Supplemental Information

Figure S1. Greater spatial restriction of GFP expression to the DG throughout the septo-temporal axis with using two-promoter strategy (A and B) and additional morphological characterization of activated DG-TeTX mice (C and D), related to Figure 1.
(**A** and **B**) The two-promoter strategy robustly restricted GFP expression to the DG throughout the septo-temporal axis. (**A**) Coronal sections from a 12-week-old Tg1xROSA26 mouse (single-promoter strategy) stained with an antibody specific for β-galactosidase (green) and cell nuclei marker, DAPI (blue). Merged images are for DAPI and β-galactosidase. β-galactosidase, an indicator for Cre-loxP recombination, was confined to cell somas and was robustly expressed in the DG as well as modestly in the arcuate nucleus of the hypothalamus and the lateral habenular nucleus. (**B**) Coronal sections of a 12-week-old DG-GFP mouse (Tg1xTg2xTg3-GFP, two-promoter strategy) raised on Dox and followed by a 2-week Dox withdrawal, stained with an antibody specific for GFP (green) and DAPI (blue). Merged images are for DAPI and GFP. GFP was detected in somas or in the axons and dendrites. However, in contrast to the β-galactosidase expression in the Tg1xROSA26 mouse, GFP expression in the DG-GFP mouse was highly restricted to the DG throughout the septo-temporal axis. (**C** and **D**) Lack of abnormalities in the hippocampal cytoarchitecture, MF projection and cell death in activated DG-TeTX mice. (**C**) Triple immunostaining of coronal sections from a 14-week-old activated DG-GFP control mouse and an activated quadruple Tg1xTg2xTg3-GFPxTg3-TeTX (DG-TeTX/GFP) mutant mouse stained with anti-NeuN (blue), anti-GFP (green) and anti-VAMP2 (red). (**D**) Hippocampal sections stained with an anti-VGLUT1 antibody (vesicular glutamate transporter 1, a presynaptic marker for glutamatergic nerve terminals) from 14-week-old control (Tg1xTg3-TeTX) and activated DG-TeTX mice, and hippocampal sections with TUNEL staining (a marker for cell death) from control and activated DG-TeTX mice. Bottom left, a positive control in which the section was treated with DNase I prior to TUNEL staining. Scale bars in **A-B**, 1 mm; **C-D**, 500 μm.

Figure S2. MF excitability is normal in activated DG-TeTX mice, related to Figure 2.
(**A**) Afferent fiber volley (AFV) plotted as a function of stimulus strength for MF inputs in control ACSF (**A**) or in the presence of forskolin (**B**). (**C** and **D**) Same as (**A**), but for PP inputs (**C**) and RC inputs (**D**). Blue circles, control littermates raised on Dox followed by a 6-week Dox withdrawal; green triangles, repressed DG-TeTX mice (always Dox-on); red circles, activated DG-TeTX mice (Dox-on-off) raised under the Dox-on condition followed by 6-week Dox withdrawal; black squares, re-repressed DG-TeTX (Dox-on-off-on) mice, which had been raised under the Dox-on condition and then underwent a 6-week Dox withdrawal, followed by a 4-week Dox readministration. Note that these curves were generated by analysis of recordings used to monitor fEPSP data presented in **Figure 2A** to **D**. The lack of any significant difference across genotype/condition ($p>0.05$, t-test) indicates that the deficit in MF transmission of activated DG-TeTX mice (**Figure 2A** and **B**) is not due to a failure of action potential propagation through the MF pathway or the stimulation of fewer fibers. Data represent mean ± SEM.

Figure S3. Normal adult neurogenesis (A to G) and integrity of MF transmission from young adult-born GCs in activated DG-TeTX mice (H to L), related Figure 3. (**A**)
Hippocampal sections stained with anti-BrdU (red) and anti-NeuN (green) from control and activated DG-TeTX mice 1 day (Proliferation), 4 weeks and 8 weeks following BrdU injection.

(**B**) Number of BrdU-positive cells in the DG. (**C**) Number of Ki67-positive cells (a marker for proliferating cells) in the DG. Dox and BrdU injection schedules are above each panel. There was no difference between the two genotypes in the numbers of BrdU- and Ki67-positive cells (p>0.05 for each condition, t-test). (**D**) Confocal images at a single z-axis with red for BrdU, green for NeuN and blue for GFAP. Optical sections along the horizontal or vertical lines across multiple z-axes are shown on the top and right, respectively. (**E**) Dox and BrdU injection schedules for experiments in **F** and **G**. (**F**) Numbers of BrdU-positive cells in the DG. (**G**) Proportion of NeuN-positive, GFAP-positive and NeuN/GFAP-negative cells among BrdU-positive cells. There was no significant genotype-specific differences in the number of BrdU-positive cells, in the proportion of NeuN-positive, GFAP-positive cells or in the proportion of NeuN/GFP-negative cells among the BrdU-positive cells (p>0.05 for each cell type, t-test). (**H**) A Moloney viral vector encoding Cre/loxP-dependent wheat germ agglutinin (WGA). A Cre-loxP recombination occurring between 1 and 2 weeks after viral injection (Figure S4). (**I**) Dox diet and viral injection schedules. (**J**) DG areas of sections stained with anti-WGA (red) and co-stained with anti-NeuN (green) from control and activated DG-TeTX mice. (**K**) Confocal images of representative neurons from the hilar regions of control and activated DG-TeTX mice. Neurons (top) colored with red (WGA), green (NeuN) and gray (DAPI). The same neurons (bottom) colored with red (WGA) and gray (DAPI). Note that WGA is localized immediately adjacent to DAPI-stained nuclei. (**L**) All presynaptic terminals labeled with anti-VAMP2 (red) remained at some distance away from DAPI-positive nuclei, whereas WGA signals in (**K**) were in close proximity of DAPI-positive nuclei, suggesting that the WGA observed in (**K**) is not at the presynaptic terminals but has crossed synapses and has accumulated in postsynaptic cells, perhaps associated with the endoplasmic reticulum. Scale bars in **A**, 250 μm; **D**, 10 μm; **J**, 250 μm; **K**, 5 μm. Data represent mean ± SEM.

Figure S4. Time course of Cre-loxP recombination along the maturation of adult-born GCs in POMC-Cre (Tg1) mice, related to Figure 4 and S3. (**A**) A Cre-dependent Moloney viral vector (virus 1) encoding GFP. (**B**) A Cre-independent Moloney viral vector (virus 2) encoding mCherry to assess successful virus injection. (**C**) A schedule of analysis relative to the viral injection. (**D** to **L**) Images of the DG from POMC-Cre (Tg1) mice in which viruses 1 and 2 were co-injected into the DG at 1 week (**D** to **F**), 2 weeks (**G** to **I**) and 3 weeks (**J** to **L**) prior to analyses. Hippocampal sections were stained with anti-GFP (green; **D**, **G** and **J**) and anti-mCherry (red; **E**, **H** and **K**). (**F**, **I** and **L**) Merged images. (**M** to **O**) Images of the DG from a wild type mouse co-injected with viruses 1 and 2 into the DG at 3 weeks prior to analyses. The blue color is for DAPI (**D** to **O**). Note that GFP signals were first detected at 2 weeks after viral injection (**G**). mCherry signals were detected at all time points in Tg1 and wild type mice, but no GFP signal was observed in the wild type mouse or in the Tg1 mouse at 1 week after viral injection, indicating that Cre-loxP recombination occurred in the adult-born DG GCs between 1 and 2 weeks of cellular age. Scale bars **D-O**, 250 μm

Figure S5. Activated DG-TeTX mice exhibited no change in the light/dark transition (A), elevated plus maze (B), open field (C), rotarod (D) and hot plate tests (E), related to Figure 5. (**A**) Performance in the light/dark transition test of activated DG-TeTX mice (red) and their control littermates (blue). There was no significant difference between the two genotypes in the distance travelled in each compartment (p>0.05 for both light and dark compartments, t-test), in the total time spent in each compartment (p>0.05 for both light and dark compartments, t-test) or in the

number of transitions between the light and dark compartments (p>0.05, t-test; n=12 per genotype). (**B**) Performance in the elevated plus maze test. No significant difference was observed between the two genotypes in the number of entries into each arm (p>0.05 for both closed and open arms, t-test), in the percentages of time spent in each arm (p>0.05 for both closed and open arms, t-test) or in the total distance travelled on the maze (p>0.05, t-test; n=12 per genotype). (**C**) Performance in the open field test for a 30-min session in a novel chamber. There was no significant difference between the two genotypes in the total distance travelled (ANOVA, genotype, $F_{(1,22)}$=0.013, p=0.912) or in the time spent at the arena center (ANOVA, genotype, $F_{(1,22)}$=0.007, p=0.932; n=12 per genotype). (**D**) Performance in the rotarod test along six trials of activated DG-TeTX mice (n=15) and their control littermates (n=13). No significant difference was detected between the two genotypes (ANOVA, genotype, $F_{(1, 26)}$=0.440, p=0.513). (**E**) Performance in the hot plate test. There was no significant difference between genotypes in the latency to lift the front paws (p>0.05, t-test; n=12 per genotype). Data represent mean ± SEM.

Figure S6. Contextual fear memory acquired during Dox-on condition does not decay during a subsequent 4 week-long Dox off treatment in DG-TeTX mice, related to Figure 7. (**A**) Freezing of DG-TeTX mice and their control littermates (n=12 per genotype) during the five days of fear conditioning under the Dox-on condition (CS; 3 min contextual exposure per day, US; a single footshock per day for 2s. 0.65mA). No difference was observed in freezing levels between genotypes during the five days of fear conditioning (ANOVA, genotype, $F_{(1,22)}$=1.725, p=0.203). (**B**) The conditioned mice were kept in their home cages for 4 weeks under the Dox-off condition and then subjected to context-dependent memory recall by being returned to the original context (no shock) for 3 min while freezing was scored. There was no difference in freezing levels between genotypes (p>0.05, t-test). Data represent mean ± SEM.

Figure S7. Additional data for the spatial pattern completion experiment, related to Figure 7. (A and **B)** During the Morris water maze training of repressed (i.e. Dox-on) DG-TeTX mice (n=23) and their control littermates (n=24), there was no genotype-specific difference in the total distance travelled to reach the hidden platform (**A**, ANOVA, genotype, $F_{(1,45)}$=1.806, p=0.186) or in the swim speed (**B**, ANOVA, genotype, $F_{(1,45)}$=1.450, p=0.235). (**C** and **D**) Latency for the first crossing of the phantom platform during the probe trial with the full set of cues (P1, **C**, p>0.05 between genotypes, t-test) or no cue (P5, **D**, p>0.05 between genotypes, t-test). (**E**) No genotype-specific difference in swim speed during the probe trial with the full set of cues (P4, p>0.05, t-test). (**F** and **G**) The amount of time (sec) the mice spent during the 90s-long probe trial under one (**F**, P2) and the full set of cues (**G**, P4) in the circular (diameter 13 cm) platform location in the target quadrant (TA) and the corresponding locations in each of the other three quadrants (OP, opposite; L, left; R, right). No genotype-specific difference with either one cue or four (i.e. a full set of) cues (ANOVA in TA of P2 and P4, genotype x cue, $F_{(1,45)}$=0.321, p=0.574; genotype, $F_{(1,45)}$=0.947, p=0.336; cue, $F_{(1,45)}$=0.457, p=0.503). Data represent mean ± SEM.

Table S1: Summary of electrophysiological properties of adult generated granule cells.

	Control Mice GFP+ cells 21-28 dpi	DG-TeTx Mice GFP+ cells 21-28 dpi
Intrinsic Membrane Properties	(n = 29 cells)	(n = 15 cells)
Resting potential (mV)	-63 ± 2	-63± 3
Input resistance (MΩ)	389 ± 69	402 ± 51
Time constant (ms)	27 ± 2	31 ± 3
Membrane capacitance (pF)	95 ± 9	82 ± 6
Frequency at 2X threshold (Hz)	15 ± 1	13 ± 1
Spike threshold (mV)	-34 ± 1	-31 ± 2
Spike amplitude (mV)	67 ± 3	67 ± 4
Spike half-width (ms)	1.1 ± 0.07	1.2 ± 0.10
Spike Maximal decay (mV/ms)	-72 ± 5	-64 ± 6
IPSC Properties	(n=6 cells)	(n=7 cells)
sIPSC Amplitude (pA)	-49 ± 8	-44 ± 3
sIPSC Frequency (Hz)	2 ± 0.5	1.4 ± 0.6
sIPSC τ_{decay}	9.7 ± 1.0	9.2 ± 0.4
$GABA_{tonic}$ (pA)	-36 ± 6	-19 ± 5
EPSC Properties	(n=6 cells s/eEPSCs, 8 cells LTP)	(n=6 cells s/eEPSCs, 6 cells LTP)
sEPSC Amplitude (pA)	-10 ± 0.5	-10 ± 0.9
sEPSC Frequency (Hz)	1.5 ± 0.3	1.6 ± 0.9
sEPSC τ_{decay}	5.7 ± 0.6	6.8 ± 0.7
eEPSC AMPA/NMDA ratio	1.4 ± 0.1	1.6 ± 0.2
eEPSC PPR	1.0 ± 0.1	1.1 ± 0.1
$eEPSC_{NMDA}$ τ_{decay}	83 ± 6	85 ± 2
eEPSC TBS LTP (% Control)	171 ± 26	154 ± 14

Table S1. Comparison of passive membrane, firing and synaptic properties of young adult-born GCs in control and DG-TeTX mice, related to Figure 2. Pooled data showing the mean ± SEM (n as indicated) for membrane and synaptic properties of GFP-positive DG GCs in control and activated DG-TeTX mice 3-4 weeks post-injection. No significant difference between genotypes was noted for any parameter with t-tests.

Table S2-1

Measurement for R1	CA1		CA3	
	Control (N=11, n=106)	DG-TeTX (N=16, n=190)	Control (N=9, n=58)	DG-TeTX (N=10, n=78)
Mean firing rate (Hz)	1.25 ± 0.10	1.15 ± 0.06	0.63 ± 0.06	0.93 ± 0.09
Peak rate (Hz)	9.10 ± 0.49	8.27 ± 0.35	5.63 ± 0.49	7.68 ± 0.64*
Field size (% of sampled space)	18.64 ± 1.49	19.91 ± 1.03	13.64 ± 1.16	14.52 ± 1.15
Mean Infield firing rate (Hz)	4.18 ± 0.22	3.84 ± 0.17	2.55 ± 0.22	3.42 ± 0.28
Spatial Information (bits/spike)	1.24 ± 0.06	1.23 ± 0.05	1.57 ± 0.09	1.33 ± 0.07*
Complex spike index (%) (bursting)	20.17 ± 1.14	19.25 ± 0.87	33.44 ± 1.99	29.06 ± 1.74*

* Significantly different from control within region (Mann-Whitney U-test, $p<0.05$)

Table S2-2

Control CA1

		Average Rate (Hz)	Peak Rate (Hz)
Cell 1	Square	1.33	**10.31**
	Circle	0.91	5.07
Cell 2	Square	0.96	5.28
	Circle	0.4	**6.68**
Cell 3	Square	1.7	**11.89**
	Circle	0.52	5.38
Cell 4	Square	1.28	12.47
	Circle	1.4	**13.7**
Cell 5	Square	0.87	4.14
	Circle	1.29	**10.99**

DG-TeTX CA1

		Average Rate (Hz)	Peak Rate (Hz)
Cell 1	Square	1.43	6.98
	Circle	1.63	**9.08**
Cell 2	Square	1.01	**13.9**
	Circle	0.57	5.74
Cell 3	Square	0.36	2.67
	Circle	0.89	**6.98**
Cell 4	Square	1.42	**11.71**
	Circle	1.38	8.51
Cell 5	Square	1.22	**10.44**
	Circle	0.46	4.25

Control CA3

		Average Rate (Hz)	Peak Rate (Hz)
Cell 1	Square	0.98	**9.05**
	Circle	1.51	7.07
Cell 2	Square	1.34	11.68
	Circle	1.42	**19.88**
Cell 3	Square	0.28	**5.43**
	Circle	0.34	2.25
Cell 4	Square	0.44	2.45
	Circle	1.33	**6.23**
Cell 5	Square	0.54	**4.68**
	Circle	0.13	1.77

DG-TeTX CA3

		Average Rate (Hz)	Peak Rate (Hz)
Cell 1	Square	0.74	**5.84**
	Circle	0.21	1.71
Cell 2	Square	1.01	**8.7**
	Circle	1.05	3.2
Cell 3	Square	0.68	4.7
	Circle	1.35	**9.1**
Cell 4	Square	0.02	0.18
	Circle	1.07	**10.72**
Cell 5	Square	0.11	2.5
	Circle	0.59	**6.38**

Table S2. Basic properties of pyramidal cells recorded from CA1 and CA3 during exploration of the familiar arena (RUN1) on day 4. Values are mean ± SEM. N, number of mice; n, number of cells, related to Figure 6. An average firing rate threshold of 0.2 Hz in at least one of the two arenas was used. A total of 190 CA1 and 78 CA3 pyramidal cells from 18 mutant mice and 106 CA1 and 58 CA3 pyramidal cells from 14 control littermates met the criteria. The mean firing rates, mean infield firing rates, and field sizes were similar across genotypes in both CA1 and CA3 (Table S2-1). However, DG-TeTX mutant mice had a higher peak rate as well as slightly lower spatial information (bits/spike) and complex spike indices in CA3 ($p<0.05$ for both, Mann-Whitney U-test). Table S2-2 contains the peak and mean firing rates of the examples used in Figure 6E. Data represent mean ± SEM.

Supplemental Experimental Procedures

All procedures relating to animal care and treatment conformed to Institutional and NIH guidelines. All behavioral experiments were conducted by operators blind to genotype and Dox treatment.

Generation of mice

*α*CamKII-loxP-STOP-loxP-tTA (Tg2) and TetO-TeTX (Tg3-TeTX) transgenic mice were generated in the same manner as previously described (Nakashiba et al., 2008) and maintained in a C57BL/6 genetic background. We selected different Tg2 and Tg3-TeTX transgenic lines (Tg2 line 2 and Tg3-TeTX line 2) from those used in CA3-TeTX mice (Nakashiba et al., 2008) due to difficulties in efficiently obtaining triple-transgenic DG-TeTX mice. The POMC-Cre transgenic line is the same as previously described (McHugh et al., 2007). To obtain DG-TeTX mice, heterozygous Tg1xTg3-TeTX (*POMC-Cre/+, TetO-TeTX line 2/+*) mice were crossed to generate homozygous double transgenic mice (*POMC-Cre/POMC-Cre, TetO-TeTX line 2/TetO-TeTX line 2*). Male homozygous mice were then bred with the female Tg2 line 2 (*αCamKII-loxP-STOP-loxP-tTA line 2/+*). Half of the resultant progeny was thus heterozygous triple-transgenic mice (*POMC-Cre/+, TetO-TeTX line 2/+, αCamKII-loxP-STOP-loxP-tTA line 2/+*), herein referred to as DG-TeTX mice. The other half of the progeny was thus heterozygous double transgenic mice (*POMC-Cre/+, TetO-TeTX line 2/+, +/+*), which did not express TeTX and therefore served as control mice. DG-GFP mice were generated in a similar manner by using Tg3-GFP mice (Nakashiba et al., 2008) instead of TetO-TeTX line 2 during the breeding procedure. In the Tg3-GFP line, a bi-directional TetO promoter (pBI from Clontech) drives GFP and NR3B gene. Tail DNA from all offspring was genotyped by PCR to detect the presence of each transgene separately. PCR primers and conditions were as previously described (Nakashiba et al., 2008).

Doxycycline (Dox) treatment

DG-GFP mice, DG-TeTX mice and their control littermates were raised with drinking water containing 10 μg/ml Dox (Sigma) supplemented with 1% sucrose (Sigma) from the time of conception to weaning (3 weeks old) and then with food containing 10 mg Dox per kg (Bioserve) from weaning to adulthood. This protocol was sufficient to repress GFP expression in DG-GFP mice as assessed by immunohistochemistry with the GFP antibody (Figure 1) and TeTX expression as assessed with the VAMP2 antibody (Figure 1).

General histology

All mice were transcardially perfused with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), and brains were post-fixed by the same fixative overnight. For vibratome sections, the brains were sliced (50 μm thick) after overnight post-fixation. For cryostat sections, the brains were further processed in 30% sucrose, embedded in OCT compound (SAKURA) and frozen on dry ice before slicing (50 μm thick). For GFP/Prox-1/NeuN triple staining, cryostat sections were first blocked with TNB (TSA System, PerkinElmer) containing 3% normal goat serum for 30 min and incubated with primary antibodies (rat anti-GFP, 1/500, Nacalai USA; rabbit anti-Prox 1, 1/400, Millipore and mouse anti-NeuN, 1/100, Millipore) diluted in the same blocking solution at 4˚C overnight. After rinsing sections with TNT (100 mM Tris-HCl, 150 mM NaCl and 0.3% Triton-X100), sections were incubated with secondary antibody solution (Alexa

488-conjugated anti-rat IgG, Alexa 568-conjugated anti-rabbit IgG and Alexa 647-conjugated anti-mouse IgG; all were diluted 1/200 with TNB containing 3% normal goat serum) for 2 hours at room temperature. The sections were then rinsed with PBS, incubated with DAPI (Invitrogen) and mounted on glass slides.

For GFP/doublecortin (DCX)/NeuN triple staining, vibratome sections were treated in the same manner as above, except goat anti-DCX (1/250, Santa Cruz Biotechnology) and normal donkey serum were used in place of anti-Prox1 and normal goat serum, respectively. DCX was visualized with Alexa 568-conjugated anti-goat IgG (1/200, Invitrogen). For VAMP2 staining, vibratome sections were first incubated with rabbit anti-VAMP2 (1/250, Synaptic Systems) primary antibody and then with Alexa 568-conjugated anti-rabbit IgG (1/200, Invitrogen) secondary antibody. For VGLUT1 staining, vibratome sections were first incubated with guinea pig anti-VGLUT1 (1/1000, Millipore) primary antibody and then with Alexa 488-conjugated anti-guinea pig IgG (1/200, Invitrogen) secondary antibody. For TUNEL staining, the *in situ* cell detection kit (Roche) was used following the manufacturer's instructions. As a positive control for DNA fragmentation, some sections were treated with 10 units/ml DNase I (Promega) for 1 hour prior to TUNEL staining. Images were taken with a SPOT camera (Nikon) or with confocal microscopy (Leica Microsystems, TC2-SP2 AOBS).

***In vitro* slice physiology**

Hippocampal slices (300 μm thick) were prepared from 3- to 6-month-old DG-TeTX mice and their control littermates. Initially, to characterize the activated (Dox-on-off) DG-TeTX mice, one activated DG-TeTX mouse and one control littermate were dissected sequentially each day, and recordings from slices obtained from both mice were interleaved with the experimenter blind to the genotype. Recordings for repressed DG-TeTX mice and re-repressed DG-TeTX mice were subsequently carried out in parallel. All mice were anesthetized with isoflurane, and brains were dissected in partial sucrose artificial cerebrospinal fluid (ACSF) containing (in mM): 80 NaCl, 3.5 KCl, 1.25 H_2PO_4, 25 $NaHCO_3$, 4.5 $MgSO_4$, 0.5 $CaCl_2$, 10 glucose and 90 sucrose, equilibrated with 95% O_2 and 5% CO_2. The brains were hemisected, and transverse slices were cut using a VT-1000S vibratome (Leica Microsystems). The slices were then incubated in the above solution at 35˚C for 30 min and then kept at room temperature in the same solution until use.

For recordings, slices were transferred to a recording chamber and perfused (3-5 ml/min, 32-35˚C) with ACSF composed of (in mM): 130 NaCl, 24 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO4, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 10 glucose, 0.05 ± dl-AP5 and 0.01 bicuculline methobromide, saturated with 95% O_2 and 5% CO_2, pH 7.4. Field excitatory postsynaptic potentials (fEPSPs) were recorded using electrodes (2-3 MΩ) pulled from borosilicate glass (World Precision Instruments) filled with oxygenated ACSF and connected to a multiclamp 700A amplifier (Axon Instruments, Foster City, CA). For MF→CA3 fEPSPs, the recording electrode was placed in the s. lucidum, whereas the recording electrode was placed in CA3 s. radiatum to record PP→CA3 and RC→CA3 fEPSPs. Synaptic responses were evoked at 0.1 Hz by stimulation (150-μs duration, 0.05- to 0.25-mA intensity) via a constant current isolation unit (A360, World Precision Instruments, Sarasota, FL) connected to glass electrode filled with oxygenated ACSF placed in the DG cell layer, the lateral PP or the CA3 s. radiatum to stimulate MF, PP and RC inputs,

respectively. Data acquisition (filtered at 3 kHz and digitized at 20 kHz) and analysis were performed using a PC equipped with pClamp 9.2 software (Axon Instruments).
Input-output (I/O) relations for each pathway were obtained by stepping the stimulus intensity from 0.05 mA to 0.25 mA. For MF→CA3 recordings, an I/O relation was obtained in control or forskolin (20 μM, Sigma)-supplemented ACSF and then again in the presence of DCGIV (2 μM, Tocris). Averaged waveforms (10 consecutive sweeps) obtained in DCGIV at each stimulus intensity were digitally subtracted from the corresponding averaged waveform (i.e., same intensity) obtained in control ACSF or forskolin to obtain pure MF→CA3 fEPSPs (Kamiya et al., 1996). For analysis, the area of the pure MF→CA3 fEPSPs was determined in the first 2.5 ms after the end of the AFV determined prior to the digital subtraction. Area was used rather than peak or slope because MF fEPSPs have complicated waveforms that may confound peak or slope measurements, particularly in the activated DG-TeTX mice (e.g., see waveforms in Figure 2A and B). AFV amplitude for MF→CA3 recordings was measured directly from non-DCGIV-subtracted traces. For PP→CA3 and RC→CA3 recordings, the AFV and fEPSP peaks were measured directly from averaged waveforms (minimum of 10 consecutive) obtained at each stimulus intensity.

To characterize the electrophysiological properties of adult-born granule cells (GCs), slices were prepared from mice 3-4 weeks after injection of Moloney virus encoding GFP. GFP-expressing GCs were then targeted for whole cell patch clamp recording. Slices were perfused (3-5 ml/min) with extracellular solution composed of (in mM) 130 NaCl, 24 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.5 $MgCl_2$, and 10 glucose, saturated with 95% O_2 and 5% CO_2 (pH 7.4). Recordings were performed at 32-34°C with electrodes (3-5 MΩ) pulled from borosilicate glass (World precision instruments) filled with either (in mM) 150 K-gluconate, 3 $MgCl_2$, 0.5 EGTA, 2 MgATP, 0.3 Na_2GTP and 10 HEPES plus 2 mg/ml biocytin for characterization of membrane properties and LTP experiments or 130 CsCl, 8.5 NaCl, 0.5 EGTA, 4 MgATP, 0.5 Na_2GTP, 5 QX-314Cl and 10 HEPES for basic postsynaptic current (excitatory and inhibitory) characterization. Whole-cell patch clamp recordings were made using a Multiclamp 700A or 700B amplifier (Molecular Devices, Sunnyvale, CA) in current- or voltage clamp-mode. The signals were filtered at 3 kHz (Bessel filter; Frequency Devices, Haverhill, MA) and digitized at 20 kHz (Digidata 1322A or 1440A and pClamp 9.2 or 10.2 Software; Molecular Devices). Recordings were not corrected for a liquid junction potential.

The resting membrane potential was noted immediately upon achieving a whole-cell configuration. The membrane potential was then biased to -60 mV by constant current injection. The input resistance (R_m) was measured using a linear regression of voltage deflections (± 15 mV from the resting potential, ~60 mV) in response to 2-s current steps of 6-10 different amplitudes in 5-pA steps. The membrane time constant (τ_m) was calculated from the mean responses to 20 successive hyperpolarizing current pulses (-20 pA; 400 ms) and was determined by fitting voltage responses with a single exponential function. Action potential (AP) threshold was defined as the voltage at which the slope trajectory reached 10 mV/ms (Stuart and Häusser, 1994) and AP amplitude was defined as the difference in membrane potential between the threshold and the peak. Spike half-width was defined as the duration of the AP at half of the determined amplitude. These properties were measured for the first two action potentials elicited by a depolarizing 800 ms current pulse of amplitude that was just sufficient to bring the cell to threshold for AP generation. Firing frequency was calculated from the number of spikes

observed during the 800 ms window during a current injection twice this amount. All intrinsic electrophysiological parameters were measured in pClamp or using procedures written in Igor 6 (Wavemetrics, Portland, OR).

Spontaneous inhibitory postsynaptic currents (sIPSCs) were pharmacologically isolated by the addition of DNQX (10 μM) and dl-AP5 (50 μM) to the perfusing medium and recorded as inward currents at a holding potential of -70 mV with the chloride reversal potential set to 0 mV (CsCl based internal). Events were detected using a template matching algorithm and analyzed in pClamp. At the end of the recordings, bicuculline (10 μM) was added to the perfusing medium to determine the tonic GABAergic current. Spontaneous and evoked excitatory postsynaptic currents (s/eEPSCs) were pharmacologically isolated by the addition of bicuculline to the perfusing medium. sEPSCs were recorded at a holding potential of -70 mV and detected by a template matching strategy and analyzed in pClamp. eEPSCs were elicited at 0.1 Hz as paired pulses (20 Hz) by low-intensity microstimulation (100 μsec duration; 10-30 μA intensity) via a constant-current isolation unit (A360, World Precision Instruments, Sarasota, FL) connected to a patch electrode filled with oxygenated extracellular solution placed in the molecular layer. The AMPA receptor-mediated component was determined from the peak of the eEPSC (the first event of paired pulses) observed at a holding potential of -70 mV, and the NMDA receptor-mediated component was measured 25 ms after the peak at a holding potential of +40 mV. Paired pulse ratios (PPRs) were calculated as the mean P2/mean P1, where P1 was the amplitude of the first evoked current and P2 was the amplitude of the second synaptic current obtained for consecutive individual traces.

For LTP experiments, bicuculline was added to the perfusing medium, and AMPAR mediated EPSCs were monitored in voltage clamp mode (V_h= -70mV) before (2-3 minutes) and after (15-20 minutes) LTP induction with a theta burst stimulation (TBS) protocol (Schmidt-Hieber et al., 2004). TBS was performed in current clamp mode with the membrane potential biased to -60 mV via constant current injection. TBS consisted of 10 trains of stimuli at 5 Hz, with each train consisting of 10 stimuli at 100 Hz. The duration of each train was paired with a postsynaptic depolarizing current injection of 300-400 pA that was sufficient to evoke a burst of postsynaptic action potentials. This was repeated four times at a frequency of 0.1 Hz, after which the recording configuration was switched back to voltage clamp mode, and AMPAR mediated EPSCs were monitored as described above for a minimum of 20 minutes post-TBS.

BrdU injection and histology

For BrdU experiments to characterize adult neurogenesis, 14- to 20-week-old DG-TeTX and their control littermates received four injections of BrdU (75 mg/kg body weight per injection, Sigma) separated by 2 hours. The mice were then sacrificed at the time points indicated in Supplementary Figure S3. Cryostat sections (50 μm thick) were first treated with 2N HCl in PBS containing 0.1% Triton-X100 (PBST-0.1%) for 30 min at 37˚C. After washing with PBS three times, the sections were blocked and incubated with primary antibodies (rat anti-BrdU, 1/100, Accurate Chemical; mouse anti-NeuN, 1/100, Millipore; rabbit anti-GFAP, 1/500, Dako) in PBST-0.1% containing 3% normal goat serum at 4˚C overnight. The sections were then rinsed with PBS and incubated with secondary antibodies (Alexa 488-conjugated anti-mouse IgG, Alexa 568-conjugated anti-rat IgG and Alexa 633-conjugated anti-rabbit IgG, all diluted 1/200, Invitrogen) in PBS for 2 hours at room temperature. The number of BrdU-positive cells (*n* = 8-

10 mice per genotype/condition) was counted in every sixth section throughout the entire extent of the DG.

Virus generation, injection and histological characterization

We used a Moloney viral vector (Molar, a kind gift from Dr. Carlos Lois) containing an internal promoter derived from the Rous sarcoma virus. Viral vectors were modified as follows. For co-expression of synaptophysin-GFP and mCherry-VAMP2 fusion proteins, mCherry was fused in frame to the cDNA of VAMP2 by PCR to generate mCherry-VAMP2. This was subcloned downstream from the encephalomyelocarditis virus internal ribosomal entry site (IRES) to generate IRES-mCherry-VAMP2, which was then subcloned downstream from synaptophysin-GFP cDNA (from Dr. Carlos Lois). The resultant bi-cistronic synaptophysin-GFP-IRES-mCherry-VAMP2 was then cloned under the promoter in the Molar vector. For expression of wheat germ agglutinin (WGA) in a Cre-dependent manner, a DNA cassette carrying two pairs of incompatible lox sites (loxP and lox2272) was synthesized, and the WGA cDNA (Addgene) was inserted between loxP and lox2272 sites in the reverse orientation. The resulting double-floxed reverse WGA cassette was cloned downstream from the promoter in the Molar vector. WGA cDNA was generated by PCR with 15 amino acids deleted at the C-terminus (Yoshihara et al., 1999). The viral vector for the expression of GFP in a Cre-dependent manner was generated in an identical manner to the WGA vector. A viral vector for Cre-independent mCherry expression was obtained from Dr. Carlos Lois.

We examined which neurons made direct synaptic contact to young, adult-born GCs using rabies monosynaptic retrograde labeling from defined cells (Wickersham et al., 2007). This virus can retrogradely cross synapses through the rabies envelope protein (G). The rabies virus was modified to replace the rabies G gene with a fluorescent marker and thus became incompetent for retrograde labeling unless rabies G is provided in infected cells. To restrict rabies viral infection to a desired population of cells, the rabies virus was further pseudotyped with EnvA, an avian viral envelope protein, whose receptors mammals lack. This modified rabies virus (EnvAΔG) can infect cells expressing the EnvA receptor TVA. If TVA-expressing cells also express rabies G, the EnvAΔG virus can utilize the cell-provided rabies G protein and thus be transferred to the presynaptic cells in a retrograde manner. However, the transfer is limited to monosynaptic connections, as trans-synaptically infected cells do not express the rabies G protein. In order to express TVA and rabies G glycoprotein in young, adult-born GCs, a Cre-dependent Moloney virus was generated. A double-floxed DNA cassette containing GFP, TVA and rabies G glycoprotein fused with 2A signals was obtained from Addgene (Wall et al., 2010) and cloned downstream from the promoter in the Molar vector in the reverse orientation.

Moloney viral particles were produced via calcium phosphate co-transfection of 293T cells with the viral vectors, Eco and VSV (from Dr. Carlos Lois) in the same procedure as previously described (Lois et al., 2002). Supernatants were collected starting from 36 hours after the transfection for 36 hours and concentrated by ultracentrifugation at 25,000 rpm for 90 minutes. The resulting viral pellet was resuspended in PBS at $1/500^{th}$ of the original volume, and the viral aliquot was then frozen. Viral titers were approximately 10^9 infectious units/ml. DG-TeTX mice and their control littermates (12-16 weeks old) were anesthetized with avertin and stereotaxically injected at two sites targeting the right DG (0.9 μl of viral aliquot per site). The stereotaxic coordinate for the first site was 2.06 mm posterior from bregma, 1.25 mm lateral from the

midline and 1.75 mm ventral from the brain surface. The second site was 2.70 mm posterior from bregma, 2.00 mm lateral from the midline and 1.75 mm ventral from the brain surface. The scalp incision was sutured, and post-injection analgesics were given to aid recovery (0.1 mg/kg, buprenex). For the viral co-injection described in Figure S4, equal amounts of the Cre-dependent GFP virus and the Cre-independent mCherry virus were mixed, and 0.9 μl of the viral mixture was injected at the same location as above. For rabies monosynaptic retrograde labeling, a Cre-dependent Moloney virus encoding GFP, TVA and rabies G glycoprotein was first injected into the DG at the same sites as above (0.9 μl of viral aliquot per site). Three weeks later, the EnvA-pseudotyped and G-deleted rabies virus containing RFP (9.3×10^8 infectious units/ml) was injected at the same sites (0.45 μl of viral aliquot per site).

At the time points after virus injection indicated in the figures, all mice were transcardially perfused with 4% PFA in PBS and brains were post-fixed by the same fixative overnight. Brain sections were prepared in the same manner described above. For double immunostaining for GFP and mCherry, vibratome sections (50 μm thick) were used with rat anti-GFP (1/500, Nacalai USA) and rabbit anti-RFP (1/500, Rockland immunochemicals) primary antibodies and Alexa 488-conjugated anti-rat IgG (1/200, Invitrogen) and Alexa 568-conjugated anti-rabbit IgG (1/200, Invitrogen) secondary antibodies. Although the transgene construct for the Tg3-TeTX was initially generated as a fusion protein with GFP, we failed to detect any GFP-TeTX fusion protein in the DG-TeTX mice with this immunostaining procedure (data not shown). Therefore, we concluded that signals detected with this procedure were derived solely from synptophysin-GFP fusion proteins. For WGA and NeuN double staining, immunostaining for WGA was first performed. Cryostat sections (50 μm thick) were treated with 3% H_2O_2 in PBS for 15 min, followed by treatment with 3% normal horse serum (NHS) in PBS containing 0.2% Triton-X100 (PBST-0.2%) for 30 min. After rinsing twice with PBS, the sections were treated with the Avidin/Biotin blocking kit (Vector laboratories) following the manufacturer's instructions. The sections were then incubated with goat anti-WGA (1/3000, Vector Laboratories) in PBST-0.2% containing 3% NHS for 2 hours at room temperature. After rinsing three times with PBST-0.2%, the sections were incubated with biotinylated anti-goat IgG (1/1000, Vector Laboratories) for 1 hour at room temperature. Next, the sections were rinsed once with PBST-0.2% and twice with PBS before incubation with streptavidin-biotin/horseradish peroxidase complex (ABC, 1/200, Vector Laboratories) for 30 min. After rinsing three times with PBS, the sections were incubated with Biotin-tyramide (1/50, PerkinElmer) at room temperature for 10 min. Following a subsequent wash with PBS three times, the sections were incubated with ABC again for 30 min and then rinsed three times with PBS. WGA signals were visualized with Cy3-tyramide (1/200, PerkinElmer) by 10 min of incubation at room temperature. The sections were then further processed for NeuN staining. After blocking with PBST-0.1% containing 3% normal donkey serum, the sections were incubated with mouse anti-NeuN (1/100, Milipore) and visualized with Alexa 488-conjugated anti-mouse IgG (1/200, Invitrogen). Finally, the sections were incubated with DAPI (Invitrogen) and mounted on glass slides. WGA signal was not observed when the virus was injected to non-Cre transgenic mice (data not shown). For rabies viral experiments, the primary antibodies used were rat anti-GFP (1/500, Nacalai USA), rabbit anti-tRFP (1/10000, Evrogen) and anti-NeuN (1/100, Milipore). Images were captured with a SPOT camera (Nikon) or with confocal microscopy (Leica Microsystems, TC2-SP2 AOBS).

Contextual discrimination fear conditioning between contexts

Fear conditioning was performed with male mice between 14 and 22 weeks of age during the light cycle in the animal facility. Activated DG-TeTX mice (n=12) and their control littermates (n=12) were assessed in a contextual discrimination fear conditioning between a pair of similar contexts. This procedure was based on that described previously (McHugh et al., 2007). Mice were trained to discriminate between two contexts through repeated experience in each context. The same four identical conditioning chambers (30 x 25 x 25 cm; Med-Associates, Inc.) were used for both context A (the context paired with a shock) and context B (never paired with a shock). The conditioning chambers were placed in a sound-attenuating cubicle with a 60 dB background noise provided by a fan. The floor of each chamber was made of 32 stainless steel rods (2-mm diameter) spaced 6 mm apart with alternating heights (4-mm offset) wired to a shock generator and scrambler (Med-Associates, Inc.) to deliver the footshock. Each chamber was wiped down with 70% ethanol before conditioning and between animals. A metal pan containing a thin film of Windex was placed underneath the grid floors and was replaced between animals. The only feature that differentiated the two contexts was that the A context had a plastic A-frame insert (two black plastic panels joined at the top and sloping down to the side of the conditioning chamber at a 60º angle to the floor), whereas context B did not. This was done to make the discrimination as difficult as possible so that the pattern separation function of the DG could be assessed. On each conditioning day, animals were brought to a holding room in their home cages and left undisturbed for a minimum of 30 min. Animals were then transported from the holding room to the conditioning chambers in their home cages.

Contextual fear acquisition: Animals received context conditioning on Days 1 through 3. Animals were placed in context A for 3 min and allowed to explore. A 2-s 0.65 mA shock was then presented, and the animals were removed 1 min later. Freezing was measured during the 3 min preceding the shock. *Generalization test:* On Day 4, animals were placed in context A or B for 3 min and then placed in the opposite context for 3 min 1.5-2 hours later, with the order counterbalanced such that half of the animals were placed in context A first and half were placed in context B first. Day 5 followed this same procedure with the order reversed. No shock was delivered in A or B during the generalization test. Freezing was measured during the 3 min.

Discrimination training: Days 6-17 consisted of discrimination training in which animals were placed in both contexts on each day. Context A was again paired with a shock, but context B was not. The animals were placed in each context for a total of 4 min and 2 s. In context A, the animals received a 2-s 0.65 mA shock after 3 min and were left in the chamber for 1 min following the shock. In context B, the animals were placed in the chamber for an equivalent 4 min and 2 s. Freezing was measured during the 3 min preceding the shock on all days in which the shock was administered in context A and the equivalent period of time in context B during discrimination training. Freezing was measured using Video Freeze software (Med-Associates, Inc.) recorded at 30 frames per second with the freezing threshold set at 19 and the minimum freeze duration set at 1 s. The order of training followed a double alternation schedule: Day 6, B→A; Day 7, A→B; Day 8, A→B; Day, 9 B→A; etc. For statistical analysis and graphical presentation, the data were collapsed into consecutive 2-day blocks so that each block consisted of one day of A→B and one day of B→A. Discrimination training was analyzed in a manner that allowed us to conservatively state when a group differentiated between A and B. For each trial block within each genotype using Scheffé's method to correct for multiple comparisons, with a protection significance level of p=0.05. We chose the Scheffé method because we had no *a*

priori prediction about when discrimination would emerge and Scheffé's method retains a 0.05 confidence level regardless of the number of comparisons that are made. Only trial blocks with an effect of context that exceeded this correction were considered significant.

Contextual discrimination between a pair of very distinct contexts was performed using the same experimental schedule for the first 5 days as the one described above with activated DG-TeTX mice (n=12) and their control littermates (n=12) between 14 and 22 weeks of age. The chambers (context A), measuring 30 x 25 x 21 cm, had plexiglass fronts and backs and were located in one room lit with overhead fluorescent lamps. The chamber floors consisted of 36 stainless steel rods placed at the same height and spaced 7.9 mm apart. The chambers were cleaned prior to an introduction of an individual mouse with 70 % alcohol, and a solution of 0.25% benzaldehyde (in 100% ethanol) was placed beneath the chambers during the experiment to provide a dominant odor. The chambers (context D) had the same dimension as context A but were located in another room adjacent to the first one, which was lit with dim red light. In addition, the chambers had a curved plastic roof, and the floor consisted of 19 stainless steel rods spaced 16 mm apart with alternating heights. The chambers were cleaned with quatricide prior to the introduction of each mouse, and a solution of 1% acetic acid was placed beneath the chambers during the experiment to provide a dominant odor. The lighting, chamber materials and odor employed in context D provided a context that was quite distinct from context A.

In Figure 5H to O, irradiation was performed with male mice between 9 and 12 weeks of age. Repressed DG-TeTX mice (n=28) and their control littermates (n=28) were anesthetized with a mixture of 100 mg/kg ketamine and 7 mg/kg xylazine. Half of the mice were focally irradiated by X-ray, and the other half were sham-operated (i.e., they were anesthetized but not subjected to irradiation). The mice were irradiated with three fractions of 5Gy/day on days 1, 3, and 5 for a total dose of 15 Gy using a Gulmay Medical RS320 Irradiation System X-ray unit at 300kV (Gulmay Medical Ltd., Camberly, Surrey, UK). The beam was filtered using 1.5 mm Cu and 3 mm Al giving a HVL of 3 mm Cu at a dose rate of 1.975 Gy/min. Multiple experiments using Harshaw TLD-100H (LiF:Mg, Cu, P) and film (GAFCHROMIC EBT2, International Specialty Products, Wayne, NJ) dosimetry were performed and calibrated against a clinical cobalt-60 irradiator (Theratron-1000, MDS Nordion, Ontario) indicating an inhomogeneity of the radiation doses within the field was less than 6.5%. As previously described (Santarelli et al., 2003), the mice were placed on the stereotaxic frame and protected from X-ray with a lead shield covering the entire body except the area above the hippocampus (interaural -2.64 mm to +0.58 mm). After irradiation, the Dox diet was replaced with a regular diet. Six weeks later, the mice were subjected to the same contextual discrimination fear conditioning as above using a different context pair (A and C). There were three different features (odor, background noise and interior) that differentiated these contexts. The context A had background fan noise and was scented with Windex, whereas the context C did not have background noise and was scented with Simple Green. In addition, a plastic A-frame insert was placed in context C. After the completion of discrimination training, half of the mice received BrdU injections twice (75 mg/kg body weight). The mice were then transcardially perfused with 4% PFA in PBS on the following day. BrdU-positive cells in the DG were counted in the same manner as described above, and BrdU-positive cells in the subventricular zone were counted in the lateral walls of the lateral ventricle between +1.70 and -0.10 from bregma.

Delayed non-matching to place task in an eight-arm radial maze

The delayed non-matching to place (DNMP) task in an eight-arm radial maze was performed in activated DG-TeTX mice (n=12) and their control littermates (n=12) between 14 and 22 weeks of age during the light cycle. The mice were transported from the colony to a holding room adjacent to the behavioral suite containing the eight-arm radial maze, where they sat undisturbed in their home cages for 30 min prior to the experiment. All mice were food restricted for 1 week prior to subjecting them to the task and maintained at 85% body weight throughout the experiment. Water was supplied *ad libitum*. After reaching the criteria, the mice were habituated to an eight-arm radial maze (Harvard Apparatus) for 2 days by allowing them to explore the maze foraging for food pellets. On the first day, mice from the same cage (two to four mice) explored the maze together for 1 hour. On the second day, they explored the maze separately for approximately 5 min. On the following day, the mice were subjected to the DNMP task, four trials per day, for 12 consecutive days. Each trial consisted of a sampling phase and a choice phase and was separated by at least 2 hours between trials. In the sample phase, the mice were placed in a start arm of the maze, in which doors for the start arm and a sample arm (rewarded) were open. After retrieving food pellets in the sample arm, the mice were returned to the home cage and kept there for 20 s. During this 20-s period, the maze was cleaned with quatricide and set up for the choice phase, in which doors for the start arm and the sample arm (non-rewarded) and a third correct arm (rewarded) were open. The mice returned to the maze 20 s after the completion of the sample phase and were allowed to retrieve the food pellets. Mice that visited the correct arm (rewarded) were considered to have made correct choices, whereas mice that visited the sample arm (non-rewarded) were considered to have made incorrect choices and were allowed to visit the correct arm to retrieve the food pellets before being returned to the home cage. The correct arms were separated from the sample arm by 45°, 90°, 135° and 180°. The mice were subjected to four trials per day, receiving at least one trial from each separation degree every day in a pseudo-random manner. The start arm location for each trial was also determined in a pseudo-random manner.

Pre-exposure-mediated contextual fear conditioning (PECFC)

PECFC was conducted with male mice between 14 to 22 weeks of age during the light cycle. The mice were transported from the colony to a holding room, where they sat undisturbed for 30 min prior to the experiment. The conditioning chamber (A), measuring 30 x 25 x 21 cm, had plexiglass fronts and backs and were located in a room lit with dim red light. The chambers had a curved plastic roof, and the floor consisted of 19 stainless steel rods spaced 16 mm apart with alternating heights. The chambers were cleaned with quatricide prior to the introduction of an individual mouse, and a solution of 1% acetic acid was placed beneath the chambers during the experiment to provide a dominant odor. Repressed mice (i.e., Dox-on) were brought into the conditioning chamber (Figure 7B, C, D and F) or a very distinct chamber (D) composed of an opaque plastic box (30 x 20 x 20 cm) without a roof (Figure 7E). The (pre-) exposure session consisted of a free exploration of the chambers for 10 min. The mice were then transported back to their home cages. Mice were given (pre-) exposure sessions for 5 consecutive days, 10 min per day, and the Dox diet was then replaced with a Dox-free diet (Figure 7B, C, E and F) or unchanged (Figure 7D). Four weeks later, the mice were transported individually to the conditioning chamber and then received a single 1.5-mA footshock (2-s duration) 10 s (Figure 7B, D and E) or 3 min (Figure 7C) after being placed in the chamber or did not receive a footshock 10 s after being placed in the chamber (Figure 7F). The mice remained in the chamber

for a further 30 s and were then transported back to their home cages. On the next day, the mice were returned to the conditioning chamber for a 3-min test. During all of these sessions, the activity of the animals in the chamber was recorded using the FreezeFrame software. Freezing behavior was assessed from the video image of the mouse using the FreezeView software (Med-Associates, Inc.).

Morris water maze

The Morris water maze (MWM) task was conducted with DG-TeTX mice (*n*=23) and control littermates (*n*=24) between 14 and 24 weeks of age. Repressed mice (i.e., Dox-on) were kept in a temperature-controlled room on a constant 12-hour light/dark cycle. All experiments were conducted at approximately the same time of day. The mice were transported from the colony to a holding area, where they sat undisturbed for 30 min prior to the experiment. The test was performed in a rectangular dimly lit room (340 cm x 297 cm) with a circular pool (160-cm diameter) filled with opaque water made with color paints (White 5130, Berghause; Peach 2906, Pearl Tempera) maintained at 19°C. Four large objects illuminated with floor lamps were hung on black curtains surrounding the pool as extramaze cues. A hidden circular platform (13 cm in diameter) was placed 1 cm below the water surface, and the mice were trained to find the platform with four trials per day for 12 days, with an inter-trial interval of approximately 60 min. During training, the mice were released from four pseudorandomly assigned start locations (N, S, E and W) and were allowed to swim for 90 s. If a mouse did not find the platform within 90 s, it was manually guided to the platform and allowed to rest on the platform for 15 s. A probe trial was conducted on Day 13 under the full-cue condition (P1; Figure S7C). The mice were released at the center of pool and were allowed to swim for 90 s in the absence of the platform. Following the probe trial, the mice received four training trials in the presence of the platform to avoid memory extinction that may have occurred during the probe trial. The mice then underwent 5 weeks of Dox withdrawal in the colony. Subsequently, the mice received four probe trials with extramaze cue manipulations, one probe trial per day, without re-training between probe trials. For the one-cue probe trial (P2; Figure 7J), one cue located more distally from the platform was kept, and the other three cues were removed from the surrounding curtains. For the two-cue probe trial (P3; Figure 7J), one cue located close to the platform and the cue used in the one-cue probe trial remained, but the other two cues were removed from the surrounding curtains. For the no-cue probe trial (P5; Figure S7D), all four extramaze cues were removed. Data for training and probe trials were collected and analyzed with the HVS Image Water 2020 software. Escape latency and distance traveled to the hidden platform and swim speed were measured during training, and the latency to the first crossing of the phantom platform location and the amount of time mice spent during the 90s in the circular platform surface were measured during probe trials. These data were then averaged over mice of each genotype.

***In vivo* recording**

Activity of hippocampal neurons (“place cells”) depends on an animal’s location in the environment (O’Keefe and Dostrovsky, 1971), and shifting a rodent from one context to another similar but distinct context in the same room results in firing rate changes of place cells without significantly changing the firing field locations (“rate remapping”), particularly in CA3 (Leutgeb et al., 2004; Leutgeb et al., 2007). In addition, we previously showed that a deficit of CA3 rate

remapping correlated with an impairment of behavioral pattern separation in mice devoid of NMDA receptors in DG GCs (McHugh et al., 2007).

Surgery: Male mice (DG-TeTX and littermate controls, 16-24 weeks of age) were implanted in the right hippocampus with a microdrive array consisting of 6-12 independently adjustable tetrodes as previously described (McHugh et al., 1996) (stereotaxic coordinates from bregma (mm): CA1: AP 1.8, ML 1.6; CA1/CA3: AP 1.6, ML 1.4). Prior to implantation, tetrodes were coated with DiI (Invitrogen) to enable tracking of their locations. At the completion of the experiment, mice were given a lethal dose of anesthetic and a small electrical current (20-50 μA) was run down each tetrode for 5-10 s to create a small lesion at the tip. Mice were then perfused transcardially with 4% PFA in 0.1 M sodium phosphate buffer. The brains were sectioned (50 μm) and stained with nuclear red or neurotrace green fluorescent Nissl stain. The recording position of each tetrode was verified by examining the location of the lesion and the tracks of the electrodes under fluorescence or light microscopy.

Apparatus and recording protocol: All experiments were performed by researchers blind to the genotypes of the animals. Mice were familiarized to one of two arenas [a low-walled black square arena (43 cm x 43 cm x 10 cm high) with a white cue card (23 cm x 8 cm high) or a low-walled white circular arena (48 cm diameter x 10 cm high) with a black cue card (23 cm x 8 cm high)] in a free exploration task for 15 min/day for 3 consecutive days. The presentation of the black square and white circle was counterbalanced between animals. Diffuse room lighting was provided by low intensity LEDs focused onto four distal salient visual cues located on black curtains that surrounded the elevated recording arena. On day 4, the pattern separation recording sessions consisted of two "RUN" epochs (15 min each). Before and after each run session, the mice were placed in a small high-walled box outside of the behavioral environment for 20 min ("SLEEP" sessions). RUN1 was conducted in the familiar environment in the same room and conditions as days 1-3. For RUN2, familiar arena was replaced by the novel arena (see Figure 6D). Both environments were similar in terms of area (~1800 cm^2). The presentation order of the black square and white circle did not influence the results; thus, the two recording conditions were pooled.

Unit recording and tracking: The position of the animals was tracked at ~30 Hz using a pair of LEDs placed on the animal's headstage. As animals explored the arenas, unit activity was amplified 5,000-30,000 times and bandpass filtered at 0.3-6 kHz. Spike waveforms above a threshold of 65 μV were time stamped and digitized at 32 kHz. A total of 1 ms of data was stored for each waveform. A single EEG channel was sampled from each tetrode continuously at ~3 kHz.

Data analysis: Spike sorting was performed off-line using custom clustering software, and action potentials were assigned to individual cells based on a spike's relative amplitude across the four recording wires of the tetrode as previously described (McHugh et al., 1996). Cluster boundaries were compared across run and sleep sessions to determine whether clusters were derived from the same cell or not and whether recordings were stable. Hippocampal cells and interneurons were distinguished by their firing pattern (i.e., complex spike index (CSI)), spike shape, average firing rate, and spatial firing pattern. Pyramidal cells were accepted for further analysis if the average rate was > 0.2 Hz and the peak firing rate was > 5 Hz in at least one run. Interneurons

(mean firing rate > 5 Hz and CSI < 5% in both RUN1 and RUN2) were not included in any analysis.

To characterize the consequence of the specific blockade of the vast majority of GCs on the activity of CA3 and CA1 pyramidal cells, we measured several properties, including: 1) the mean firing rate; 2) the peak firing rate; 3) the place field size, defined as the size of the largest group of contiguous pixels with a firing rate above 20% of the peak rate (Brun et al., 2002); 4) the mean infield firing rate, defined as the total number of spikes emitted by a cell while the mouse was in the place field divided by the total time spent in the field and 5) spatial information, or the amount of information (in bits) conveyed about spatial location by a single action potential emitted by a single cell (Markus et al., 1994), defined as $I = \Sigma P_i(\lambda_i/\lambda) \log_2(\lambda_i/\lambda)$, where λ_i is the mean firing rate in each pixel, λ is the overall mean firing rate, and P_i is the probability of the animal to be in pixel i (i.e., dwelling time in each pixel / the total dwelling time).

To assess the similarity in ensemble activity between RUN1 and RUN2 (pattern separation recording session, day 4), we calculated two different rate remapping indices for each pyramidal cell meeting our minimum criteria and averaged those values for each region and genotype. First, we calculated the Rate Difference ((high rate-low rate)/(high rate+low rate)) (McHugh et al., 2007) as well as the Rate Overlap (low rate/high rate) (Leutgeb et al., 2004). The Rate Differences for each hippocampal region and genotype were then compared to the Estimated Rate Difference (ERD) values expected from independent firing rates in each region. For this calculation, the Rate Difference was calculated between cells from RUN1 and randomly chosen cells from RUN1 or RUN2. The means from 10,000 permutations were then calculated and used to represent the ERD for each region/genotype.

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