Supporting Information

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SI Materials and Methods

Animals. *ChAT-IRES-Cre* [B6;129S6-*Chat*$^{tm2(cre)lowl}$/J] and *Som-IRES-Cre* [*Sst*$^{tm2.1(cre)Zjh}$] knockin mice were originally obtained from the Jackson Laboratory and were bred in-house at the animal facility. We established the Chrna-cre mouse [Tg(Chrna2cre)OE29Gsat/Mmucd] colony at the National Institute of Environmental Health Sciences from founders obtained from the MMRRC. Mice were tail tattooed and genotyped at postnatal day 21 before being weaned. All animal procedures were performed according to the animal study protocols approved by the Animal Care and Use Committee and in compliance with the NIH Humane Care and Use of Animals.

Monosynaptic Tracing. Before all virus injections, mice were deeply anesthetized with 100 mg/kg ketamine and 7 mg/kg xylazine administered i.p. and were fixed to the stereotaxic instrument (Stoelting/Neurostar). Virus injections were made at a rate of 100 nL/min with a 5-μL Hamilton syringe. The AAV helper virus that carries the glycoprotein G, the retroviral receptor TVA, and GFP (AAV9-CaMKIIa-T2A-TVA-E2A-B19G, subcloned from Addgene #44187, titer 1.31×10^{13} genome copies per milliliter; Viral Vector Core titer) was injected into the ECV of 10- to 12-wk-old *ChAT-IRES-Cre* mice at the following coordinates: anteroposterior (AP) −4.25, mediolateral (ML) ±3.45, dorsoventral (DV) −4.44. After a minimum of 4 wk of recovery after the helper AAV injection, the attenuated G-deleted rabies virus that expresses mCherry (EnvA G-deleted Rabies-mCherry, titer 5.9×10^{7} transduction units/mL, Viral Vector Core titer) was injected into the ECV. Seven to 10 days after the rabies injection, mice were transcardially perfused for immunohistochemical staining.

Septo-Hippocampal Coculture System. The septo-hippocampal coculture was made from 7- to 8-d-old *ChAT-IRES-Cre* mice. A 350-μm-thick coronal slice containing the medial septum was placed near the CA3 region of a horizontal slice containing the hippocampus, which was cultured for at least 2 wk before being used for electrophysiological recordings. Horizontal slices containing ventral, intermediate, or dorsal hippocampus were used for the coculture system. It has been shown that neurons in the culture system continue to differentiate and develop connections in vitro (1, 2). Therefore, 2- to 3-wk-old cultures of 1-wk-old slices show circuits comparable to those in 3- to 4-wk-old mice (1). The culture medium (1 L) contained the following: 5.52 g Basal Medium Eagle (Sigma), 30 mL Earle's Balanced Salt Solution 10× (Sigma), 20 mM NaCl, 5 mM $NaHCO_3$, 0.2 mM $CaCl_2$, 1.7 mM $MgSO_4$, 48 mM glucose, 26.7 mM Hepes, 50 mL horse serum, 10 mL penicillin-streptomycin-glutamine (Gibco), and 1.32 mg insulin. NGF 2.5 S (250 nM, 6.7 ng/mL) was added every 60 h to the culture medium to increase the survival of cholinergic neurons. Virus was injected into slices using a microinjector (Nanoject 3-00-203-X; Drummond) through a glass pipette targeting the area of interest.

For expression of ChR2 in cholinergic neurons or somatostatin-expressing neurons, the AAV9 that carries Cre-inducible ChR2-mCherry [AAV9-EF1a-double floxed-hChR2(H134R)-mCherry-WPRE-HGHpA] was injected into cocultured slices from *ChAT-IRES-Cre* and *Som-IRES-Cre* mice. At least 10 d of incubation time was allowed after the virus injection before the cocultured slices were used for electrophysiological recordings. The optogenetic experiment using the cocultured slices that do not contain any mCherry-positive ChR2-expressing cholinergic cells that project to the hippocampus suggests that blue light illumination itself, without ChR2 expression, does not cause a significant change in eEPSC amplitude in ECV neurons [from 176.5 ± 66.6 to 204.5 ± 77.8 pA, Student's paired *t* test, $P = 0.136$, $t(3) = -2.028$, $n = 4$].

For the rabies virus-dependent expression of ChR2 for slice electrophysiology experiments, the AAV helper virus that carries the glycoprotein G, the retroviral receptor TVA, and GFP (AAV9-CaMKIIa-T2A-TVA-E2A-B19G, subcloned from Addgene #44187, titer 1.31×10^{13} genome copies per milliliter, Viral Vector Core titer) was injected into the ECV of cocultured slices. One to two weeks after the helper virus injection, the attenuated G-deleted rabies virus that expresses mCherry and ChR2 (EnvA G-deleted Rabies-ChR2-mCherry, titer 6.80×10^{7} transduction units per milliliter, Salk Gene Transfer Targeting and Therapeutics Core) was injected to the ECV. After 1–2 wk of incubation, the slices were used for electrophysiological recordings.

In Vivo Fiber Photometry. A homemade fiber photometry system similar to that previously described (3) was used for in vivo measurements of GCaMP6f fluorescence in ECV neurons. The 5:1 mixture of the AAV9 viral solution that carries GCaMP6f (AAV9- CaMIIα -GCaMP6f; subcloned from Addgene #40755; titer, 4.68×10^{12} genome copies per milliliter, Viral Vector Core) and tdTomato (AAV9-CaMIIα-tdTomato-WPRE; subcloned from Addgene #51506; titer 6.23×10^{13} genome copies per milliliter, Viral Vector Core) was injected to the ECV of 2- to 5-mo-old *ChAT-IRES-Cre* mice at the following coordinates: AP −4.15, ML ±3.45, DV −4.44. In addition, AAV9 carrying double-floxed Arch (AAV9-FLEX-Arch-GFP; Addgene #22222; titer 1.63×10^{13} genome copies per milliliter, Viral Vector Core) was injected into the medal septum at the coordinates AP 0.95, ML 0.02, and DV −4.5 with a needle angled 10° toward the midline. For chemogenetic inhibition of OLM interneurons, AAV9 carrying double-floxed inhibitory DREADD [AAV9-hSyn-DIO-hM4D(Gi)-mCherry] was injected into the following coordinates: AP −3.00, ML ±2.75, DV −2.05. After a minimum of 4 wk of recovery after intracerebral microinjection of virus injection, optical probes were lowered into the EC (coordinates: AP −4.15, ML ±3.45, DV −4.44) and medial septum (AP 0.95, ML 0.02, DV −4.5, angled 10°) during a stereotaxic surgery. The optical probe in the EC (to measure the GCaMp6f and tdTomato signals) was made of a ferruled multimode optical fiber pigtail with 105-μm core and 125-μm cladding diameters and a numerical aperture (NA) of 0.22. A larger-diameter optical probe (200-μm core/230-μm cladding diameters, NA 0.39) was used for optogenetic inhibition of cholinergic neurons at a site in the medial septum. The distal end of the probe was a 5-mm-long free fiber with a flat-cleaved end. The proximal end of the probe was a polished end with a 1.25-mm o.d. ceramic ferrule. The animals were allowed to recover for at least 2 wk after the fiber implantation surgery before the measurements began. For in vivo fiber photometry measurements, the optical probe in the EC was connected through a ceramic mating sleeve to a 2-m-long multimode fiber patch cable with 105-μm core and125-μm cladding and a 0.22 NA. Only one of the probes in the EC (either left or right) was recorded; probe selection was based on the intensity of GCaMP6f signal. The probe in the medial septum was connected to a fiber patch cable with a200-μm core/230-μm cladding and a 0.22 NA. Before recording, animals were placed in a new environment, a 21.6 × 17.8 cm arena (ENV-307W-CT; Med Associates) with dim house light illumination. Fiber photometry measurements began after 5 min of habituation to the patch

cables. The GCaMP6f and tdTomato fluorescence signal was acquired at 50 Hz using a custom-built transistor–transistor logic (TTL) generator. At least a 5-min baseline recording of GCaMP6f and tdTomato fluorescence was acquired before optogenetic or pharmacological treatments began. For optogenetic inhibition of cholinergic neurons, green light (561 nm, laser output at the tip 1–1.5 mW) was continuously applied to the probe in the medial septum for 10 min. For pharmacological inhibition of muscarinic AChRs, PBS was first injected i.p. after a minimum of 5-min baseline recording. Ten minutes after the PBS injection, scopolamine (1 mg/kg body weight as a 1 mg/10 mL solution) was injected i.p. For chemogenetic inhibition of OLM interneurons, the CNO solution (3 mg/kg body weight as a 3 mg/10 mL solution) was injected i.p. The stock CNO solution was made in DMSO, which was diluted 1:200 in PBS on the day of the experiment. GCaMP6f fluorescence was calculated by total photon counts between 500 nm and 540 nm. TdTomato fluorescence was calculated by photon counts between 575 nm and 650 nm. To correct for movement-induced artifacts, the ratio of GCaMP6f/tdTomato ($R_{G/td}$) was used to represent in vivo calcium signals. The ratio was further normalized within each mouse in the format of $(R_{G/td} - R_{G/td\ average})/R_{G/td\ average}$, where $R_{G/td\ average}$ was the mean of all $R_{G/td}$ acquired during the measurement. Mini Analysis 6.0.9 (Synaptosoft) was used to analyze calcium transients.

Electrophysiology. All electrophysiological recordings were performed using a Multiclamp 700B or Axopatch 200B patch-clamp amplifier (Molecular Devices). The recording data were acquired using a Digidata 1550A or 1332A system (Molecular Devices) and pClamp software (Molecular Devices). Electrophysiological recordings were performed on slices in a recording chamber that was continuously perfused with oxygenated artificial cerebrospinal fluid (aCSF: 126 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 11 glucose, 2 $CaCl_2$, and 1.3 $MgCl_2$). Using borosilicate glass (1.65 μm o.d., 1.2 μm i.d.; KG-33; King Precision Glass), whole-cell patch-clamp recordings were performed. Patch electrodes with a tip resistance of 3–6 MΩ were made using a horizontal micropipette puller (P-97; Sutter Instruments). To record AChR currents in OLM interneurons and eEPSCs in ECV neurons, cells were held at −60 mV. The internal solution contained the following (in mM): 130 K-gluconate, 2 $MgCl_2$, 2 Mg ATP, 0.3 Na-GTP, 10 Hepes, 1 EGTA, 10 glucose, 25 sorbitol, pH adjusted to 7.25 with KOH. For recording of eEPSCs in ECV, the CA1 pyramidal layer was electrically stimulated using bipolar stimulating electrodes (FHC) to deliver 0.03- to 0.05-ms pulses of 10–11 μA current (S88x; Grass Astro-Med, Inc.). The TFS was composed of 10 pulses at a rate of 10 Hz (0.02–0.05 mA, ≤10 μA).

To record IPSCs in CA1 pyramidal neurons, the cells were held at 0 mV in the presence of the glutamate receptor antagonists DNQX and AP5. The internal solution for the IPSC recordings contained the following (in mM): 130 Cs-gluconate, 10 CsCl, 10 Hepes, 1 $MgCl_2$, 1 EGTA, 2 Mg ATP, and 0.3 Na_2GTP. For some experiments, IPSCs were recorded at −60 mV using the CsCl-based internal solution (120 CsCl, 2 MgCl2, 1 CaCl2, 11 EGTA, 4 ATP-Mg salt, and 30 Hepes, pH adjusted to 7.2 with CsOH and osmolarity adjusted to 300 mOsmol with D-sorbitol). To record the firing activity of neurons, we used current-clamp or loose-seal recordings. We calculated the F/I slope in the current-clamp mode using the first depolarizing step (in 20-pA increments) that reliably produced action potential, as described previously (4). For loose-seal recordings, we used patch electrodes with a bigger tip size (pipette resistance between 1 and 3 MΩ), which were filled with the external solution (aCSF). Spike currents were recorded at the membrane potential with 0-pA current injection (i.e., resting membrane potential). Recordings that showed a sudden increase in the seal resistance (>50 MΩ) were excluded.

For optogenetic electrophysiological experiments, either a laser (LSM 510 META; Zeiss) or an LED system (CoolLED; Andor) was used to generate light, which projected from the objective lens. With the laser system, a region of interest was predefined using Zen 2012 (Zeiss). With the LED system, Andor iQ3 software and the digital micromirror device (Mosaic3; Andor) were used to illuminate a region of interest. The TTL outputs of the laser and LED systems were connected to the computer through the digitizer to verify the duration of illumination.

The following drugs were bath-applied during recordings: pirenzepine (3 μM; R & D Systems), atropine (10 μM; Sigma), MLA (20 nM; R & D Systems), gabazine (10 μM; R & D Systems), DNQX (20 μM; R & D Systems), and AP5 (50 μM; R & D Systems). Current amplitudes and areas were measured using Clampfit 10.5 (Molecular Devices) and Mini Analysis 6.0.9 (Synaptosoft). Spike frequencies were counted using Clampfit 10.5.

Immunohistochemistry. To acquire brain sections for immunostaining, mice were deeply anesthetized with pentobarbital (100 mg/kg) and then were transcardially perfused with 4% PFA in 0.1 M PBS with heparin (1 US Pharmacopeia unit per milliliter). After a mouse was perfused with 15–20 mL of the PFA solution, the brain was dissected out and postfixed in 4% PFA in 0.1 M PBS overnight. The brains were then washed three times with 0.01 M PBS and transferred to 20% sucrose in 0.01 M PBS. After 1–2 d, when the brains were equilibrated with the sucrose solution, the brains were mounted in the tissue-freezing medium (General Data), and 50-μm-thick coronal sections were obtained using a cryostat (Leica CM3050S) for labeling of somatostatin neurons. For the animals that were used for fiber photometry, the sucrose-equilibrated brains were divided into two parts (one rostral and one caudal) before being mounted in the tissue-freezing medium. From the rostral block, coronal sections containing the medial septum were obtained, whereas horizontal sections containing the EC were obtained from the caudal block. From these blocks, we obtained thicker (200-μm) sections to avoid the probe mark's tearing the sections. All sections were washed with 0.01 M PBS five times to remove the tissue-freezing medium before being used for immunohistochemistry.

For the examination of GFP-Arch expression in the medial septa from mice used for photometry recording, the sections were incubated in 10% normal donkey serum (NDS) in 0.01 M PBS with 0.3% Triton X-100 (PBST) for 4 h on a shaker at RT before being used for double immunostaining. The sections were then treated with anti-ChAT (1:100; AB144P; Millipore) in 2% NDS PBST overnight at 4 °C. The sections were washed with PBST five times and then were incubated with donkey anti-goat IgG-Alexa Fluor 594 (1:200; ab150136; Abcam) at RT. The sections were washed with PBST five times and then were incubated with anti-GFP (1:1,000; ab13970; Abcam) in 2% NDS PBST overnight at 4 °C. The sections were washed with PBST five times and then were incubated with donkey anti-chicken IgY-FITC (1:200; ab150136; Abcam) at RT.

For the immunostaining of mCherry for the monosynaptic tracing experiments, sections were incubated in 10% normal goat serum (NGS) in PBST for 4 h on a shaker at RT. The sections were then treated with anti-mCherry (1:2,500; M11217; Thermo Fisher Scientific) in PBST overnight at 4 °C. The sections were washed with PBST five times and then were incubated with goat anti-rat IgG-Alexa Fluor 568 (1:1,000; A11077; Thermo Fisher Scientific) in PBST for 2 h at RT.

For measuring the number of OLM interneurons, we used immunostaining against somatostatin and counted the somatostatin-positive cells in the stratum oriens. It is noteworthy that immunostaining of Chrna2 shows the expression dominantly in the projections but not in the soma, making cell counting

unfeasible. For immunostaining of somatostatin neurons, the sections were incubated in 10% NDS in PBST for 4 h on a shaker at RT. The sections were then treated with anti-somatostatin (1:200; MAB354; Millipore; Research Resource ID: AB_2255365) in PBST overnight at 4 °C. The sections were washed with PBST five times and then were incubated with donkey anti-rat IgG-Alexa Fluor 488 (1:250; ab150153; Abcam) in PBST for 2 h at RT.

For measuring the number of CA1 pyramidal neurons, sections were treated with DAPI stain (1:2,000; 508741; EMD Millipore) for 10 min at RT and then were washed five times with PBST.

After the final antibody incubation, all sections were washed with PBST five times and then were mounted on slides and coverslips with ProLong Diamond Antifade Mountant (Molecular Probes). After being cured for 24 h at 4 °C, the sections were imaged on a confocal microscope (Zeiss LSM 710 or LSM880; Carl Zeiss). The negative controls, which received the same treatments except for the omission of the primary antibody, showed no staining. To count somatostatin-positive cells, coronal sections that are between bregma −1.3 and −2.6 mm, with reference to *Paxinos and Franklin's The Mouse Brain in Stereotaxic Coordinates* (5), were used as dorsal hippocampal slices, whereas sections between bregma −2.60 and −3.6 mm were used as ventral hippocampal slices. For quantification of OLM interneurons, the numbers of somatostatin-positive cells in the deeper side of the stratum oriens were counted automatically using Imaris 8.2.0 (Bitplane).

Behavioral Assays. For behavioral studies, the AAV9 virus carrying Cre-dependent DTA (AAV9-mCherry-flex-dtA) was stereotaxically injected into the stratum oriens in the hippocampus of 6- to 18-wk-old Chrna2-cre mice. Mice recovered for at least 3 wk before being used for behavioral studies.

All mice used for behavioral tests were housed in a 12-h:12-h reverse light–dark cycle for at least 3 wk before behavior testing, which was performed during their dark cycle. Before being tested for the OLT, mice were habituated to the open-field chambers (ENV-510, 27.31 × 27.31 × 20 cm; Med Associates) for 3 d for 10 min/d. The chambers were illuminated with house lights at 10 lx. During the first day of habituation, the mice's ambulatory distance and time spent in the central zone (19.05 × 19.05 cm) were recorded using the three sets of 16 infrared arrays (ENV-256).

The OLT and NORT consisted of three phases: training, retention delay, and testing. During the 10-min training phase, mice were placed in the open-field chamber where two identical objects were placed. Mice were placed back in the same arena after a retention delay of 20 min, during which one of the objects was moved to a new location (OLT) or was replaced with a novel object (NORT). The mouse's exploration of objects was scored using the automated tracking software EthoVision XT 11.5 (Noldus). Exploration of an object was defined as when the distance between the nose point and the object was <1 cm but not when the mouse is climbing the object, which is excluded by eliminating the time points when the center point of the mouse is inside the object. The DI was calculated as follows: $(T_{novel} - T_{familiar})/(T_{novel} + T_{familiar}) \times 100$.

For the Y-maze spontaneous alternation task, the mouse was placed in a distal arm of a Y-maze (three 36.83 × 7.5 cm runaways covered with clear Plexiglas; ENV-343U) by an experimenter to whom the mouse had been habituated for at least a week. Visual extramaze cues were placed on the curtains that enclose the area that contains the maze (173 × 165 cm), which was illuminated with red light bulbs attached to the ceiling at 3–5 lx. After a 10-s delay, the guillotine door that separates the arm from the rest of the maze was opened, and the mouse was allowed to explore the maze freely for 8 min. The mouse's locations were tracked using EthoVision XT to record the sequence of arm entries. An arm entry was defined when the mouse's four paws crossed into the arm. An alternation was scored when an animal entered three different arms sequentially. Spontaneous alternations (%) were calculated by dividing the number of alternations by the maximum possible number of alternations as follows: (number of alternations)/(total number of arm entries − 2) × 100. A direct visit was scored when an animal entered the same arm sequentially. Direct visits (%) were calculated as follows: (number of direct visits)/(total number of arm entries − 1) × 100. An indirect visit was scored when an animal entered the same arm after visiting a different arm. Indirect visits (%) were calculated as follows: (number of indirect visits)/(total number of arm entries − 2) × 100. We verified that the results analyzed with EthoVision XT were comparable to the results obtained with manual scoring [spontaneous alternations, 48.9 ± 3.6% with EthoVision, 49.4 ± 2.9% with manual scoring, $n = 8$, $t(7) = 0.2673$, $P = 0.7970$, Student's t test]. For manual scoring, MATLAB R2014a (MathWorks) was used to calculate spontaneous alternations. All mice used for behavioral studies were numbered randomly, and the experimenter who handled the mice and analyzed data was not aware of the mouse genotype.

Statistics. Data were analyzed using MATLAB R2014a (MathWorks), GraphPad Prism 6 (GraphPad), and SigmaPlot 13.0 (Systat Software). Data were tested for normality with Shapiro–Wilk, Jarque–Bera, and Lilliefors tests using custom-written MATLAB scripts. For comparison of two groups, Student's t test was used for data that follow the normal (Gaussian) distribution, whereas the Mann–Whitney U test was used for data that do not follow the normal distribution. For comparisons of multiple groups, ANOVA and the Wilcoxon signed-rank test were used as a parametric and nonparametric test, respectively. Pairwise comparisons were performed using Sidak's multiple-comparisons and Tukey's tests. All data were expressed as mean ± SEM. $P < 0.05$ was considered significant.

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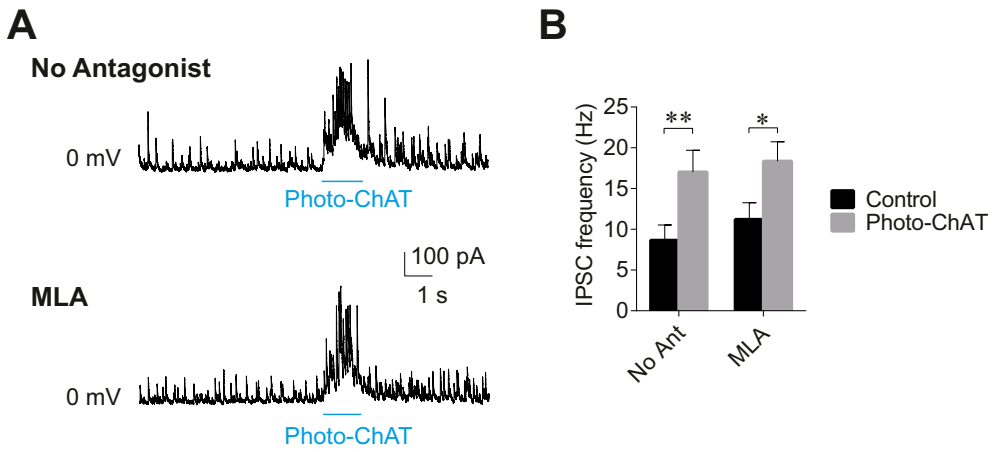

Fig. S1. Cholinergic stimulation of GABAergic synaptic inputs to CA1 pyramidal neurons is not dependent on α7 nAChRs. (*A*) Representative traces showing that photostimulation of ChAT neurons caused an increase in IPSC frequency in CA1 pyramidal neurons which was still present in the presence of the selective α7 nAChR antagonist MLA (20 nM). (*B*) Summary bar graph showing the ChAT neurons-induced increase in IPSC frequency with or without MLA [no antagonist, from 8.647 ± 1.891 to 17.033 ± 2.655 Hz, $n = 5$ neurons, $t(8) = 4.227$, $P = 0.0058$; MLA, from 11.213 ± 2.048 to 18.367 ± 2.375 Hz, $n = 5$ neurons, $t(8) = 3.605$, $P = 0.0138$, repeated-measures two-way ANOVA with Sidak's multiple-comparisons test]. $^*P < 0.05$; $^{**}P < 0.01$.

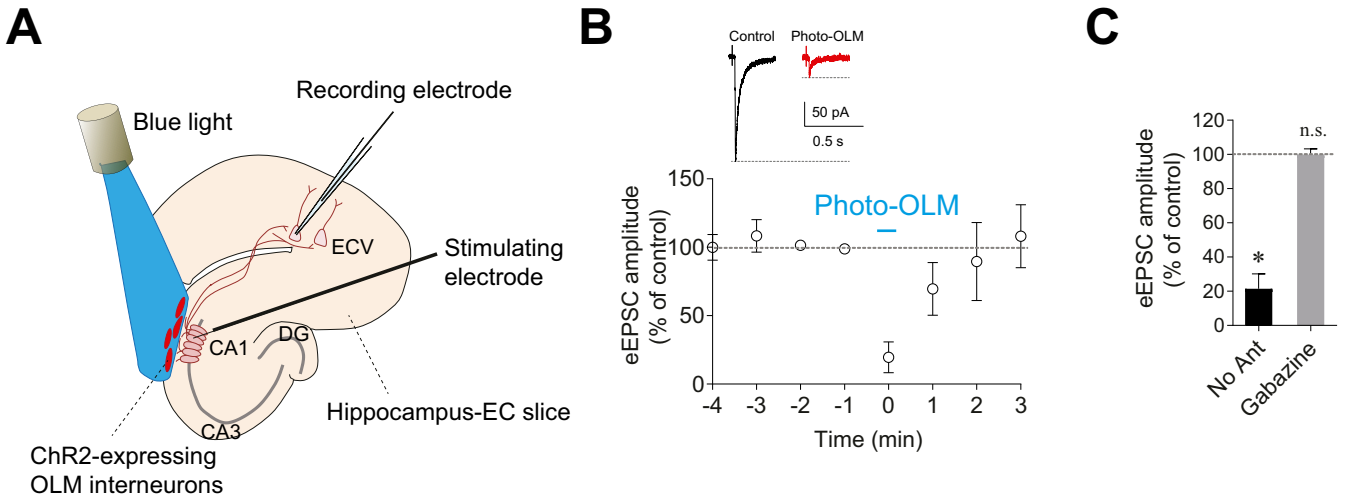

Fig. S2. OLM interneurons suppress the CA1–EC circuit. (*A*) The effect of photostimulation of OLM interneurons on electrically evoked EPSCs in ECV was examined. OLM interneurons were stimulated by blue laser light (488 nm, 50–700 μW) during and before the electrical stimulation of the CA1 pyramidal layer. (*B*) Photostimulation of OLM interneurons (Photo-OLM) caused a decrease in eEPSC amplitude in ECV. Time course mean-normalized eEPSC amplitude showing a decrease in eEPSC amplitude in ECV neurons induced by photostimulation of OLM interneurons. (*C*) Summary of the OLM-induced decrease in eEPSC amplitude [no antagonist (No Ant), 21.5 ± 8.7% of control, $n = 8$ neurons, $t(9) = 2.826$, $P = 0.0393$, two-way ANOVA followed by Sidak's multiple-comparisons test], which was blocked by gabazine [99.8 ± 3.4% of control, $n = 3$ neurons, $t(9) = 0.05007$, $P = 0.9985$, two-way ANOVA followed by Sidak's multiple-comparisons test]. $^*P < 0.05$.

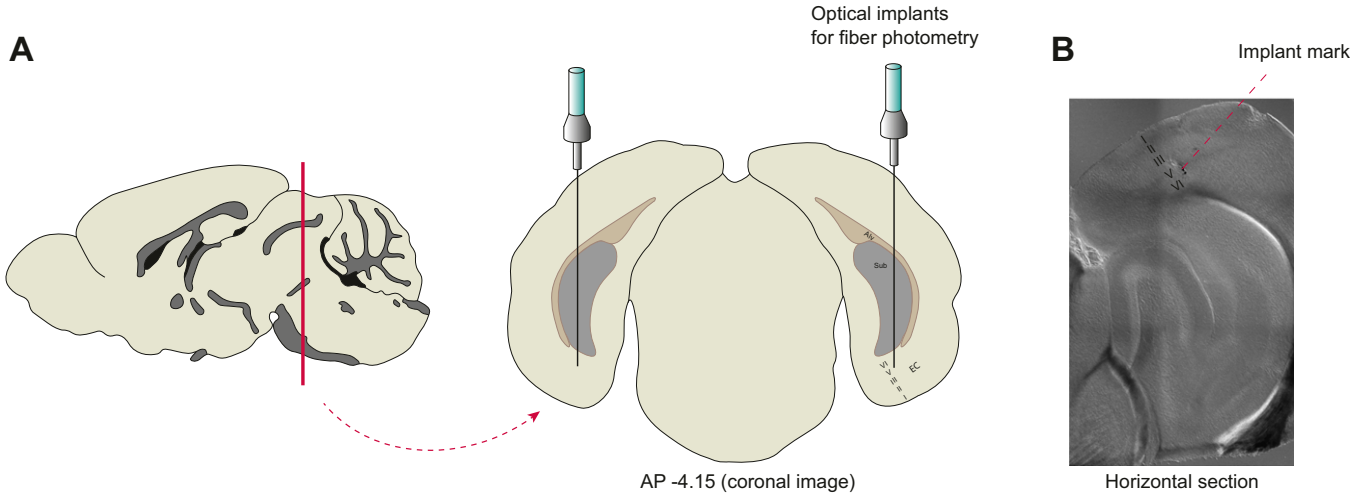

Fig. S3. Localization of optical probes in ECV for fiber photometry. (*A*) To record neuronal activity of ECV neurons, optical probes were implanted to the following coordinates: AP −4.15, ML ±3.45, DV −3.94. (*B*) The horizontal section of the brain showing the location of the optical probe placement.

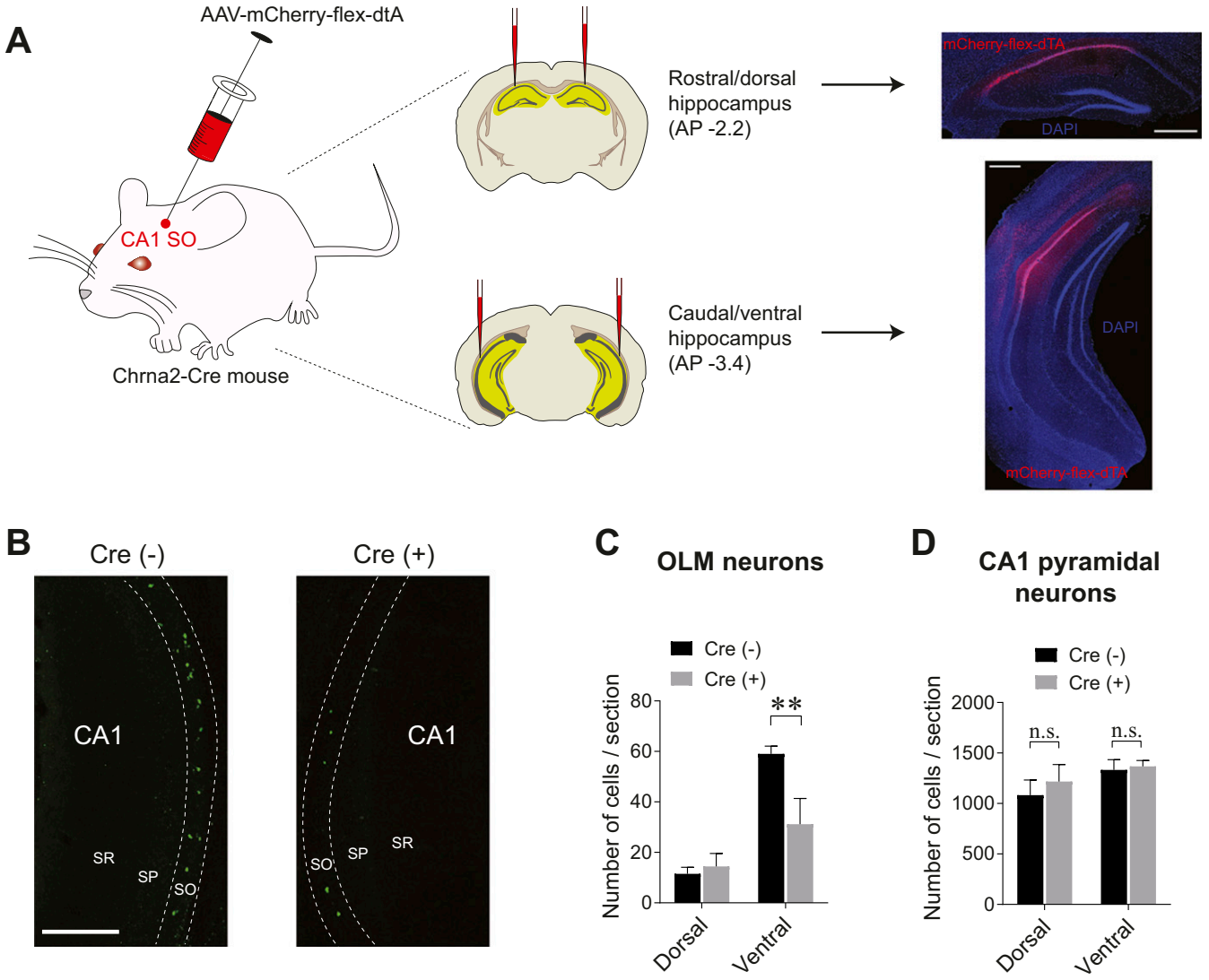

Fig. S4. DTA-induced ablation of OLM interneurons. (*A*) The Cre-inducible viral vector AAV-mCherry-flex-dtA was injected into the brain of Chrna-Cre mice, targeting two rostral and two caudal parts of the hippocampus. (*Left*) Schematic drawings of the injection sites. (*Right*) The expression of mCherry-flex-dtA showed that the AAV injection successfully targeted the stratum oriens of the CA1 region. (Scale bars: 500 μm.) (*B*) The number of OLM interneurons was quantified by performing immunohistochemical staining against somatostatin and counting immune-positive cells specifically in the stratum oriens (SO). Injection of the Cre-inducible viral vector caused a decrease in the number of OLM interneurons in the ventral hippocampus. SP, stratum pyramidale; SR, stratum radiatum. (*C*) Summary bar graph showing the DTA-induced decrease in the number of OLM cells in the ventral hippocampus [Cre−, 59.0 ± 3.1 cells per section, $n = 6$ mice; Cre+, 31.2 ± 11.2 cells per section, $n = 6$ mice, $t(20) = 3.242$, $P = 0.0081$, two-way ANOVA followed by Sidak's multiple-comparisons test], which was absent in the dorsal hippocampus [Cre−, 11.5 ± 2.6 cells per section, $n = 6$ mice; Cre+, 14.4 ± 5.1 cells per section, $n = 6$ mice, $t(20) = 0.3462$, $P = 0.9286$, two-way ANOVA followed by Sidak's multiple-comparisons test]. (*D*) On the contrary, the AAV-mediated DTA expression had no significant effect on the number of CA1 pyramidal neurons in the dorsal hippocampus [Cre−, 1,083.3 ± 149.1 cells per section, $n = 6$ mice; Cre+, 1,216.8 ± 138.5 cells per section, $n = 6$, $t(18) = 0.7329$, $P = 0.7224$, two-way ANOVA followed by Sidak's multiple-comparisons test] or ventral hippocampus [Cre−, 1,331.8 ± 102.8 cells per section, $n = 6$ mice; Cre+, 1,367.0 ± 53.5 cells per section, $n = 6$, $t(18) = 0.1931$, $P = 0.9772$, two-way ANOVA followed by Sidak's multiple-comparisons test]. $^{**}P < 0.01$.

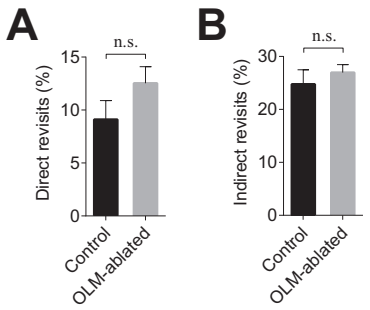

Fig. S5. The effect of OLM cell ablation on behavior on a Y-maze. (*A*) Compared with the control group, OLM-ablated mice tend to show higher direct visits, but the difference did not reach statistical significance [control, 9.1 ± 1.8%, $n = 8$ mice; OLM-ablated, 12.5 ± 1.6%, $n = 12$ mice, Student's unpaired t test, $t(18) = 1.420$, $P = 0.173$]. (*B*) Indirect visits of OLM-ablated mice were not statistically different from the control group [control, 24.8 ± 2.7%, $n = 8$; OLM-ablated, 27.0 ± 1.5%, $n = 12$ mice, Student's unpaired t test, $t(18) = 0.7676$, $P = 0.453$].

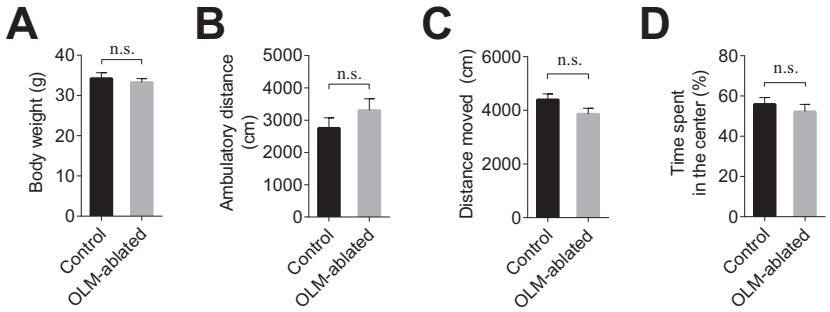

Fig. S6. Ablation of OLM interneurons did not have any significant effect on body weight gain (*A*), locomotion (*B* and *C*), or anxiety (*D*). (*A*) Average weight of OLM-ablated mice was not significantly different from that of littermate controls [control, 34.2 ± 1.4 g, $n = 10$ mice; OLM-ablated, 33.3 ± 0.9 g, $n = 16$ mice, $t(24) = 0.5975$, $P = 0.5558$, Student's unpaired t test]. (*B*) Average ambulatory distance of OLM-ablated mice in an open-field box, which was measured during the first day of habituation for OLT, was not different from the control group [control, 2,752 ± 321 cm, $n = 10$ mice; OLM-ablated, 3,304 ± 359 cm, $n = 10$ mice, $t(18) = 1.147$, $P = 0.2655$, Student's unpaired t test]. (*C*) Locomotion of OLM-ablated mice in a Y-maze during the spontaneous alternation task was not different from the control group [control, 4,396 ± 216 cm, $n = 8$ mice; OLM-ablated, 3,864 ± 216 cm, $n = 12$, Student's unpaired t test, $t(18) = 1.674$, $P = 0.1114$]. (*D*) The anxiety levels of OLM-ablated mice were not significantly different from the controls, as shown by the time spent in the center of an open-field box [control, 55.9 ± 3.4%, $n = 10$ mice; OLM-ablated, 52.3 ± 3.6%, $n = 11$ mice, $t(19) = 0.7359$, $P = 0.4708$, Student's unpaired t test].