Critical Roles of Embryonic-Born Dorsal Dentate Granule Neurons for Activity-Dependent Increases in BDNF, Adult Hippocampal Neurogenesis, and Anti-Anxiety-like Behaviors

Supplement 1

Supplemental Methods and Materials

Animals and mouse breeding

Neurod6-Cre (neurogenic differentiation 6-Cre) mice were kindly provided by Dr. KA Nave (1). CaMKII-Cre (calcium/calmodulin-dependent protein kinase II alpha-Cre) (Stock No. 005359), Pomc-Cre (pro-opiomelanocortin-alpha-Cre) (Stock No. 005965), HSA-Cre (human alpha-skeletal actin-Cre) (Stock No. 006149), NestinCreER^{T2} (Stock No. 016261), Ai3 mice (Stock No. 007903) and Ai9 (Stock No. 007907) mice were purchased from the Jackson laboratory. Ocn-Cre;Ai9, Neurod6-Cre;Ai9, CaMKII-Cre;Ai9, Pomc-Cre;Ai9, Nestin-cre^{ER};Ai9 and HSA-Cre;Ai9 mice were generated by crossing Ai9 mice with Ocn-Cre, Neurod6-Cre, CaMKII-Cre, Pomc-Cre, Nestincre^{ER} and HSA-Cre mice, respectively. Ocn-Cre;Ai3 mice were generated by crossing Ai3 mice with Ocn-Cre mice. All the mouse lines were maintained in C57BL/6 strain background for >6 generations, and confirmed by genotyping analysis with PCR. Mice were maintained on a 12-h light-dark cycle with ad libitum access to water and food. Male mice were used for all the studies.

Antibodies

The information of primary antibodies used was as follows: anti-Akt1/2/3 (Santa Cruz, sc-81434, 1:500), anti-p-Akt1/2/3 (Santa Cruz, sc-514032, 1:500), anti-Erk1/2 (Cell Signaling, 9102S, 1:1000), anti-p-Erk1/2 (Cell Signaling, 4370S, 1:1000), anti-c-fos (Santa Cruz, sc-

271243, 1:500), anti-Mcm2 (BD Biosciences, 610700, 1:500), anti-DCX (Santa Cruz, sc-8066, 1:200), anti-NeuN (Millipore, MAB377, 1:1000), anti-BrdU (Accurate chemical & scientific corporation, OBT0030, 1:500), anti-GFP (aves, GFP-1020, 1:500), anti-mCherry (Takara, 632496, 1:1000), anti-CaMKII (Cell Signaling, 50049, 1:200), anti-TrkB (R&D systems, AF1494-SP, 1:500), anti-p-TrkB (Millipore, ABN1381, 1:300), anti-BDNF (Millipore, AB1779SP, 1:500), anti-β-actin (Abcam, ab8227, 1:5000). All the corresponding conjugated secondary antibodies (1:1000) were purchased from Invitrogen. Nuclei was stained with 4′,6-diamidino-2-phenylindole (DAPI) (1:1000, Roche).

Drug administration

5-Bromo-2'-deoxyuridine (BrdU, a thymidine analogue that is incorporated into DNA of dividing cells) and 5-ethynyl-2'-deoxyuridine (EdU, a modified thymidine analogue that is incorporated into the DNA of dividing cells) were purchased from sigma (#B5002) and ABP Biosciences (#A012), respectively. To label the birthdates of Ocn-Cre^{+} DGCs, pregnant mice and pups were injected with BrdU (10 mg/ml in PBS, i.p., 50 mg/kg body weight) and/or EdU (10 mg/ml in PBS, i.p., 50 mg/kg body weight) as the diagram described in Figure 4. To analyze the neural stem cell proliferation, adult mice were injected with BrdU (10 mg/ml in PBS, i.p., 50 mg/kg body weight) as the diagram described in Figure 6, 8 and S11. For inducible Cre expression, Nestin-CreER^{T2} mice were injected with Tamoxifen (Sigma, T5648, 20mg/ml in corn oil, i.p., 100 mg/kg body weight) at the age of P60, and sacrificed at P90. Clozapine-N-oxide (CNO) was purchased from Sigma (#C0832). For acute treatment, mice were injected with CNO (1mg/ml in PBS, i.p., 2 mg/kg or 5 mg/kg body weight) for 1 h before behavioral test and biochemical analyses. For chronic treatment, CNO was dissolved in the drinking water at a concentration of 0.03

mg/ml. At this concentration, the mice (~30g) received approximately 5 ml water per day, suggesting a dose of 5 mg/kg/d of CNO treatment (preliminary data).

Behavioral analysis

For all behavioral experiments, mice were transferred to the testing room 4 h before any test to acclimate to the environment. All behavioral instruments were cleaned with 70% ethanol prior to each trial.

Open field test (OFT), each mouse was placed in a chamber (L x W x H = 50 x 50 x 20 cm) and movement was monitored for 10 min using an overhead camera. Light intensity was about 150 lux. The video was analyzed by a tracking software (Etho Vision, Noldus). Total distance and center (25 x 25 cm) duration time were quantified.

Elevated plus maze test (EPMT), the EPM was placed 50 cm above the ground. Each mouse was initially placed in the center square facing one of the open arms (L x W = 60 x 5 cm). Light intensity was about 100 lux. Mice movement was recorded for 5 min using an overhead camera and tracking software (Etho Vision, Noldus). Time spent in the open arms and the number of open arm entries were quantified.

Light/dark transition test (LDT), mouse was firstly placed in the dark compartment, overhead camera was turned on, and the door between lit and dark chambers was opened. Light intensity was about 200 lux in the lit chamber. 10 min of movement was recorded using a tracking software ((Etho Vision, Noldus)). The time spent in the lit chamber and the number of transitions were quantified.

Elisa

Blood samples were obtained from mice that were exposed to anxiogenic or non/mild anxiogenic/locomotive environments for 10 min and put back to home cage for 30 min. All the samples were centrifuged for 10 min at 3000 rpm, and the plasma were collected. Mouse plasma corticosterone level was measured by using Corticosterone ELISA kit (ab108821).

Electrophysiological recording

Electrophysiological recording was performed as previously described (2). Lamellar 300 μm thickness slices of hippocampus as described elsewhere (3) using Vibratome (VT1200S, Leica Microsystems) and transferred to a storage chamber containing Choline Chloride-ACSF (in mM, 120 Choline Chloride, 2.5 KCl, 7 MgCl2, 0.5 CaCl2, 1.25 NaH2PO4, 5 Sodium ascorbate, 3 Sodium pyruvate, 26 NaHCO3, and 25 glucose.) at 32°C for 15 min, Slices were transferred and stored in regular ACSF (in mM): 124 NaCl, 2.5 KCl, 2 MgSO4, 2.5 CaCl2, 1.25 NaH2PO4, 26 NaHCO3, and 10 glucose) at room temperature (25 ± 1°C) for additional 1 h. All solutions were saturated with oxygenated (95% O2/5% CO2).

Slices were transferred to a recording chamber superfused (2 mL/min, 32-34°C) with regular ACSF. Slice was visualized with infrared optics using an upright microscope equipped with 40x water-immersion lens (BX51WI, Olympus) and infrared-sensitive CCD monochrome video camera (C2400-75, Hamamatsu).

Ocn-Cre^{+} neurons were identified by the expression of Td. The glass electrodes were pulled by a micropipette puller (P-1000, Sutter Instrument), the pipettes (resistance: 4-6 MΩ) were

filled with a solution containing (in mM): 125 K-gluconate, 5 KCl, 10 HEPES, 0.2 EGTA, 1 MgCl2, 4 Mg-ATP, 0.3 Na-GTP and 10 phosphoCreatine, at pH 7.3 (290-295 mOsm). Series resistance was below 20 MΩ. For excitability recording, action potential firing frequency was analyzed from current-clamp recording and measured by injecting a series of depolarizing current pulses or a 100-pA depolarizing current ramp in the presence of 20 μM CNQX, 50 μM DL-AP5 and 20 μM bicuculline. Recordings were performed with MultiClamp 700B amplifier and 1550B digitizer (Molecular Devices). Data were digitized at 10 kHz and low pass filtered at 3 kHz.

Production of virus and virus grafting in vivo

Production of retrovirus expressing GFP was performed as described elsewhere with slight modification (4). Briefly, retroviral plasmids were transfected into cultured 293GPG cells with polyethyleneimine, medium was collected at 48, 72 and 96 h post transfection, and filtered by a 0.2 μm filter, and then concentrated at 20000 rcf for 2 h at 4 ℃ using a SW27 rotor (Beckman). The supernatant was removed, and the retrovirus was resuspended in 100 μl PBS.

AAV5-hSyn-DIO-mCherry (#50459), AAV5-hSyn-DIO-hM3D(Gq)-mCherry (#44361) and AAV5-hSyn-DIO-hM4D(Gi)-mCherry (#44362) were purchased from addgene. AAV-mCherry-flex-DTA was purchased from UNC Vector Core. Lenti-scramble shRNA (#TR30021V) and Lenti-BDNF shRNA (#TL513468V) were purchased from Origene.

Virus injection was performed as described previously with a slight modification (5). In brief, male mice (6-week-old) were anesthetized with Ketamine/Xylazine (HENRY SCHEIN #056344), and head was fixed in a stereotaxic device (David Kopf Instruments).

After antiseptic treatment, skull was exposed and cleaned using 1% H_2O_2. Holes were drilled into the skull and viruses were bilaterally injected into DG at the coordinates relative to bregma: caudal: −2.06 mm; lateral: + /−1.3 mm; ventral: −1.75 mm. After injection, needle was left in place for 5 min to allow for diffusion of injected viruses before being slowly withdrawn. For the following 5 days, mice were daily injected with Meloxicam to reduce pain. Injection locations were validated in each mouse after experiments.

Immunohistochemistry

Immunostaining was performed as described previously with a slight modification (6). Briefly, mice were deeply anesthetized with isoflurane and perfused with 50 ml PBS followed by 50 ml 4% paraformaldehyde (PFA). Brains were post-fixed in 2% PFA overnight at 4℃ and cut into 40 μm slices using a vibratome (Leica VT1000 S). Brain sections were washed 3 times with PBS (5 mins each) and treated with blocking buffer (10% Donkey Serum + 0.5% Triton X 100) for 1 h at room temperature, and then incubated with primary antibody overnight at 4 °C. Sections were washed 3 times with PBS and incubated with corresponding conjugated secondary antibody for 2 h at room temperature. DAPI was used for nucleus staining.

Stereological cell counting

Stereological quantification of cells was performed as previously described (5). Briefly, 40-μm thick brain sections were immunostained with related antibodies. One in every eight serial sections starting at the beginning of hippocampus (Bregma −1.06 mm) to the end of hippocampus (Bregma −3.80 mm) were examined with a Zeiss confocal system (LSM880), and each section was separated into ten z-plane images by a 4-μm step. About eight sections

of each DG were counted, and total counts of eight examined sections were multiplied by 8 to estimate the total number of markers positive cells per DG. To quantify the cell density (number/area), we first measured the area of each section by using Image J software, and then calculated the cell number per mm^2. Finally, data were presented as the mean of each counted section.

Quantitative real time-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from hippocampus by using RNeasy Mini Kit (QIAGEN, Cat No. 74104), and purified RNA (5μg) was used for cDNA synthesis with Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, # K1621). cDNA products were subjected for subsequent qPCR using SYBR green (QIAGEN) in CFX96 real-time system (Bio-Rad). Primers used were as follows: c-fos, forward: 5'GGGAATGGTGAAGACCGTGTCA3', reverse: 5'GCAGCCATCTTATTCCGTTCCC3'; BDNF, forward: 5'GGCTGACACTTTTGAGCACGTC3', reverse: 5'CTCCAAAGGCACTTGACTGCTG3'; VEGFa, forward: 5'CTGCTGTAACGATGAAGCCCTG3', reverse: 5'GCTGTAGGAAGCTCATCTCTCC3'; FGF2, forward: 5'AAGCGGCTCTACTGCAAGAACG3', reverse: 5'CCTTGATAGACACAACTCCTCTC3'; BMP, forward: 5'AACACCGTGCGCAGCTTCCATC3', reverse: 5'CGGAAGATCTGGAGTTCTGCAG3'; Shh, forward: 5'GGATGAGGAAAACACGGGAGCA3', reverse: 5'TCATCCCAGCCCTCGGTCACT3'; GAPDH, forward: 5'CATCACTGCCACCCAGAAGACTG3', reverse:

5'ATGCCAGTGAGCTTCCCGTTCAG3'. Each sample was repeated at least for 3 times, and the mRNA level was normalized to GAPDH using the $2^{-\triangle\triangle Ct}$ method.

Western blot

Western blot was performed as described previously with slight modification (7). In brief, Hippocampus was dissected and homogenized in lysis buffer (20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM EDTA, 5 mM sodium fluoride, and 2 mM sodium orthovanadate and supplemented with a protease inhibitor cocktail (Roche)). Lysates were centrifuged at 12,000 rcf, 4°C for 15 mins. The supernatants were taken and mixed with loading buffer. Based on the protein molecular weight, we used 15% or 10% SDS-PAGE gels to separate proteins and then transferred them onto Nitrocellulose Blotting Membranes. After that, Nitrocellulose membranes were blocked with 5% low fat dried milk (in 1X TBST) for 1 h at room temperature. Antigen specific primary antibodies were diluted to incubate membrane overnight at 4°C. Subsequently, species-specific horseradish-peroxidase-conjugated secondary antibodies were used for recognizing the primary antibody (1:5,000, Thermo), and ECL kit (Pierce, Rockford, IL) was used for visualizing the target proteins. For quantitative analysis, detected protein bands were analyzed using Image J software. Bands of interested proteins were normalized to loading control (β-actin).

RNA scope

RNA scope was performed in mouse brain sections by using RNAscope® Multiplex Fluorescent Detection Kit (PN323110) and mouse BDNF probe (ACD, Cat No. 424821). Briefly, mice were deeply anesthetized with isoflurane and perfused with 50 ml RNase-

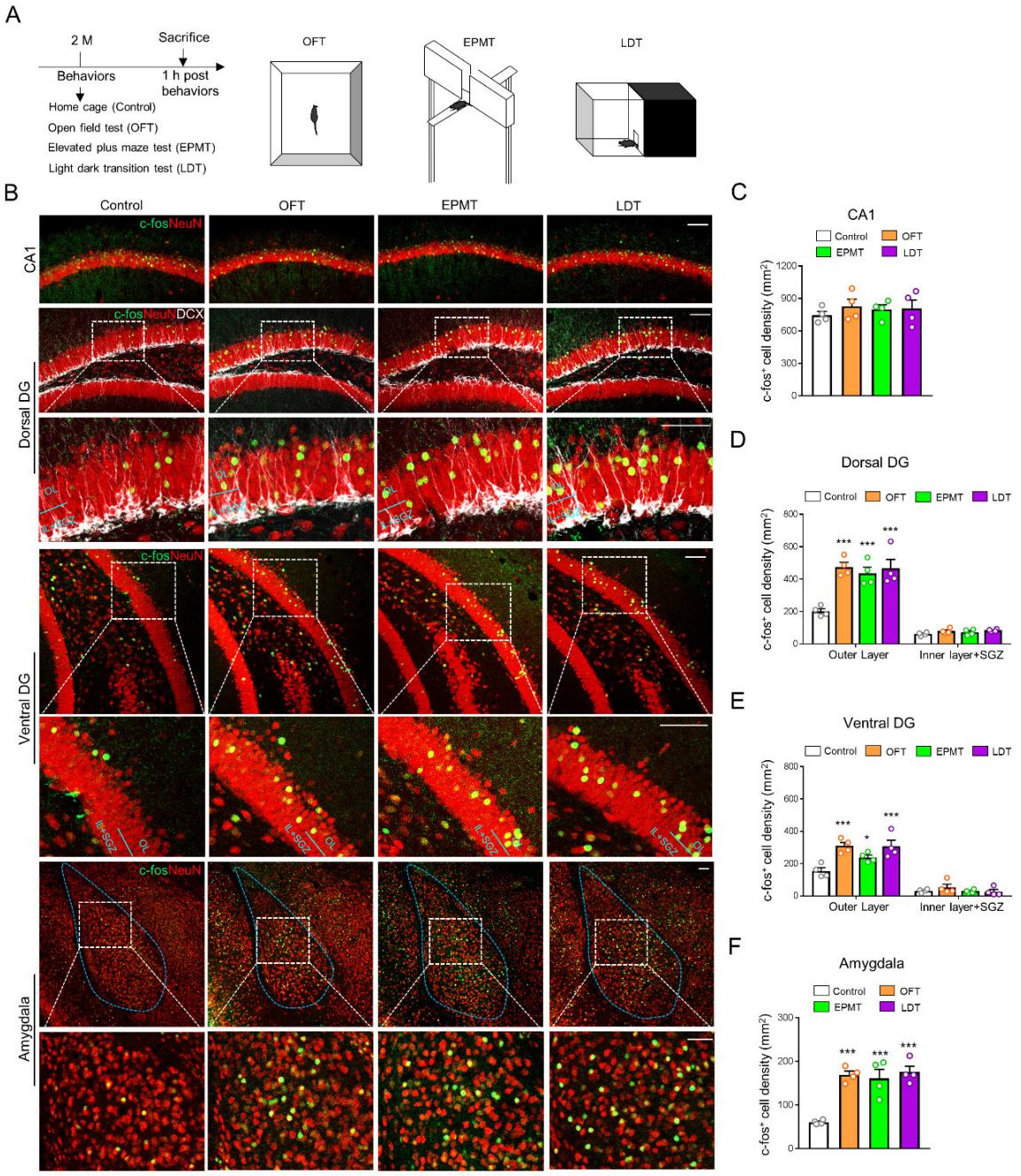
free PBS followed by 50 ml 4% paraformaldehyde (PFA). Next, brains were dissected and post-fixed with 4% PFA for 2 h and dehydrated with 30% sucrose for 1 day, and then cut into 10 μm sections using a freezing microtome (Leica). Subsequent processes followed RNAscope® Multiplex Fluorescent Reagent Kit v2 User Manual. Finally, images were taken using a Zeiss confocal system (LSM880).

Statistical analysis

All results presented in this study were from at least three independent experiments. GraphPad Prism 7 software was used for statistical analyses. For two groups of samples comparisons, unpaired student's t-test was used to evaluate statistical significance. For multiple comparisons of three or more groups of samples, ANOVA was used. All data were presented as mean ± standard error of the mean (SEM) and described in the figure legends. Statistical significance was defined as $P < 0.05$.

Supplemental Figures

Figure S1. Increased neuronal activity in mouse DG following exposure to anxiogenic environment



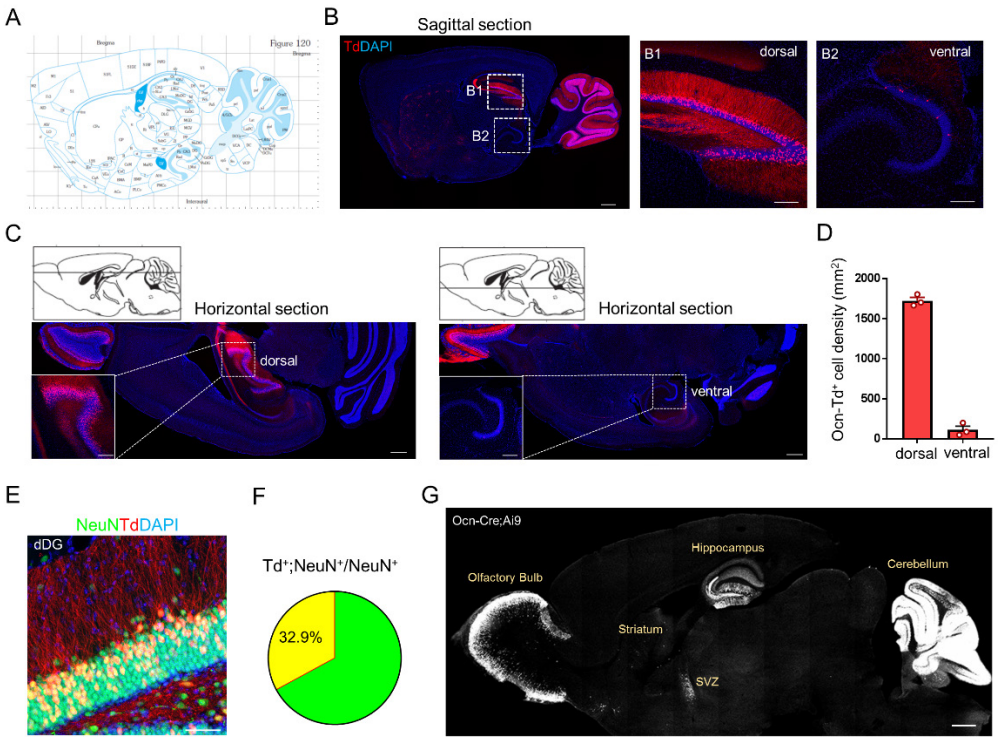
(A) Schematic diagram of behavioral tests in mice: Open field test (OFT), Elevated plus maze test (EPMT) and light dark transition test (LDT); mice in home cage are controls.

(B) Co-immunostaining of c-fos (green), DCX (white) and NeuN (red) in CA1, DG and amygdala of mice following behavioral tests and control mice. DAPI (blue) indicates cell nucleus. OL: outer layer, IL+SGZ: inner layer + SGZ. Scale bar = 50 μm.

(C-F) Statistical analysis of c-fos$^+$ cell density in CA1 (C), dorsal DG (D), ventral DG (E) and amygdala (F). n = 4 mice in each group. *$p < 0.05$; ***$p < 0.001$. One-way ANOVA followed by Dunnett's post hoc test.

Data presented are the mean ± SEM

Figure S2. Ocn-Cre expressed largely in dDG, but barely in vDG



(A) Sagittal section map of adult mouse brain.

(B) A representative image of Td (red) in the sagittal brain section of Ocn-Cre;Ai9 mouse. DAPI (blue) was stained for cell nucleus. Scale bar = 500 μm. Higher magnification of the selected region in DG was shown in B1 and B2. Scale bar = 200 μm.

(C) Representative images of Td (red) in dorsal (D) and ventral (E) DG of horizontal brain sections of Ocn-Cre;Ai9 mouse. DAPI (blue) was stained for cell nucleus. Scale bar = 500 μm. Higher magnification of the selected region in DG was shown in left bottom. Scale bar = 200 μm.

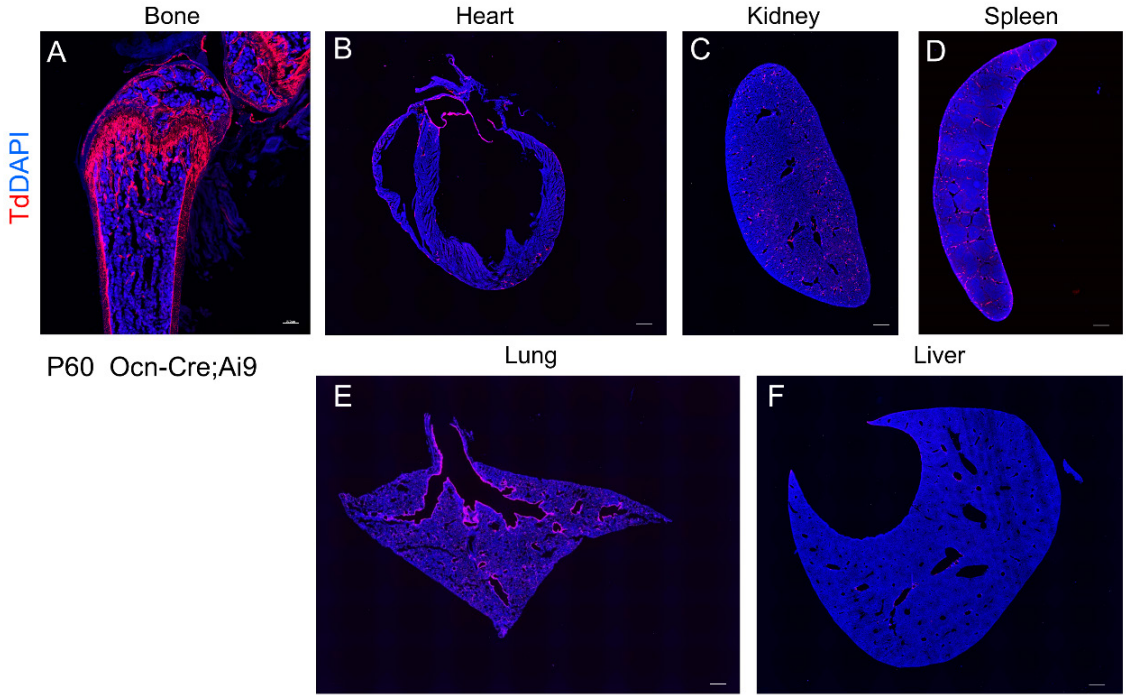
(D) Quantification of the density of Ocn-Cre$^+$ DGCs in dorsal and ventral DG. n = 3 mice. Data presented are the mean ± SEM.

(E) Co-immunostaining analysis of Td (red) with NeuN (green) in dDG of Ocn-Cre;Ai9 mice. DAPI (blue) indicates cell nucleus. Scale bar = 50 μm.

(F) Quantification of the percentage of Td^+;$NeuN^+$ over total $NeuN^+$ cells in E. n=3 mice.

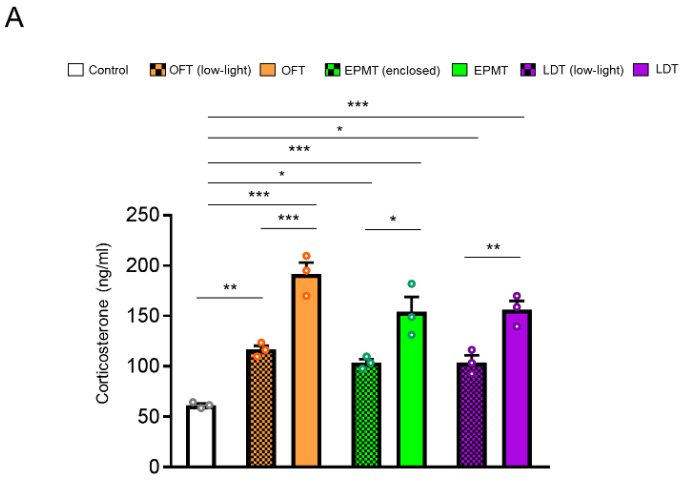
(G) A representative image of Ocn-Cre expression in the sagittal brain section of Ocn-Cre;Ai9 mouse at P30.

Figure S3. Ocn-Cre expressed in different tissues



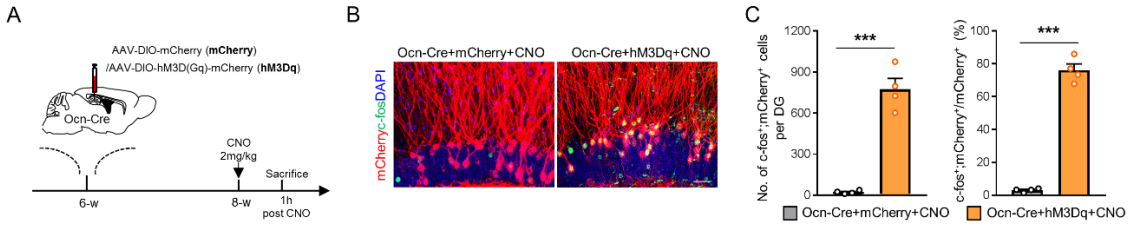
(A-F) Representative images of Ocn-Cre expression (viewed by Td) in different tissues of Ocn-Cre;Ai9 mouse at P60. A: Bone; B: Heart; C: Kidney; D: Spleen; E: Lung; F: Liver. Scale bar = 300 μm.

Figure S4. Increased plasma corticosterone levels in mice exposed to anxiogenic environments



(A) Measurement of plasma corticosterone levels in mice exposed to anxiogenic and non/mild anxiogenic/locomotive environments by Elisa assay. n=3 mice in each group. *p < 0.05; **p < 0.01; ***p < 0.001. One-way ANOVA followed by Tukey's post hoc test. Data presented are the mean ± SEM.

Figure S5. Chemogenetic activation of Ocn-Cre^{+} dDGCs by CNO treatment

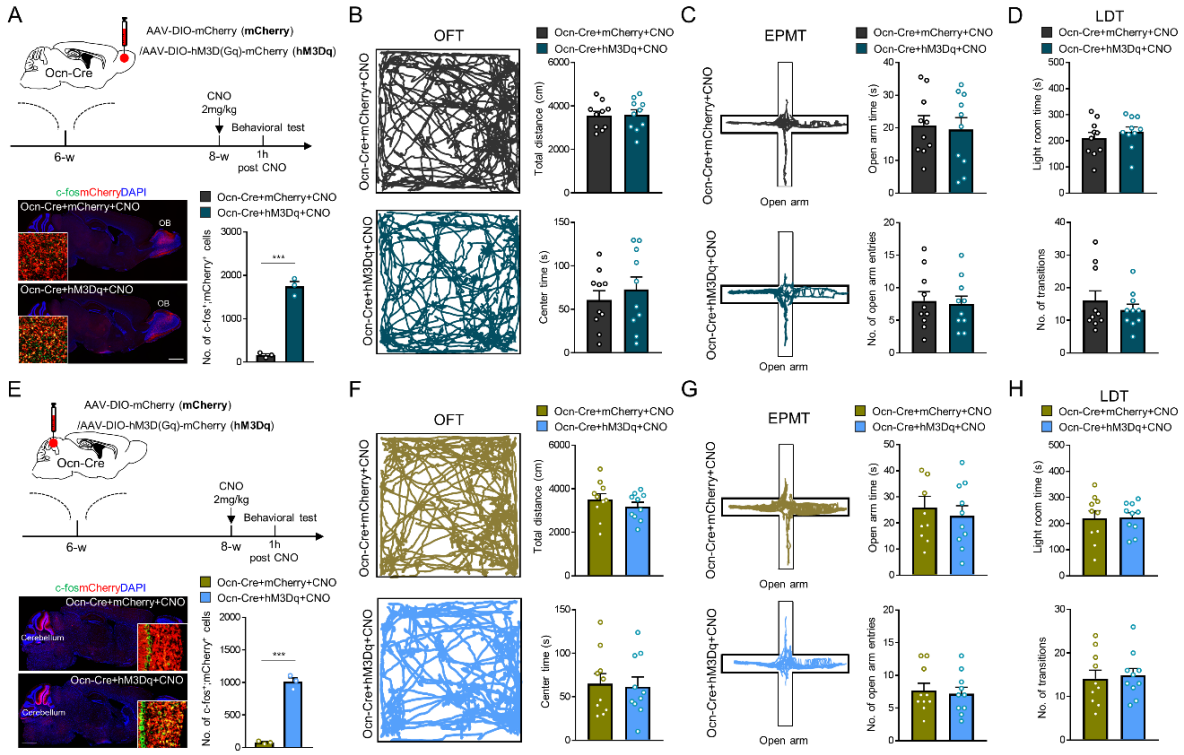


(A) Schematic diagram of AAV (mCherry or hM3Dq) injections in dDGs of Ocn-Cre mice and CNO treatment.

(B) Representative images of c-fos^{+} (green) and mCherry^{+} neurons in dDG of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM3Dq+CNO mice. DAPI (blue) indicates cell nucleus. Scale bar = 50μm.

(C) Quantifications of the data in B, the number of c-fos^{+};mCherry^{+} DGCs (left) and the percentage of c-fos^{+};mCherry^{+} cells over mCherry^{+} cells. n = 4. ***p < 0.001. Student's *t*-test. Data presented are the mean ± SEM.

Figure S6. Little effect on anxiety-like behaviors by activating Ocn-Cre^{+} neurons in OB and cerebellum



(A, E) Schematic diagram of experimental design for behavioral tests in Ocn-Cre mice injected with mCherry or hM3Dq viruses in olfactory bulb (OB) (A) and cerebellum (E); co-immunostaining of c-fos (green) and mCherry (red) in Ocn-Cre+mCherry+CNO and Ocn-Cre+hM3Dq+CNO mice. DAPI (blue) indicates cell nucleus. Scale bar = 200μm. n = 3 mice in each group. ***p < 0.001. Student's *t*-test.

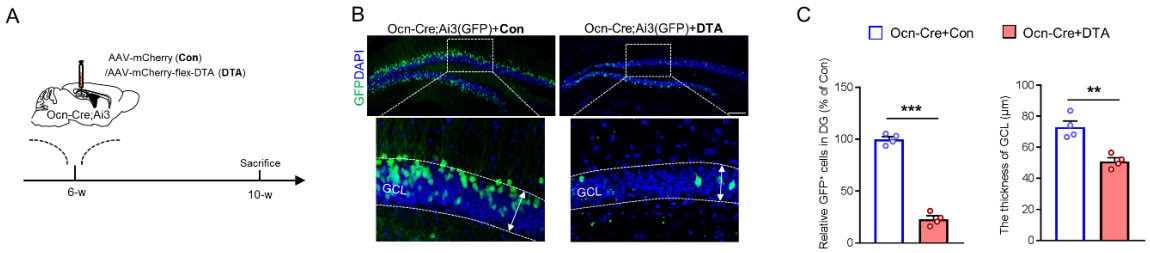
(B, F) Representative tracing images and quantifications of total distance and center duration time in the OFT of Ocn-Cre mice injected with mCherry or hM3Dq viruses in OB (B) and cerebellum (F). n = 10 mice in each group.

(C, G) Representative tracing images and quantifications of open arm duration time and entries in the EMPT of Ocn-Cre mice injected with mCherry or hM3Dq viruses in OB (C) and cerebellum (G). n = 10 mice in each group.

(D, H) Quantifications of the time in the light room and the number of transitions into the light room in the LDT of Ocn-Cre mice injected with mCherry or hM3Dq viruses in OB (D) and cerebellum (H). n = 10 mice in each group.

Data are presented as the mean ± SEM.

Figure S7. Ablation of Ocn-Cre$^+$ dDGCs by DTA

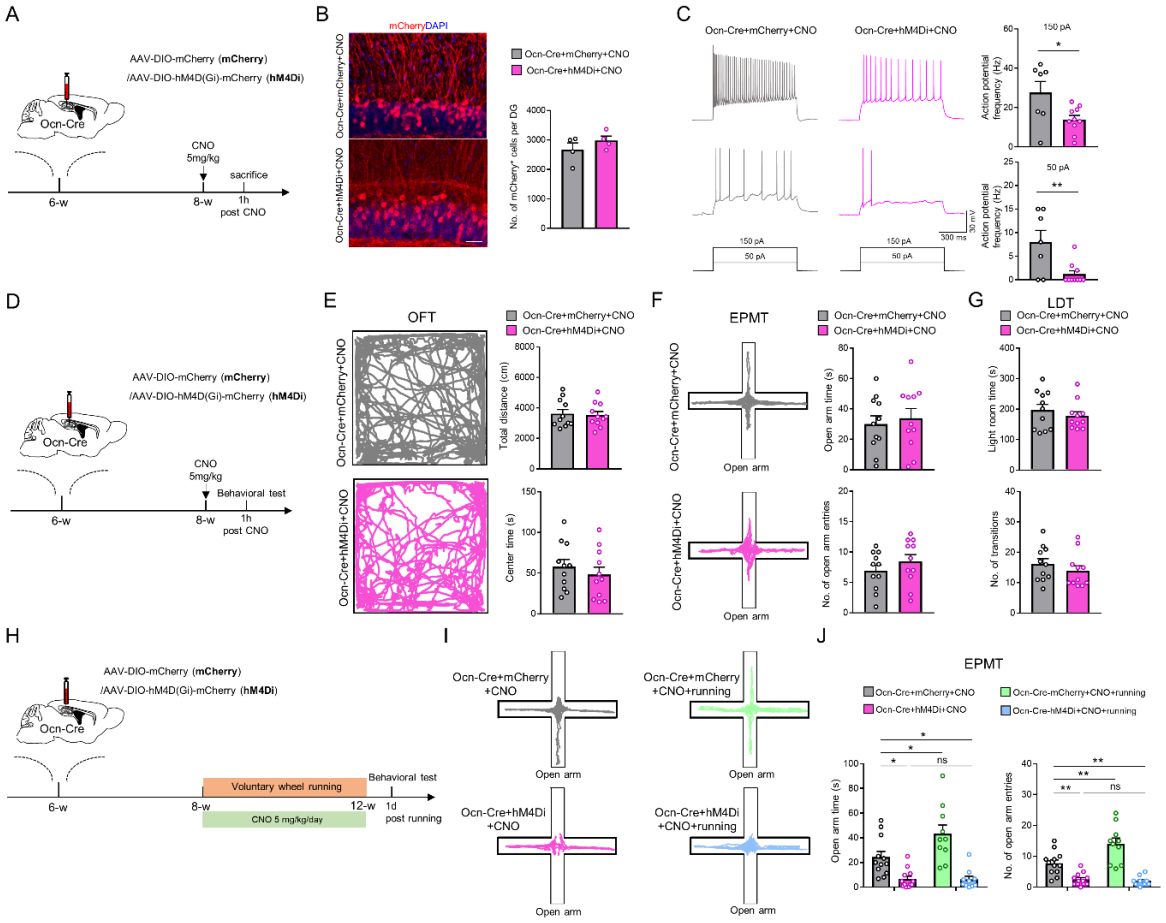


(A) Schematic diagram of AAV (Con or DTA) injections in dDGs of Ocn-Cre mice for 4 weeks.

(B) Representative image of Ocn-GFP$^+$ neurons in dDG of Ocn-Cre+Con and Ocn-Cre+DTA mice. Higher magnification of the selected region in DG was shown in the bottom. Scale bar = 100μm

(C) Quantifications of the data in B, the relative number of Ocn-GFP$^+$ dDGCs and the thickness of GCL. n = 4 in each group. **p < 0.01; ***p < 0.001. Student's *t*-test. Data presented are the mean ± SEM.

Figure S8. Chronic, but not acute, inhibition of Ocn-Cre$^+$ dDGCs exacerbated anxiety-like behaviors



(A) Schematic diagram of experimental design for virus injections (mCherry or hM4Di) in dDGs of Ocn-Cre mice.

(B) Representative images (left) and quantification of the number of mCherry$^+$ (red) neurons (right) in dDG of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM4Di+CNO mice. DAPI (blue) indicates cell nucleus. Scale bar = 50μm.

(C) Representative action potential (AP) firing (left) and quantification of the AP frequency (right) in Ocn-Cre+mCherry+CNO (n=7) and Ocn-Cre+hM4Di+CNO (n=10)

DGCs evoked by 50-pA or 150-pA depolarizing current pulse injection. Scale bar: 300 ms, 30 mV. *p < 0.05; **p < 0.01. Student's *t*-test. Data presented are the mean ± SEM.

(D) Schematic diagram of experimental design for behavioral tests in Ocn-Cre mice injected with mCherry or hM4Di viruses.

(E) Representative tracing images and quantifications of total distance and center duration time in the OFT of Ocn-Cre mice injected with mCherry or hM4Di viruses in dDG. n = 11 mice in each group.

(F) Representative tracing images and quantifications of open arm duration time and entries in the EMPT of Ocn-Cre mice injected with mCherry or hM4Di viruses in dDG. n = 11 mice in each group.

(G) Quantifications of the time in the light room and the number of transitions into the light room in the LDT of Ocn-Cre mice injected with mCherry or hM4Di viruses in dDG. n = 11 mice in each group.

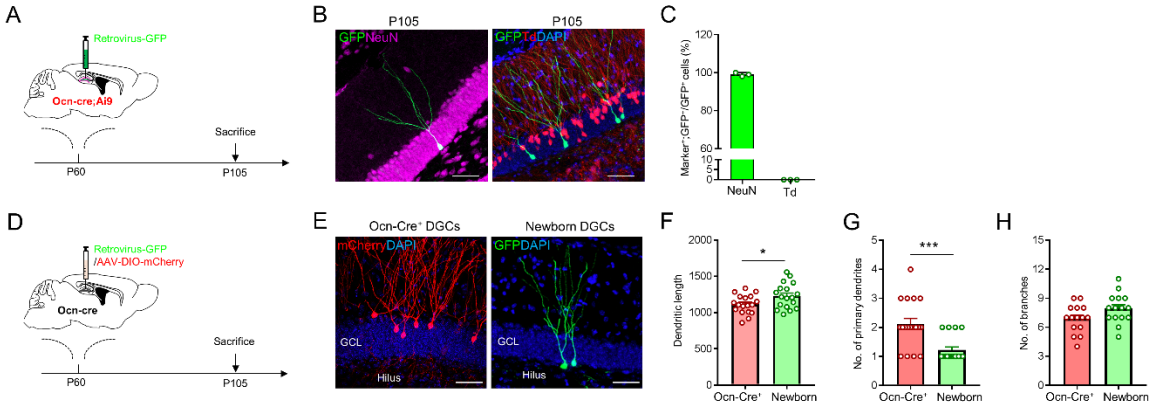
(H) Schematic diagram of virus injections (mCherry or hM4Di) in dDGs of Ocn-Cre mice and voluntary wheel running for behavioral test.

(I-J) Representative tracing images (I) and quantifications of open arm duration time and entries in the EMPT (J) of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM4Di+CNO mice with or without running. Ocn-Cre+mCherry+CNO (n=12); Ocn-Cre+hM4Di+CNO (n=12); Ocn-Cre+mCherry+CNO +running (n=10); Ocn-Cre+hM4Di+CNO+running (n=10). *p < 0.05; **p < 0.01; ns = no significant difference. Two-way ANOVA test for open arm duration time, interaction: $F_{(1, 40)}$=4.628, p=0.0375; running factor: $F_{(1, 40)}$=4.282, p=0.045; virus factor: $F_{(1, 40)}$=37.44, p<0.0001. Two-way ANOVA test for open arm entries,

interaction: $F_{(1, 40)}$=8.825, p=0.005; running factor: $F_{(1, 40)}$=6.404, p=0.0154; virus factor: $F_{(1, 40)}$=57.63, p<0.0001.

Data in (B, C, E, F, G and J) are presented as the mean ± SEM.

Figure S9. Ocn-Cre^{+} dDGCs displayed distinct features from that of adult born dDGCs



(A) Schematic diagram shows that retroviruses expressing GFP were injected into dDG of Ocn-Cre;Ai9 mice for 45 days.

(B) Co-immunostaining analysis of GFP (green) with NeuN (purple) or Td (red) in DG. DAPI (blue) was stained for cell nucleus. Scale bar = 50 μm.

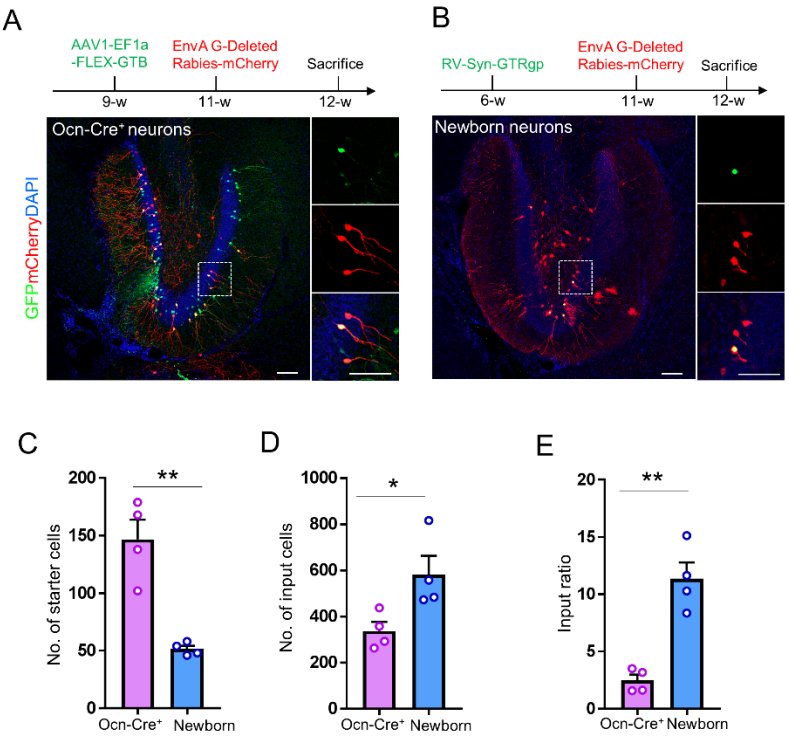
(C) Quantification of the data in (B), the percentage of marker^{+};GFP^{+} over total GFP^{+} cells. n=3 in each group.

(D) Schematic diagram shows that AAV-DIO-mCherry or retroviruses expressing GFP were injected into dDG of Ocn-Cre mice for 45 days.

(E) Immunostaining analysis of GFP (green) and mCherry (red) in DG. DAPI (blue) was stained for cell nucleus. Scale bar = 50 μm.

(F-H) Quantifications of dendrites length (F), the number of primary dendrites (G) and branches (H) in Ocn-Cre^{+} dDGCs and adult newborn dDGCs. n=18 neurons in each group. *P < 0.05; ***P < 0.001. Student's *t*-test. Data presented are mean ± SEM.

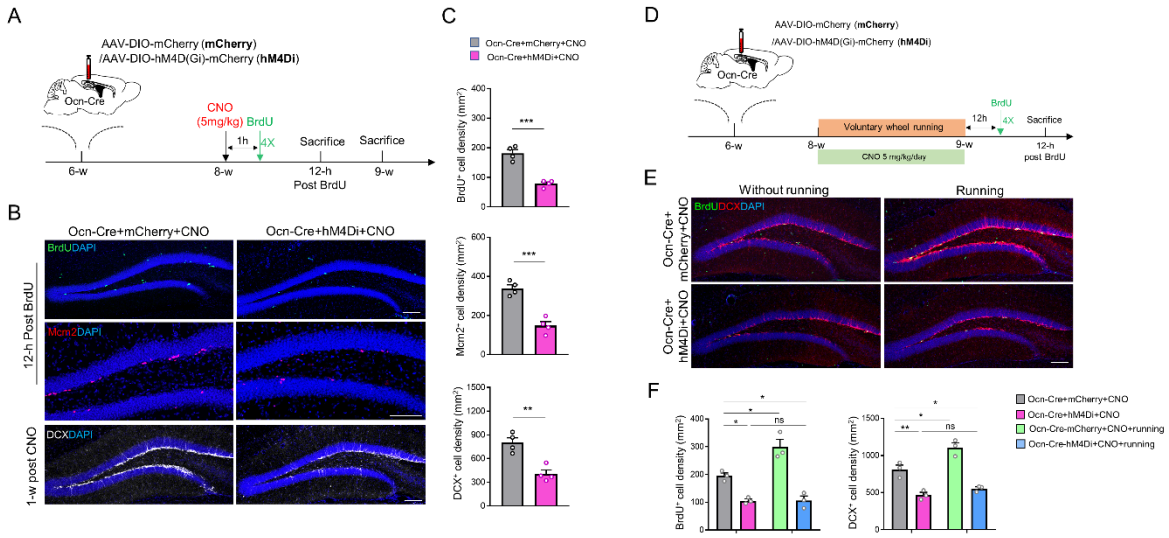
Figure S10. Decreased monosynaptic inputs to Ocn-Cre$^+$ dDGCs as compared with that of newborn dDGCs



(A and B) Schematic diagram and representative images of monosynaptic retrograde tracing in Ocn-Cre$^+$ dDGCs (A) and newborn dDGCs (B). Scale bar = 50μm.

(C-E) Quantitative analyses of the data in (A and B), the number of starter cells (C), input cells (D) and input ratio (E). Data presented are mean ± SEM (n = 4). *P < 0.05; **P < 0.01. Student's *t*-test.

Figure S11. Decreased adult DG neurogenesis by inhibition of Ocn-Cre$^+$ dDGCs



(A) Schematic diagram of experimental design for analyzing adult DG neurogenesis after inhibition of Ocn-Cre$^+$ dDGCs.

(B) Representative images of BrdU (green), Mcm2 (red) and DCX (white) in Ocn-Cre+mCherry+CNO and Ocn-Cre+hM4Di+CNO mice. DAPI (blue) indicate cell nucleus. Scale bar = 100 μm.

(C) Quantitative analyses of the data in (B), the density of BrdU$^+$ (top), Mcm2$^+$ (middle) and DCX$^+$ (bottom) cells. **p < 0.01. ***p < 0.001. Student's *t*-test. n=4 in each group.

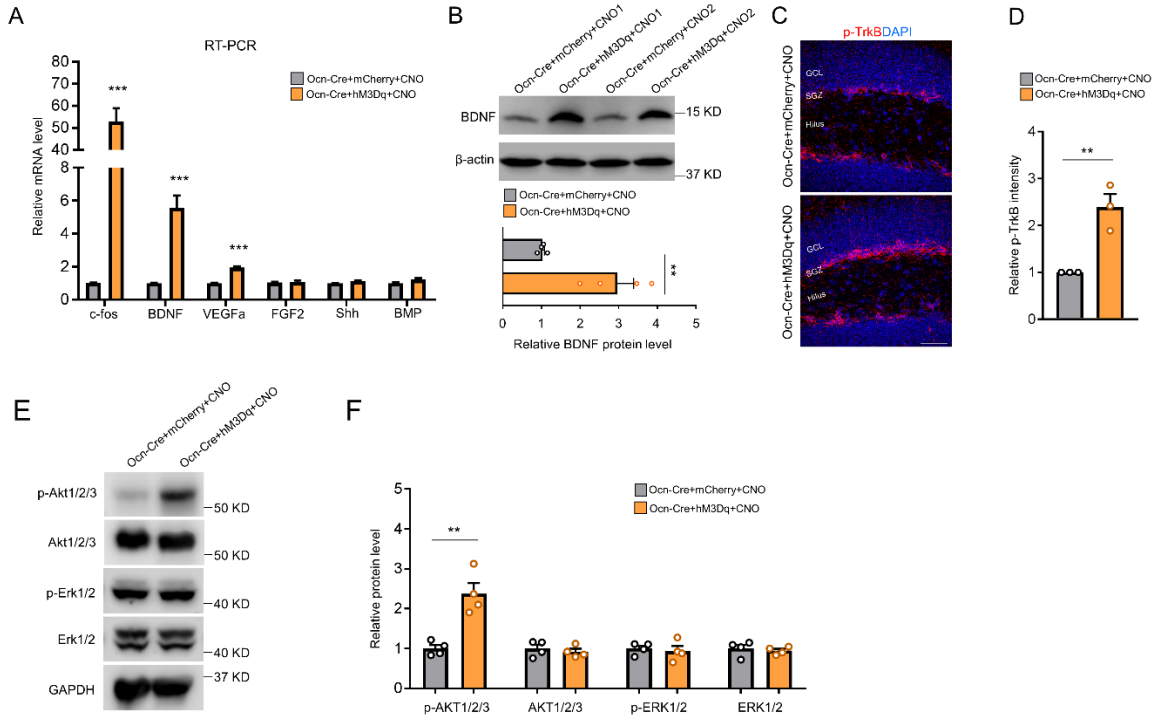
(D) Schematic diagram of virus injections (mCherry or hM4Di) in dDGs of Ocn-Cre mice and voluntary wheel running for analyzing adult DG neurogenesis. CNO was delivered in drinking water.

(E) Co-immunostaining analysis of BrdU (green) and DCX (red) in dDG of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM4Di+CNO mice with or without running. DAPI (blue) indicates cell nucleus. Scale bar = 100 μm.

(F) Quantifications of the data in (E), the density of $Brdu^+$ (left) cells and DCX^+ (right) cells. n=3 mice in each group. *p < 0.05; **p < 0.01; ns = no significant difference. Two-way ANOVA test for $BrdU^+$ cell density, interaction: $F_{(1, 8)}$=9.119, p=0.0166; running factor: $F_{(1, 8)}$=9.513, p=0.015; virus factor: $F_{(1, 8)}$=68.07, p<0.0001. Two-way ANOVA test for DCX^+ cell density, interaction: $F_{(1, 8)}$=5.728, p=0.0436; running factor: $F_{(1, 8)}$=12.81, p=0.0072; virus factor: $F_{(1, 8)}$=90.03, p<0.0001.

Data in (C and F) are presented as the mean ± SEM.

Figure S12. Increased BDNF, p-TrkB and p-Akt1/2/3 in hippocampus after activating Ocn-Cre^{+} dDGCs



(A) Real-time PCR (RT-PCR) analysis of relative gene expression in Ocn-Cre+hM3Dq+CNO mice (Normalized to Ocn-Cre+mCherry+CNO mice). ***p < 0.001. Student's *t*-test. n=5 mice in each group.

(B) Western blot analysis of BDNF protein level in hippocampus of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM3Dq+CNO mice. **p < 0.01. Student's *t*-test. n=4 mice for each group.

(C) Immunostaining analysis of p-TrkB (red) in dDG of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM3Dq+CNO mice. DAPI (blue) indicates cell nucleus. Scale bar = 50 μm.

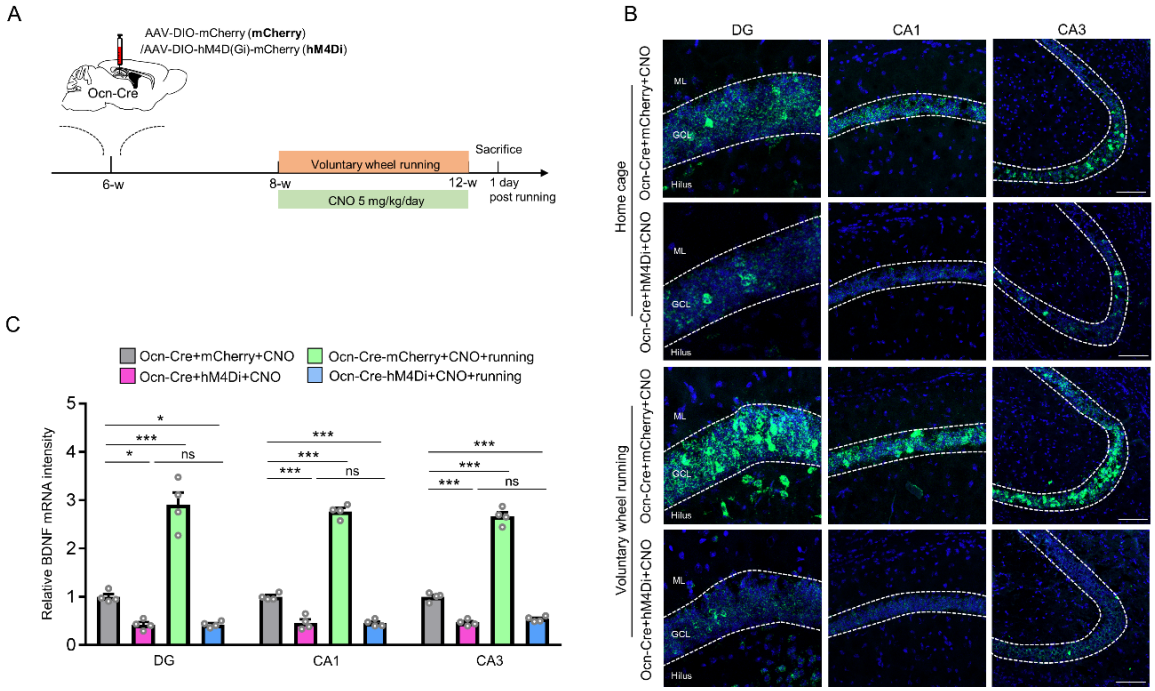
(D) Quantification of the data in (C), the relative p-TrkB levels. **p < 0.01. Student's *t*-test. n=3 mice in each group.

(E) Western blot analysis of Akt1/2/3, p-Akt1/2/3, Erk1/2 and p-Erk1/2 protein levels in DG of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM3Dq+CNO mice.

(F) Quantifications of the data in (E), the relative protein level of Akt1/2/3, p-Akt1/2/3, Erk1/2 and p-Erk1/2. **$p < 0.01$. Student's *t*-test. n=4 mice for each group.

Data in (A, B, D and F) are presented as the mean ± SEM.

Figure S13. Abolished activity-induced BDNF increase by inhibiting Ocn-Cre^{+} dDGCs



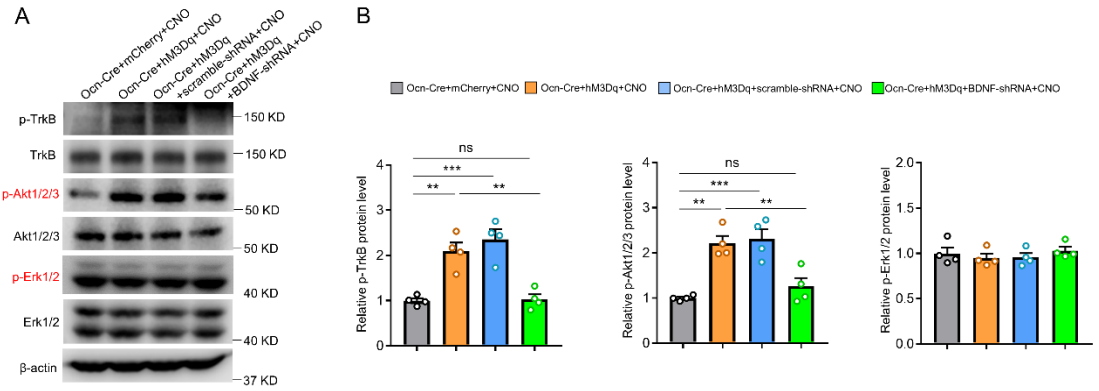
(A) Schematic diagram of virus injections (mCherry or hM4Di) in dDGs of Ocn-Cre mice and voluntary wheel running.

(B) Detection of BDNF mRNA by RNA scope in DG, CA1 and CA3 of Ocn-Cre+mCherry+CNO and Ocn-Cre+hM4Di+CNO mice with or without running. Scale bar = 100 μm.

(C) Quantification of the data in (B), the relative BDNF mRNA level. *p < 0.05; ***p < 0.001. ns = no significant difference. n=4 mice in each group. Two-way ANOVA test, for DG region, interaction: $F_{(1, 12)}$=48.87, p<0.0001; running factor: $F_{(1, 12)}$=49.51, p<0.0001; virus factor: $F_{(1, 12)}$=126.3, p<0.0001. For CA1 region, interaction: $F_{(1, 12)}$=254.5, p<0.0001; running factor: $F_{(1, 12)}$=250.4, p<0.0001; virus factor: $F_{(1, 12)}$=654.6, p<0.0001. For CA3

region, interaction: $F_{(1, 12)}$=218.1, p<0.0001; running factor: $F_{(1, 12)}$=260.9, p<0.0001; virus factor: $F_{(1, 12)}$=607.5, p<0.0001. Data are presented as the mean ± SEM.

Figure S14. Akt, but not Erk, signaling was activated by activation of Ocn-Cre^{+} dDGCs



(A) Western blot analyses of TrkB, p-TrkB, Akt1/2/3, p-Akt1/2/3, Erk1/2 and p-Erk1/2 protein level in DG of Ocn-Cre+mCherry+CNO, Ocn-Cre+hM3Dq+CNO, Ocn-Cre+hM3Dq+scramble-shRNA+CNO and Ocn-Cre+hM3Dq+BDNF-shRNA+CNO mice.

(B) Quantifications of the data in A, the relative p-TrkB (left), p-Akt1/2/3 (middle) and p-Erk1/2 (right) protein levels. **p < 0.01; ***p < 0.001; ns = no significant difference. One-way ANOVA followed by Tukey's post hoc test. n=4. Data presented are the mean ± SEM.

Supplementary References

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