °C. The following primers were used for qPCR:

Heat shock factor 1 (*Hsf1*) forward (5′-GGACAGGAACAGCTCCTTGA-3′), reverse (5′-TCCTGTTTCCCCTTCATCAG-3′)

Sialyltransferases II (St8siaII) forward (5′-GGCTGTGGCCAGGAGATTG-3′), reverse (5′-GGCATACTCCTGAACTGGAGCC-3′)

Sialyltransferases IV (St8siaIV) forward (5′-GCACCAAGAGACGCAACTCATC-3′), reverse (5′-CAGAGCTGTTGACAAGTGATCTGC-3′)

Neural cell adhesion molecule 1 (*Ncam1*) forward (5′-GGATGCCTCCATCCACCTC-3′), reverse (5′-GGCCGTCTGATTCTCTACATAGG-3′)

Gapdh forward (5′-AGGTCGGTGTGAACGGATTTG-3′), reverse (5′-TGTAGACCATGTAGTTGAGGTCA-3′)

18S rRNA forward (5′-GCAATTATTCCCCATGAACG-3′), reverse (5′-GGCCTCACTAAACCATCCAA-3′)

Amplification of a single PCR product was confirmed by monitoring the dissociation curve and by electrophoresis on 1.5% agarose gels stained with ethidium bromide. All measurements were performed in triplicate. *Gapdh* mRNA levels were used to normalize the relative expression levels of target mRNAs. When a significant difference was observed between experimental groups, the data were confirmed further by normalizing to the other internal control, 18S rRNA. RT-PCR was performed using Platinum Taq DNA polymerase (Invitrogen) according to the manufacturer's protocol. The PCR conditions were 1 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 30 s at 72 °C. Amplification of single PCR products was visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

Western Blotting. Western blotting was performed as described previously (1, 2). The medial prefrontal cortex tissues were pooled from two or three animals. Twenty or fifty micrograms of protein were separated on 7% or 12% Tris-Acetate gels and transblotted onto polyvinylidene difluoride membranes (GE Healthcare Bio-Sciences). The membranes were incubated with antibodies directed against heat shock factor 1 (HSF1) (1:500; ref. 2), polysialic acid–neural cell adhesion molecule (PSA-NCAM) (1:1,000) (Chemicon), postsynaptic density protein 95 (PSD95) (1:1,000, Thermo Scientific), GAPDH (1:4,000, Santa Cruz Biotechnology), or HA-tag (1:10,000) (Abcam). After incubation with an appropriate HRP-conjugated secondary antibody, the blots were developed with an ECL-Plus detection kit (GE Healthcare Bio-Sciences). Densitometric analysis was performed using Inquiry software (Neuroscience Inc.).

Nissl Staining. Adult (8-wk-old) mice were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde in 1× PBS. Brains were postfixed overnight in 4% paraformaldehyde and cryoprotected in 30% sucrose in 1× PBS. The brains were sectioned (30 μm) using a cryostat and stained for Nissl using a standard thionin staining protocol.

BrdU Labeling. BrdU (100 mg/kg, i.p.) (Sigma) was administered to the mice. To analyze cell proliferation, 8-wk-old mice were killed 2 h after a single injection of BrdU. To analyze cell survival and cell type, 8-wk-old mice were given four injections of BrdU (once daily for 4 consecutive days) and killed after 4 wk.

Immunofluorescent Staining. Mice were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde in 1× PBS. Their brains were postfixed overnight in 4% paraformaldehyde and cryoprotected in 30% sucrose in 1× PBS. The brains were sectioned (30 μm) using a cryostat, and single, double, or triple immunocytochemistry was performed on free-floating sections. Primary antibodies included the mouse monoclonal antibodies neuronal nuclei (NeuN) (1:1,000) (Chemicon), glial fibrillary acidic protein (GFAP) conjugated with Alexa Fluor 568 (1:500) (Cell Signaling), and PSA-NCAM (1:500) (Chemicon). A rat polyclonal antibody was conjugated with BrdU (1:500) (Abcam) and a rabbit polyclonal antibody with GFP (1:500) (Invitrogen). A goat polyclonal antibody against doublecortin (DCX) (1:400) was obtained from Santa Cruz Biotechnology. Secondary antibodies were conjugated with Alexa Fluor 350, 488, or 568 (1:500) (Molecular Probes). Images were acquired using an LSM 510 META laser confocal microscope (Zeiss) with multichannel excitation and detection options including optimal factory-recommended filter configurations to minimize spectral bleed-through. To analyze cell proliferation, $BrdU^+$ cells in every sixth bilateral section throughout the subgranular zone were counted. To analyze cell survival, $BrdU^+$ cells in every sixth bilateral section throughout the granule cell layer were counted. The number of $BrdU^+$ cells then was multiplied by 6 to estimate the total number of $BrdU^+$ cells. To correct for overestimations linked to counting the same nucleus on two adjacent sections, the following equation was applied: $n = (n \times t)/(t + d)$, where t is the section thickness, d is the nuclear diameter, and n is the number of counted nuclei (3). To analyze cell-type markers, $BrdU^+$ cells in the granule cell layer of each mouse were examined by confocal microscopy (LSM 510 META; Zeiss). The quantification of DCX^+ cells was performed similarly to the quantification of BrdU labeling.

Analysis of Dendritic Length and Spine Density. Golgi staining was performed using the FD Rapid Golgi Stain kit according to the manufacturer's protocol (FD NeuroTechnologies). In brief, freshly dissected brains were immersed in solutions A and B for

1 wk at room temperature and transferred to solution C for 24 h at 4 °C. The brains were sliced using a vibratome at a thickness of 100 μm. Bright-field microscopic (Keyence) images (at 20× or 60× magnification) of granule neurons in the dentate gyrus and of CA3 pyramidal neurons (80 neurons per genotype) were obtained. Only fully impregnated neurons displaying dendritic trees without obvious truncations and isolated from neighboring impregnated neurons were retained for analysis. The total dendrite length was quantified by tracing entire granule neurons. Quantification of spine density was limited to dendrites 100–150 μm from the soma. Spine density is expressed as the number of spines per micrometer of dendritic length.

Behavioral Procedures. In the present study, behavioral tests generally were performed in a single cohort in the following order: social investigation test, novelty-suppressed feeding test, forced swim test, sucrose preference test, and social resident–intruder test. Hidden food tests, open field tests, and Rotarod tests were performed in a different set of experiments. All experiments were conducted blind to the treatment condition of the animal.

Novelty-suppressed feeding test. Animals were housed individually, and food pellets were removed from their cages. Water remained available ad libitum. Twenty-four hours after food removal, the animals were transferred to a clean holding cage in the testing room. The testing apparatus consisted of a square open field chamber (30 cm long × 30 cm wide × 20 cm high). A piece of chow was placed in the center of the testing apparatus. Each subject was placed in a corner of the testing apparatus, and the latency to the first feeding episode was recorded for 5 min.

Open field test. Mice were placed in the center of a square open field chamber (50 cm long × 50 cm wide × 40 cm high) surrounded by walls. The total length of the path the mouse traveled (locomotor activity) and the time it spent in the 30 cm × 30 cm center square (% center) were measured over the course of 5 min using an automatic monitoring system (O'Hara Co., Ltd.).

Forced swim test. Animals were placed in a water tank (25 cm high × 15 cm in diameter filled with 23 °C water to a depth of 15 cm) for 5 min, and the duration of floating (i.e., the time during which the animal made only the small movements necessary to keep its head above water) and the latency to the first immobility bout were scored. The animals then were dried and returned to their home cage.

Sucrose preference test. Animals were habituated to drink water from two bottles for 7 d. Then they were subjected to 72 h of forced exposure to 1% sucrose solution to avoid neophobia. After the stress sessions, the animals were subjected to water deprivation for 16 h before the sucrose preference test was performed. Two preweighed bottles, one containing tap water and the other containing 1% sucrose solution, were presented to each animal for 4 h. The position of the water and sucrose bottles (left or right) was switched every 2 h. The bottles were weighed again, and the weight difference represented the animal's intake from each bottle. The sum of water plus sucrose intake was defined as the total intake, and sucrose preference was expressed as the percentage of sucrose intake relative to the total intake.

Social investigation. Mice were separated by transferring them to a new standard cage with fresh bedding material 2 h before starting the experiment. A social interaction session consisted of a 3-min exposure to a juvenile conspecific in the adult's cage. During the exposure, the duration of active investigatory behavior (mainly sniffing and licking of the anogenital region, mouth, ears, trunk, and tail of the juvenile and close following of the juvenile) was recorded, and this session was repeated three times on consecutive days using the same juvenile mouse. On day 4, an unfamiliar juvenile mouse was introduced into the adult's cage, and the duration of active social investigatory behavior by the adult mouse was recorded. As control experiments, we used an anesthetized mouse as the unfamiliar mouse, and the social interaction times of subject animals were measured as described above.

Social resident–intruder test. To assess the offensive aggressiveness of the animals, experimental mice were housed individually for 4 wk. Then an ICR male intruder mouse, which was housed in a group of five, was introduced into the cage of the experimental resident mouse. The latency to the first attack bout (attack latency) of the resident mouse was measured.

Hidden food test. Mice were deprived of food overnight before testing. A small piece of food was buried in the home cage under 3 cm of bedding. The location of the food and the starting position of the mouse were the same for each animal. The latency to feeding (i.e., when the mouse had found the food and grasped it with both paws) was recorded for 5 min.

Rotarod test. Mice were placed on a rotating drum (3 cm in diameter; O'Hara Co., Ltd.). The drum initially was rotated at a speed of 4 rpm and was gradually accelerated to 40 rpm over the course of 5 min. The amount of time that the mouse remained on the accelerating rod (running time) was measured as an indicator of motor performance. This test was performed three times consecutively.

Generation of DNA Constructs. The cDNA of a constitutively active form of HSF1 (caHSF1) was amplified by PCR with the phHSF1ΔRDT expression vector (2) and constructed by inserting Kozak and HA-tag sequences followed by an initiation codon into the pcDNA3.1 vector (Invitrogen). The plasmid DNA pcDNA3.1-EGFP was constructed by insertion of the EGFP cDNA fragment from the pEGFP-C1 plasmid (Clontech) into the pcDNA3.1 vector.

In Vivo Plasmid Transfection. caHSF1 or control EGFP expression vectors were transfected directly into the hippocampus or medial prefrontal cortex by polyethylenimine (PEI)-mediated gene delivery. Plasmid DNA/PEI complexes were prepared according to the manufacturer's protocol (in vivo-jetPEI; PolyPlus Transfection). In brief, 10 μg pcDNA3.1-caHSF1 or pcDNA3.1-EGFP plasmid DNA was diluted in a sterile solution of 5% glucose to a final volume of 16.8 μL and complexed with 3.2 μL of linear PEI. Pups at postnatal day 1 or 2 were cryoanesthetized on ice for 5–10 min and placed in a stereotaxic frame (Narishige). A dot was drawn on the skin at the insertion point using a fine felt-tipped marker. For injections into the hippocampus of the neonates, the insertion point of the 30-gauge injection needle was 0.7 mm lateral to the superior sagittal sinus and 2.0 mm rostral to the lambda. The needle was inserted to a depth of 1.8 mm from the surface of the skin. Five hundred nanoliters of PEI-plasmid complex were injected using a 5-μL Hamilton syringe. After 5 min, the needle was withdrawn slowly. The pups were placed on a heating pad with their original nesting material for 3–5 min and then were returned to their mother for further recovery. The accuracy of the coordinates was determined in pilot experiments in which methylene blue dye was infused in place of the PEI/DNA complex and brains were sliced to determine the distribution of the dye. Three days after surgery, successful transduction of the foreign genes was confirmed by immunohistochemistry and Western blotting for EGFP (rabbit anti-GFP; Invitrogen) and HA (rabbit anti-HA; Abcam), respectively. Additionally, we confirmed that successful transduction of caHSF1 lasted for 7 d, but not for 14 d, after the injection of the PEI/ca*Hsf1* complex. On postnatal day 21, the mice were weaned and placed in cages (three or four mice per cage). For adults, PEI/*Egfp* or PEI/ca*Hsf1* complexes were injected bilaterally into the dentate gyrus region of the hippocampus at a rate of 0.2 μL/min using the following stereotaxic coordinates: anteroposterior = −2.0 mm, mediolateral = ±1.6 mm, dorsoventrall = −1.8 and −2.3 mm (relative to the dura; ref. 4). The mice were subjected to behavioral assays 3 wk after surgery.

We confirmed that the successful transduction of caHSF1 lasted for at least 21 d after the injection of the PEI/ca*Hsf1* complex.

Repeated Restraint Stress Procedure. Adult (8–9 wk old) mice were weighed and individually subjected to restraint stress (2 h/d) for 14 consecutive days by placing them into a 50-mL plastic centrifuge tube. Animals subjected to nonrestraint stress were left in their home cages and were handled for 14 consecutive days during weighing.

ChIP. The dissected tissues of the hippocampus were minced into ≈1-mm pieces that were frozen immediately on dry ice and stored at −80 °C until further use. To crosslink protein–DNA complexes, tissues were placed in 1% formaldehyde for 15 min at room temperature. Fixation was quenched by adding glycine at a final concentration of 0.125 M. The tissue was washed three times with ice-cold PBS containing protease inhibitors (Complete Tab; Roche Diagnostics) and then homogenized with 12 strokes in 10 mM Tris, 10 mM NaCl, and 0.2% Nonidet P-40. The homogenate was centrifuged at 4,500 × *g* for 5 min. The supernatant was removed, and the cell pellet then was homogenized two more times using nuclear lysis buffer (Millipore) with protease inhibitors. Each sample was sonicated on ice, resulting in genomic DNA fragments ranging in size from 200–1,000 bp. Nuclear lysates were centrifuged at 20,000 × *g* for 20 min to remove insoluble material. The resulting lysates were precleared for 2 h at 4 °C with Protein A/G PLUS agarose beads (Santa Cruz Biotechnology) and were immunoprecipitated overnight at 4 °C with antiserum against mouse HSF1 (5) or preimmune serum as a negative control. Chromatin–antibody complexes were collected with Protein A/G PLUS agarose beads and were washed sequentially with low-salt, high-salt, LiCl, and TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) (twice) buffers. Chromatin was eluted with $NaHCO_3$/SDS buffer. ChIP, input (reserved from the preclearing step), and negative control samples were incubated under high-salt conditions at 65 °C overnight for crosslink reversal. DNA fragments then were purified by treatment with RNaseA and proteinase K followed by multiple extractions with phenol/chloroform/3-methylbutan-1-ol. The purified DNA samples were subjected to semiquantitative PCR. The following primers were used:

St8siaII-P1 forward (5′-AGCTCTGAGATGCCCTCTTT-3′′), reverse (5′-CCCAAGAGTAGCACACTTTC-3′)

St8siaII-P2 forward (5′-GAGGAGCATCTCCCTTTGTG-3′), reverse 5′-CGAGTTGTTTGAGGTCATGG-3′)

St8siaII-P3 forward (5′-CTTCCTAGGCCGGGAGTG-3′), reverse (5′-GCAGTTTGACAGCTTTGCTC-3′)

St8siaIV-P4 forward (5′-ATTTGCGTGGACATCAATCC-3′), reverse (5′-ATCTCTGCAGGACAGGCAAC-3′)

St8siaIV-P5 forward (5′-GCTGATGTGGAAAGTGCAAA-3′), reverse (5′-AGCCTGGAAACCAGACTCCT-3′)

St8siaIV-P6 forward (5′-AGCTGGGATCCTCATTACCC-3′), reverse (5′′-AACATACGTAAACGCACGGG-3′)

The semiquantitative PCR products were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

Endoneuraminidase (Endo-N) Treatment. To eliminate PSA-NCAMs, the highly specific PSA-glycosidase endo-N (Abcys) was used. Heat-inactivated endo-N was used as a control. The injection of 0.35 U endo-N per hemisphere into the neonatal hippocampus was performed as described above. For injection into the adult hippocampus, mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame. The skull was exposed, and a small portion of the skull over the hippocampus was removed bilaterally with a dental drill. Endo-N (0.7 U per hemisphere) or heat-inactivated endo-N (hi-endo-N) was injected bilaterally into the hippocampus at rate of 0.2 μL/min using the following stereotaxic coordinates: anteroposterior = −2.0 mm, mediolateral = ±1.7 mm, dorsoventral = −2.0 mm from the bregma. The mice were subjected to behavioral experiments 7 d after surgery. After the end of the behavioral experiments, successful removal of PSA-NCAMs from the hippocampus was confirmed by immunofluorescence.

Statistical Analysis. Analyses of the data from the three genotypes were performed using one-way ANOVA. Analyses of the behavioral data in Fig. S4 were performed using two-way ANOVA, with genotype and stress exposure (nonrestraint, repeated restraint) as fixed factors. Grouped data obtained from the social investigation (Fig. 3*I* and Fig. S5*B*) and Rotarod test (Fig. 3*L*) were analyzed by two-way ANOVA, with the genotype and the session as fixed factors. Significant effects were followed up with Bonferroni's post hoc tests. Unpaired *t* tests were used for two-group comparisons. In all cases, *P* values were two-tailed, and comparisons were considered statistically significant at $P < 0.05$. Data are presented as means ± SEM.

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2. Fujimoto M, et al. (2005) Active HSF1 significantly suppresses polyglutamine aggregate formation in cellular and mouse models. *J Biol Chem* 280:34908–34916.
3. Abercrombie M (1946) Estimation of nuclear population from microtome sections. *Anat Rec* 94:239–247.
4. Paxinos G, Franklin KBJ (1997) *The Mouse Brain in Stereotaxic Coordinates* (Elsevier Academic, San Diego).
5. Takaki E, et al. (2006) Maintenance of olfactory neurogenesis requires HSF1, a major heat shock transcription factor in mice. *J Biol Chem* 281:4931–4937.

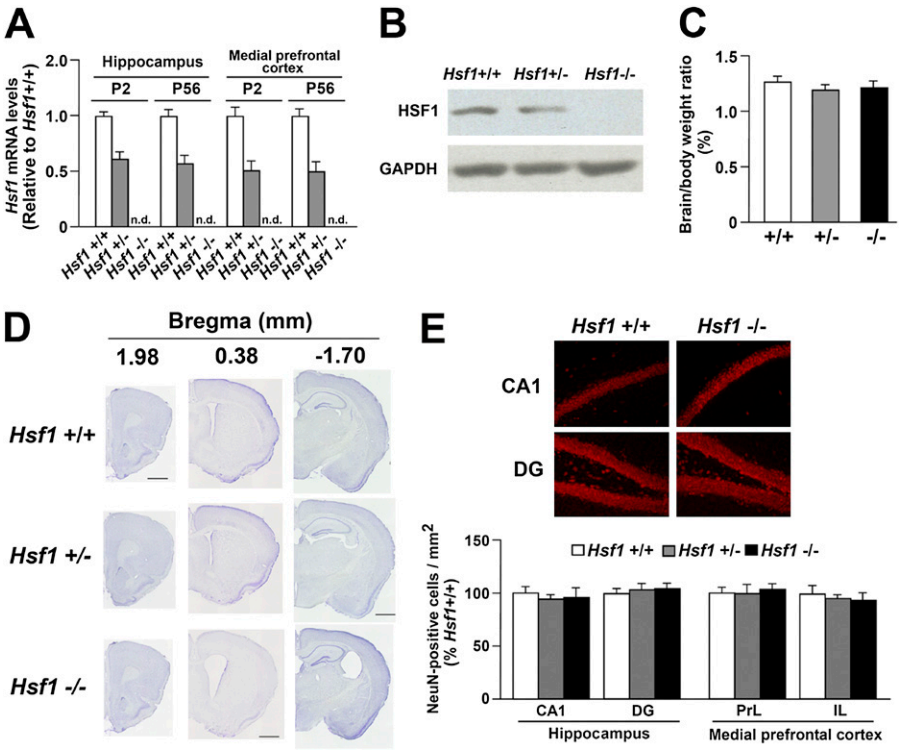

Fig. S1. Characterization of the brains of HSF1-deficient mice. (*A*) Real-time PCR analysis of the expression levels of HSF1 mRNA in the hippocampus and medial prefrontal cortex of HSF1$^{+/+}$, HSF1$^{+/-}$, and HSF1$^{-/-}$ mice on postnatal days 2 and 56 (n = 6 for each group). n.d., no data. (*B*) Western blotting of HSF1 protein levels in the hippocampus of HSF1$^{+/+}$, HSF1$^{+/-}$, and HSF1$^{-/-}$ mice at postnatal day 2. (*C*) Bar graph showing the brain/body weight ratios (percentage) of mice at postnatal day 56 (n = 5 for each group). (*D*) HSF1$^{+/+}$, HSF1$^{+/-}$, and HSF1$^{-/-}$ brain sections were Nissl stained. (*E*) NeuN staining showed no obvious changes in the densities of CA1 pyramidal neurons, dentate gyrus (DG) granule neurons, or pyramidal neurons of prelimbic (PrL) and infralimbic (IL) regions of the medial prefrontal cortex in HSF1-deficient mice at postnatal day 56 (n = 6 for each group).

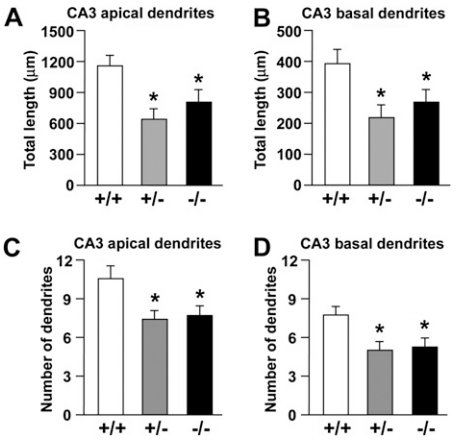

Fig. S2. Effect of HSF1 deficiency on the dendritic morphology of CA3 pyramidal neurons. Bar graphs show the total length of apical (*A*) and basal (*B*) dendrites and the total number of apical (*C*) and basal (*D*) dendrites in CA3 pyramidal neurons of HSF1$^{+/+}$, HSF1$^{+/-}$, and HSF1$^{-/-}$ mice (n = 5 for each group). *$P < 0.05$ vs. HSF1$^{+/+}$.

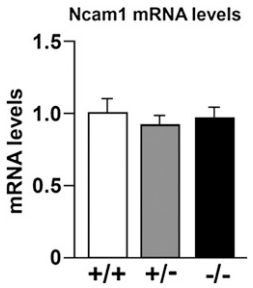

Fig. S3. Expression analysis of NCAM1 mRNA. Real-time PCR analysis showing that HSF1 deficiency had no significant effect on the mRNA expression of Ncam1 in the hippocampus of HSF1$^{+/+}$, HSF1$^{+/-}$, or HSF1$^{-/-}$ mice on postnatal day 2.

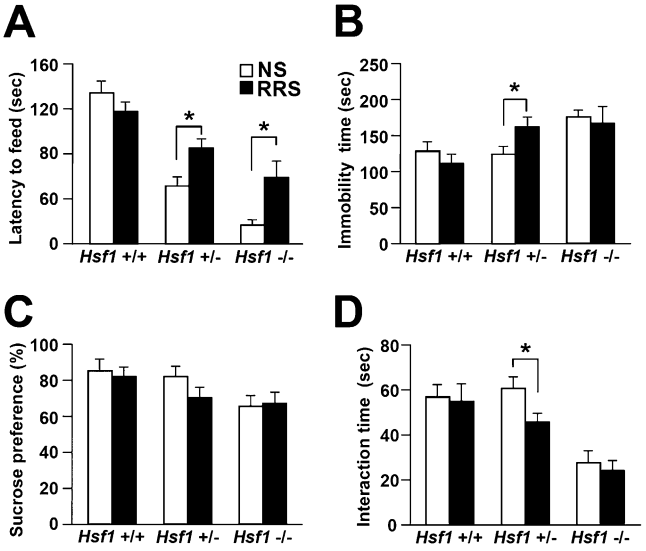

Fig. S4. Effect of HSF1 deficiency on the development of stress vulnerability. Adult mice were subjected to repeated restraint stress (RRS) or nonrestraint stress (NS) for 14 consecutive days and then assessed for anxiety- and depression-related behavior in the sucrose preference, forced swim, social investigation, and novelty-suppressed feeding tests (n = 11–14 for each group). Bar graphs show (*A*) latency to feed in the novelty-suppressed feeding test, (*B*) immobility times in the forced swim test, (*C*) sucrose preference in the sucrose preference test, and (*D*) interaction times in the social investigation test. *$P < 0.05$.

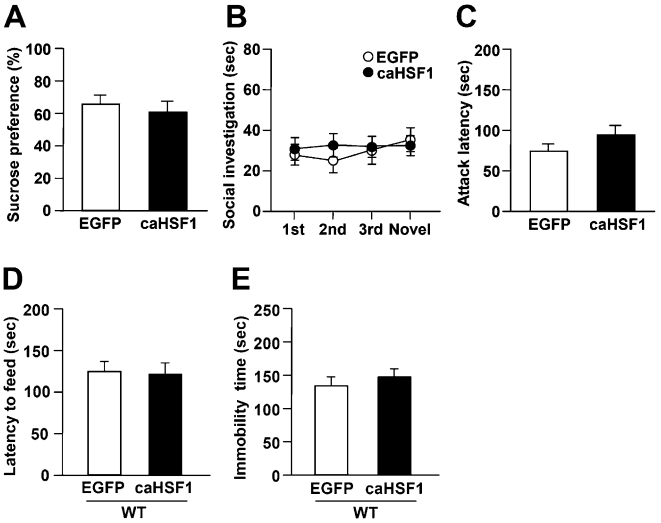

Fig. S5. Effect of rescuing the HSF1 deficiency in the neonatal hippocampus on behavioral development. Bar graphs show (*A*) sucrose preference in the sucrose preference test (n = 14–16 for each group), (*B*) interaction times in the social investigation test (n = 12–14 for each group), and (*C*) latency to first attack bout in the social resident–intruder test (n = 14–16 for each group). (*D* and *E*) Wild-type pups at postnatal days 1 and 2 were injected with PEI/*Egfp* or PEI/*caHsf1* bilaterally into the hippocampus. Behavioral assays were performed during adulthood. The bar graphs show (*D*) the latency to feed in the novelty-suppressed feeding test and (*E*) the immobility time in the forced swim test (n = 9–11 for each group).

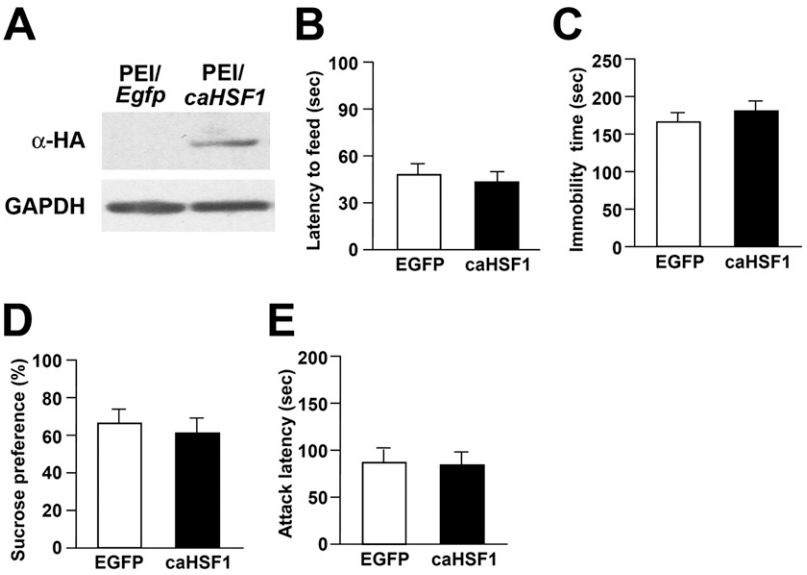

Fig. S6. Effect of rescuing the HSF1 deficiency in the adult hippocampus on behavioral development. (*A*) Western blotting with anti-HA antibody shows the transduction of HA-caHSF1 in the hippocampus of mice 2 wk after the injection of PEI/*caHsf1* complex. (*B–E*) Behavioral assays were performed 2 wk after injection of the PEI/*caHsf1* complex (n = 10–12 for each group). Bar graphs show (*B*) latency to feed in the novelty-suppressed feeding test, (*C*) immobility times in the forced swim test, (*D*) sucrose preference in the sucrose preference test, and (*E*) latency to the first attack bout in the social resident–intruder test.

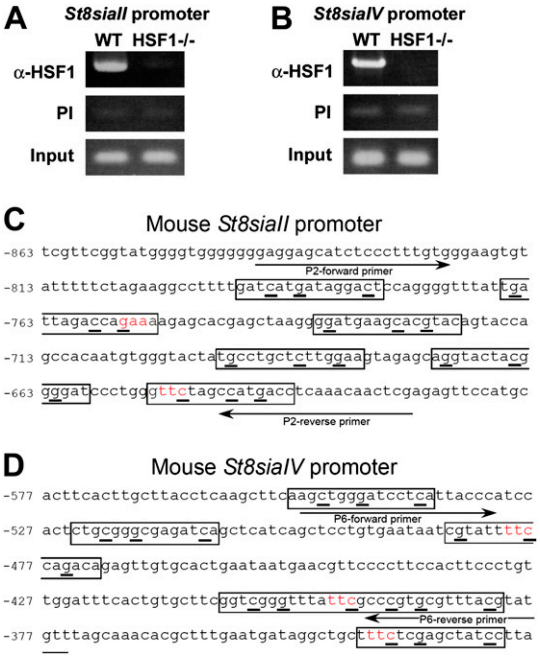

Fig. S7. HSF1 binds to the St8siaII and St8siaIV promoters. (*A* and *B*) ChIP assay showing that HSF1 binds directly to the *St8siaII* (P2 region) (*A*) and *St8siaIV* (P6 region) (*B*) promoters in hippocampal samples obtained from wild-type mice. These interactions were not observed in samples from HSF1$^{-/-}$ mice. α-HSF1, HSF1 antibody; PI, preimmune serum. (*C* and *D*) The nucleotide sequences of the mouse *St8siaII* (*C*) and *St8siaIV* (*D*) promoters are shown. Boxes indicate potential heat shock elements. Underlined nucleotides C and G indicate the conserved nucleotides in the consensus nGAAn sequence, and red characters indicate sequences that match perfectly with the consensus sequence. The PCR primers for the ChIP assay are indicated by arrows.

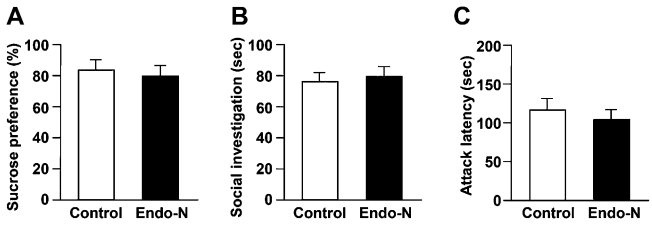

Fig. S8. Effects of enzymatic removal of PSA-NCAMs from the neonatal hippocampus on behavioral development. Bar graphs show (*A*) sucrose preference in the sucrose preference test (n = 14–16 for each group), (*B*) social interaction times in the social investigation test (n = 11 or 12 for each group), and (*C*) latency to attack in the social resident-intruder test (n = 14–16 for each group).

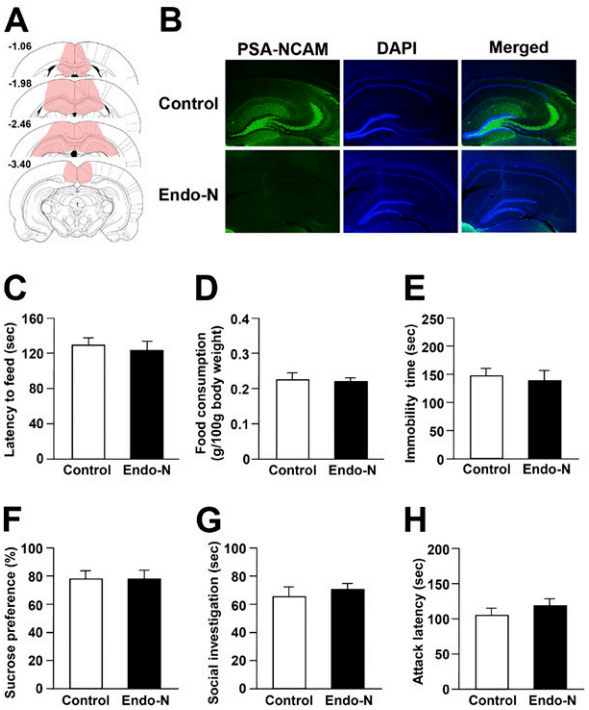

Fig. S9. Effects of enzymatic removal of PSA-NCAMs from the adult hippocampus on behavioral development. Endo-N or hi-endo-N was injected bilaterally into the hippocampus of mice at postnatal day 56. Behavioral assays were performed 7 d after injection. (*A*) Distribution of PSA-NCAM immunoreactivity 7 d after endo-N injection. Colored areas in the brain template represent diffusion of the enzyme. (*B*) Representative immunofluorescence images of PSA-NCAMs (green) and DAPI (blue) in mice injected with hi-endo-N (*Upper*) or endo-N (*Lower*). (*C*–*H*) Bar graphs showing (*C*) latency to feed and (*D*) food consumption in the novelty-suppressed feeding test (n = 14–16 for each group), (*E*) immobility times in the forced swim test (n = 14–16 for each group), (*F*) sucrose preference in the sucrose preference test (n = 11–13 for each group), (*G*) interaction times in the social investigation test (n = 10–12 for each group), and (*H*) latency to attack in the social resident-intruder test (n = 11–13 for each group).