Parvalbumin interneuron in the ventral hippocampus functions as a discriminator in social memory

Supporting information

SI Methods

Animal. PV-IRES-Cre (B6;129P2-Pvalb$^{tm1(cre)Arbr}$/J, stock number: 008069) and SOM-IRES-Cre (B6N.Cg-Sst$^{tm2.1(cre)Zjh}$/J, stock number: 018973) transgenic mice were gifted by Cao's laboratory (National Institute of Biological Sciences, Beijing) and were primarily imported from the Jackson Laboratory (JAX Mice and Services, Bar Harbor, ME, USA). All mice were maintained under a standard 12 h light/dark cycle (lights on at 8:00 A.M.) at a constant temperature of 23 ±1 ℃, with food and water available *ad libitum*. PV-Cre mice of both genders aged 2–6 months were used for this study. All experimental procedures were conducted in accordance with the regulations of the Laboratory Animal Care and Use Committees of the Institute of Psychology, Chinese Academy of Sciences.

Social Isolation. Adolescent mice (postnatal day 28 [P28]) were either housed in groups of 2–5 mice per cage (GH) or individually housed (IH) for 8 weeks, with the IH mice prevented from coming into contact with other mice. At P88, both groups were subjected to behavioral tests. Moreover, to determine the effect of social re-exposure for IH mice, some were housed in a social group (2–5 mice per cage) for 2 weeks (re-group housed [RGH]). One week after the completion of all tests, the mice were sacrificed and fixed with 4% paraformaldehyde in phosphate buffer saline (PBS) solution.

Virus Preparation. All recombinant Adeno-associated virus (AAV) vectors comprised a double-floxed inverted open reading frame (DIO) construct under the control of CMV or Ef1α promoter, which led to a Cre-dependent expression strategy. The serotype of AAV-DIO-TeNT-EGFP, AAV-DIO-hChR2(H134R)-mCherry, AAV-DIO-eNpHR3.0-EGFP, AAV-DIO-EGFP, and AAV-DIO-mCherry was AAV8, while AAV-DIO-hM3Dq-mCherry, AAV-DIO-hM4Di-mCherry, and AAV-DIO-Gcamp6(f) were serotyped with AAV9 coat proteins. All viruses used for this study were packaged by Obio Technology Co., Ltd. (Shanghai, China). The final viral vector titers ranged from 2×10^{12} to 8×10^{12} particles/ml.

Stereotaxic Surgery. The animals were anesthetized with intraperitoneal 450 mg/kg avertin (2,2,2-tribromoethanol) and then fixed in a stereotaxic frame. Virus solution was injected at a flow rate of 0.1 μl/min using a glass pipette attached to a 10 ml Hamilton microsyringe through a flexible pipe that was filled with mineral oil. After completing the injection, the needle was kept at the injection site for at least 5 min

www.pnas.org/cgi/doi/10.1073/pnas.1819133116

and then slowly withdrawn.

In cell-specific inactivation experiments, the viral vector carrying the TeNT gene fused with enhanced green fluorescence protein (EGFP; AAV-CMV-DIO-TeNT-EGFP) or EGFP alone (AAV- Ef1α-DIO-EGFP) was bilaterally injected (0.5 μl per side) into the ventral or dorsal CA1 (the vCA1 or dCA1) of the PV-Cre mice. vCA1 injections were targeted at AP: −3.16 mm, ML: ±3.20 mm, and DV: −4.50 mm; and dCA1 injections were targeted at AP: −2.00 mm, ML: ±1.50 mm, and DV: −1.50 mm.

In optogenetic stimulation experiments, 0.6 μl of AAV-Ef1α-DIO-hChR2-mCherry, AAV-Ef1α-DIO-eNpHR3.0-EGFP, AAV-Ef1α-DIO-mCherry, or AAV-Ef1α-DIO-EGFP was bilaterally injected into the vCA1 (AP: −3.16 mm, ML: ±3.20 mm, DV: −4.50 mm), and the optical fiber was implanted in the vCA1 with a tip at 0.5 mm above the injection site. A screw was placed on the skull around the implant site to provide additional stability. Dental cement was applied to secure the optical fiber implant.

In the chemogenetic experiment, 0.5 μl of AAV-hSyn-DIO-hM3Dq-mCherry, AAV-Ef1α-DIO-hM4Di-mCherry, or AAV-Ef1α-DIO-mCherry was bilaterally injected into vCA1 (AP: −3.16 mm, ML: ±3.20 mm, DV: −4.50 mm).

In the *in vivo* calcium imaging experiment, PV-Cre mice received a unilateral injection (0.8 μl) of Cre-dependent AAV carrying Gcamp6(f) into the vCA1 (AP: −3.16 mm, ML: ±3.20 mm, DV: −4.50 mm). An optical fiber was implanted at 0.1 mm above the site of the virus injection. Dental cement was applied to secure the optical fiber implant.

After surgery, the animals were returned to their cages and allowed to recover for 3−4 weeks before the experiments were initiated.

Optogenetic Manipulation. Targeted neurons were activated by a 473-nm blue laser (20 ms per pulse, 8 Hz, 15 mW), or inhibited by a 589-nm yellow laser (constant, 10 mW) via optical patch cords (AniLab Software & Instruments C., Ltd, China). Laser generators were produced at Changchun New Industries Optoelectronics Tech. Co., Ltd. (Changchun New Industries Optoelectronics Technology Co., Ltd, China). The parameters for optogenetic stimulation in the present study were in accordance with the previous studies (1, 2) in which hippocampal parvalbumin interneurons (PVI) drove intrinsic hippocampal oscillations and the hippocampal network optimally at the theta band (4–10 Hz). The patch cords were coupled with a FC fitted rotary joint (Doric Lenses Inc., Quebec, Canada) to avoid entanglement of the patch cords and keep the light stable. Stimulation frequency and pulse duration were controlled with a Master 8 stimulator (A.M.P.I. Co., Ltd., Jerusalem, Israel).

Chemogenetic Manipulation. Clozapine-N-Oxide (CNO, HY-17366, MedChemExpress) was dissolved in dimethyl sulfoxide (DMSO, D2650, Sigma) to a stocking solution of 5 mg/ml and stored at 4^{o}C. Each day before the experiment, the CNO stocking solution was diluted with saline (0.9% NaCl solution) to a final concentration of 0.5 mg/ml. PV-hM3Dq and PV-mCherry mice were injected with CNO intraperitoneally (10 mg/kg) immediately or 3 h later following a familiarization

session. PV-hM4Di mice were administrated with 5 mg/kg CNO because they became seizure-susceptible with a dose of 10 mg/kg. The mice that exhibited a seizure were excluded.

Behavioral Tests.

Social Discrimination Test. In the present study, social memory was quantified by a modified social discrimination test (SDT) (3, 4), which is a valid test based on a rodent's nature of preference for interacting with novel conspecies rather than familiar ones. The SDT apparatus was composed of a rectangle plastic box (60 cm length × 40 cm width × 30 cm height) and two vertical wire mesh cages. The background luminance was 15 lux and the light sources were well distributed around the chamber to avoid the innate preference for darkness in mice. Briefly, the social discrimination procedure comprised three sessions: familiarization, separation, and recognition, all of which corresponded to three stages of the social information process (encoding, retention, and retrieval). This design enabled the effects of manipulations on the different "stages" of social memory to be investigated.

Experimental subjects were habituated to the social discrimination chamber for several minutes for each of three successive days before testing. On the testing day (recognition session), a test mouse was placed in the testing apparatus and allowed to explore it freely for 5 min. Then, the familiar and the novel target mice were individually placed within the vertical wire mesh cages. The positions of the target animals in this test were arranged in a counterbalanced manner. All of the target mice used in this experiment were juvenile wild-type conspecifics (4–6 weeks). Between tests, the chamber and cages were cleaned with 20% ethanol before initiating the next test. Video recordings of the tests were made with a camera that was suspended above the testing apparatus, and these recordings were analyzed by a well-trained observer who was blinded to the groups. The amount of time that the test mice spent exploring target animals within a 2 cm vicinity of each cage was measured. The "social discrimination index" was calculated by the following equation:

Social discrimination index = (Duration $_{\text{Novel}}$ − Duration $_{\text{Familiar}}$) / (Duration $_{\text{Novel}}$ + Duration $_{\text{Familiar}}$)

Optogenetic delivery in recognition session: Three days before the familiarization session, the test mice were individually housed in isolated cages. At the beginning of the familiarization session, a "to-be-recognized" mouse was taken out of its home cage and placed in the experimental subject's cage. This session lasted for two different time periods (24 h and 2 weeks), which represented two levels of memory strength. The separation session was the interval between the familiarization session and behavioral recording. In this session, the target mouse that was familiar to the test mouse was removed and placed into a new cage for 30 min before the test. The laser was delivered in the recognition session. This behavioral procedure was applied for both the experiment regarding PV-specific excitation/inhibition by optogenetic stimulation and the experiment regarding chronic PVI inactivation.

Optogenetic delivery in familiarization session: The mice were group-housed (2–4 mice per cage), since previous studies revealed that individually housed mice

showed only short-lasting social recognition memory (<120 min) (3, 5). On the day of the experiment, each of the testing mice and the "to be recognized" mice were separated into new isolated new cages 2 h before familiarization. Then, a target mouse that was to be recognized was placed in a testing mouse's cage (familiarization session). This session lasted 10 minutes, during which optogenetic stimulation was delivered. Then, the familiar mice were returned to their cages, and water bottles and several food pellets were added to each cage rack. Twenty-four hours after familiarization, SDT would be conducted.

Optogenetic delivered in separation session: The pre-familiarization preparation and familiarization session was identical to that of the "*Optogenetic delivered in familiarization session*" section. The separation session was also for 24 hours, during which optogenetic stimulation was delivered at 30, 60, 90, 180, or 360 min after a single 10-min social interaction with the "to be recognized" mouse.

Three-chamber Sociability Test. The three chamber sociability test was performed as previously described (6). The apparatus comprised a Plexiglas rectangular box (60 cm length × 30 cm width × 20 cm height), divided into three compartments (20 cm × 30 cm). The protocol is shown in Fig. 2*G*. The test was composed of three consecutive sessions.

Habituation: The testing mouse was placed into the central chamber of the three-chambered apparatus and allowed to acclimate and freely explore the three chambers for 5 min.

Social interaction: At the end of the acclimation period, a novel conspecific mouse of the same sex was introduced to the "social" chamber inside a vertical wire mesh cage. In another (non-social) chamber, an identical empty cage was placed. At the beginning of each test, the testing mouse would be introduced to the social chamber, and this session lasted for 5 min. The stranger mouse was randomly placed in each test to prevent chamber bias. Furthermore, to directly compare the social function between each group, we calculated a "social interaction index" by the following equation:

Social interaction index = (Duration $_{\text{Social}}$ − Duration $_{\text{Nonsocial}}$) / (Duration $_{\text{Social}}$ + Duration $_{\text{Nonsocial}}$)

Social novelty: About 1 min after social interaction, another novel mouse was placed in the cage of the non-social chamber, which was empty during the social interaction session. The testing mouse would be initially introduced to the novel mouse chamber. The duration of investigation was also recorded for 5 min in this session. Moreover, the "social novelty index" was calculated by the following equation:

Social novelty index = (Duration $_{\text{Novel}}$ − Duration $_{\text{Familiar}}$) / (Duration $_{\text{Novel}}$ + Duration $_{\text{Familiar}}$)

Between tests, the chambers were cleaned with 20% ethanol and allowed to dry completely before initiating the next test. The time spent in the non-social, center, and social chambers was quantified using the Xeye Aba 3.2 tracking system (Beijing Macroambitior S&T Development Co., Ltd., Beijing, China).

Open Field Test. The open field test was conducted with a square plastic box (40 cm length × 40 cm width × 30 cm height) that was painted white. The box had a defined central area (20 cm length × 20 cm width). Mice were individually placed in the central area of the chamber and allowed to freely explore the entire box for 5 min. Between tests, the box was cleaned with 20% ethanol and wiped down with a clean paper towel. The tracking length and the time spent in the central area were recorded using the Xeye Aba 3.2 tracking system.

Novel Objects Recognition Test. One day after the open field test, the novel object recognition test (NORT) was performed to assess non-social recognition ability. In this test, two identical objects (green cylindrical toy bricks) were symmetrically placed at an open field (Fig. S2*A*). Subjected mice were placed into the field and allowed to explore the objects freely for 10 min for each of four consecutive days (days 1–4). On day 5, a novel object (red-blue mosaic block) randomly replaced one of the familiar objects. The test mice were then placed into the apparatus again and the time that mice spent interacting with the objects was recorded. The objects and open field were cleaned with 20% ethanol before each test. The "object discrimination index" was calculated by the following equation:

Object discrimination index = (Duration $_{\text{Novel}}$ − Duration $_{\text{Familiar}}$) / (Duration $_{\text{Novel}}$ + Duration $_{\text{Familiar}}$)

Elevated Plus Maze (EPM). The elevated plus maze was used to assess anxiety-like behavior. The white-painted maze consisted of four arms (30 cm length × 5 cm width). Two opposite open arms without walls and two opposite closed arms with 15 cm high walls formed a "+" shape. The maze was elevated 76 cm above the floor by four metal legs under each arm. Each mouse was placed at the junction of the open and closed arms, facing an open arm. The mouse was allowed to freely explore the entire maze for 5 min. The time spent in both the open and closed arms was recorded using the Xeye Aba 3.2 tracking system.

Prepulse Inhibition (PPI). Prepulse inhibition of the acoustic startle response was used to assess sensorimotor gating. The Startle Reflex Lab controlled by SR-Lab software (San Diego Instruments, USA) was employed for this test. Mice were confined to a cylindrical restraint tube inside a sound-attenuating chamber for the duration of testing. Briefly, after being exposed to 10 min of background noise (60 dB), each subject was presented with a total of 106 trials. The test session comprised startle trials (40 ms burst of 115 dB white noise), no stimulus trial (no noise was delivered except background noise), and prepulse inhibition (PPI) trials. A prepulse (20 ms burst of white noise at 66, 70, 74, or 78 dB intensity) preceded the 115 dB startle pulse (40 ms) by 100 ms. Trials were pseudo-randomly presented with an inter-trial interval of 9 to 30 s. The startle response was recorded every 1 ms for 100 ms after the onset of a startle stimulus. The maximum startle amplitude was used as the dependent variable. Baseline startle responses were calculated as the average

response to the pulse-alone trials. PPI was calculated as a percentage score for each prepulse trial type: PPI (%) = (1 – [(startle response for pulse with prepulse) / (startle response for pulse alone)]) × 100.

***In vivo* Ca^{2+} Fiber Photometry in Freely Moving Mice.** A commercialized fiber photometry system (ThinkerTech Inc., Nanjing, China) was used for recording the Ca^{2+} signals from PVIs, which has been described in detail elsewhere (7, 8). As shown in Fig. 5*A*, a beam from a 488-nm laser (OBIS 488LS; Coherent) was reflected by a dichroic mirror (MD498; Thorlabs), focused with a 10 × objective lens (NA = 0.3; Olympus), and then coupled to an optical commutator (Doric Lenses). A 2-m long optical fiber (230 μm outer diameter, NA = 0.37) guided the light between the commutator and the implanted optical fiber. The laser intensity at the tip of the optical fiber was adjusted to a range of 30–40 μW to minimize photo bleaching. The GCaMP6(f) fluorescence was filtered with a green fluorescence protein (GFP) bandpass filter (MF525-39, Thorlabs) and collected by a photomultiplier tube (PMT) (R3896, Hamamatsu). An amplifier (C7319, Hamamatsu) was used to convert the PMT current output to voltage signals, which were further filtered through a low-pass filter (40 Hz cut-off; Brownlee 440). The analog voltage signals were digitalized at 500 Hz using a Power 1401 digitizer and recorded by custom software developed in house using LabView. GCaMP signals were collected at a sampling frequency of 50 Hz. Fiber photometry recording data were exported to Matlab for further analysis. All raw data were segmented and aligned according to the onset of individual behavioral bouts. The fluorescence signal was normalized within each mouse by calculating the dF/F as $(F - F_0) / F_0$, where F_0 was the baseline fluorescence signal averaged over 3 s (heading and interaction) or 2 s (withdrawal) before the initiation of the behavioral event. Bout peaks dF/F were the average value of the largest signals within each bout.

Before the SDT, the mice were allowed to habituate the testing room for 3 days. In each habituation day, mice would explore the testing chamber for 10 min, and the implanted fiber was linked to a jumper cable but no laser on. In data analyses, we identified three types of social-associated behaviors—interaction, heading and withdrawal. For each type of behavior, we picked up 5-8 sec calcium signals around them for further analyses.

Immunohistochemical Procedure. For all cohorts, mice were anesthetized with Avertin and sequentially perfused with saline followed by 4% paraformaldehyde/phosphate buffer (PFA). Brains were then removed and post-fixed overnight in 4% PFA, and then they were immersed in 30% sucrose solution at 4 ℃ until they sunk to the bottom. Brains were sectioned into 40 μm thick slices and stored in cryoprotectant at -20 ℃ in the dark until antibody staining. Free-floating sections were washed 3 × 10 min in PBS, followed by incubation in blocking buffer (PBS containing 10% goat serum and 0.7% Triton X-100) for 2 h at room temperature. After blocking, primary antibodies were added to 5% goat serum in PBS solution and the sections were incubated overnight at 4 ℃. Primary antibodies included mouse anti-parvalbumin (Millipore, MAB1572, 1:300) and rabbit anti-c-Fos (Abcam,

ab190289, 1:500). Sections were then washed 3 × 15 min with PBS before incubation with secondary antibodies (Alexa Fluor-488 or Alexa Fluor-546 conjugated secondary antibodies, Invitrogen, 1:500) for 2 h at room temperature. Sections were again washed 2 × 10 min in PBS and then stained by 4',6-diamidino-2-phenylindole (DAPI) for 15 min, 1 × 10 min in PBS, and mounted onto slides with mounting medium (Vectashield) on glass slides. Fluorescence images were taken by fluorescence microscopy (Leica, DM5500B) using 10× and 20× objectives. The PV and c-Fos expressions were quantified by ImageJ software. The scale in ImageJ was based on the physical dimensions of the photograph recorded by the Leica microscopy system. The to-be-counted photograph was first transformed from RGB color mode into 8-bit mode, and then the "threshold" for positive signals was set in the software. Only stains that were in the settled thresholds of fluorescent intensity (FI) and size were included in the quantification.

For c-Fos analysis, mice were sacrificed by perfusion with 4% PFA 90 min after completion of the behavioral tests. As described previously (9, 10), each c-Fos-positive nucleus was classified into one of three expression levels (low, medium, and high) according to its own FI (= IntDen / Area, data from Image J). Intensity thresholds were defined as follows: low ≤ 30% × (strongest FI – weakest FI), high ≥ 70% × (strongest FI – weakest FI), and medium (the remaining 40%). Note that the comparison and classification of fluorescent intensity was performed within each brain section. Moreover, the colocalization of PV and c-Fos was determined by a well-trained observer who was blinded to the treatment groups.

Statistics. The data were analyzed using GraphPad Prism 6.0 software and Matlab. The results are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined by Student's *t*-test, Mann–Whitney U test, or two-way analysis of variance (ANOVA), which was followed by Bonferroni's multiple comparisons post hoc test. The criterion for statistical significance was $p < 0.05$.

References

1. B. Amilhon *et al.*, Parvalbumin Interneurons of Hippocampus Tune Population Activity at Theta Frequency. *Neuron* **86**, 1277-1289 (2015).
2. N. Ognjanovski *et al.*, Parvalbumin-expressing interneurons coordinate hippocampal network dynamics required for memory consolidation. *Nat Commun* **8**, 15039 (2017).
3. M. Engelmann, J. Hadicke, J. Noack, Testing declarative memory in laboratory rats and mice using the nonconditioned social discrimination procedure. *Nat Protoc* **6**, 1152-1162 (2011).
4. T. Okuyama, T. Kitamura, D. S. Roy, S. Itohara, S. Tonegawa, Ventral CA1 neurons store social memory. *Science* **353**, 1536-1541 (2016).
5. J. H. Kogan, P. W. Frankland, A. J. Silva, Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus* **10**, 47-56 (2000).
6. O. Yizhar *et al.*, Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* **477**, 171-178 (2011).
7. L. A. Gunaydin *et al.*, Natural neural projection dynamics underlying social behavior. *Cell* **157**, 1535-1551 (2014).

8. Y. Li *et al.*, Hypothalamic Circuits for Predation and Evasion. *Neuron* **97**, 911-924.e915 (2018).
9. F. Donato, S. B. Rompani, P. Caroni, Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* **504**, 272-276 (2013).
10. S. Ruediger *et al.*, Learning-related feedforward inhibitory connectivity growth required for memory precision. *Nature* **473**, 514-518 (2011).

SI Figures and Table

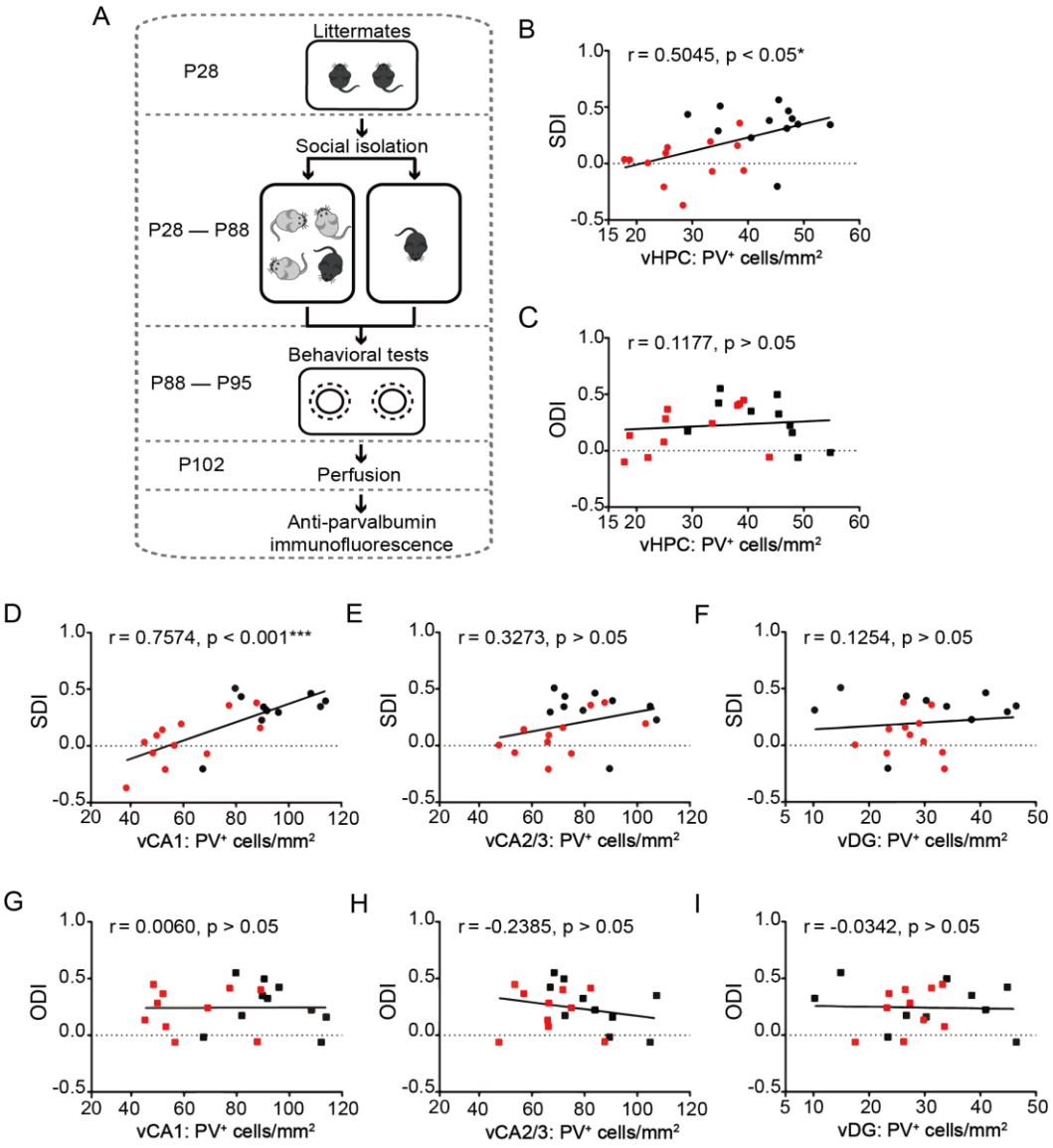

Fig. S1. Correlation analysis between SDI and vHPC-PV$^+$ counts after social isolation.
(*A*) Time schedule for social isolation and following experiments. **(*B*)** The scatter plot describing the correlation between mice's performance in SDT and the number of PV$^+$ cells in the vHPC (n = 24). **(*C*)** The scatter plot describing the correlation between mice's performance in NORT and the number of PV$^+$ cells in the vHPC (n = 21). **(*D*—*F*)** The scatter plot describing the correlation between mice's performance in SDT and the number of PV$^+$ cells in the vCA1 **(*D*)**, vCA2/3 **(*E*)** or vDG **(*F*)**, n = 22 for each correlation. **(*G*—*I*)** The scatter plot describing the correlation between mice's performance in NORT and the number of PV$^+$ cells in the vCA1 **(*G*)**, vCA2/3 **(*H*)** or vDG **(*I*)**, n = 20 for each correlation. Red and black dots represent individual-housed (IH) and group-housed (GH) mice, respectively. SDI: social discrimination index, ODI: object discrimination index. *****$p < 0.05$.

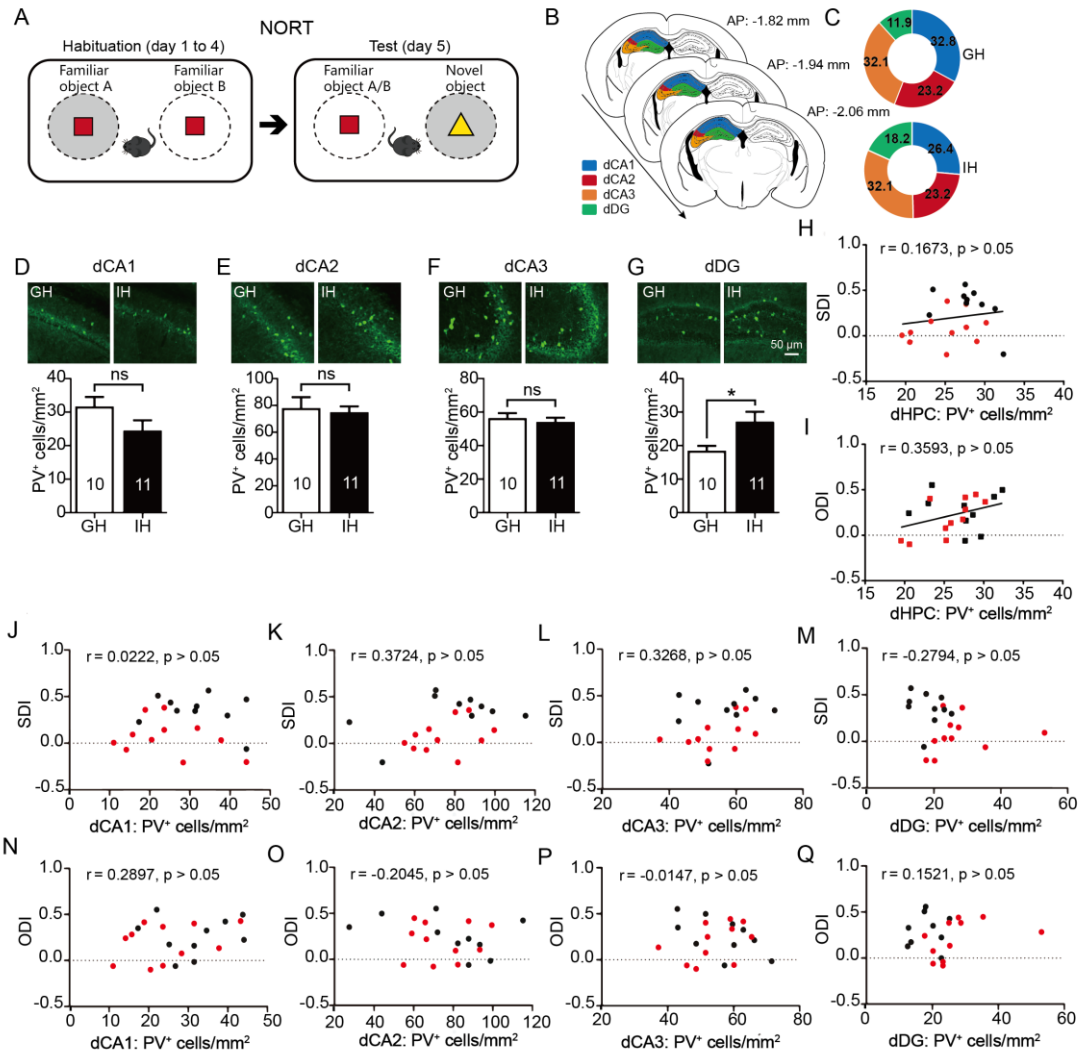

Fig. S2. PV$^+$ counts analyses in the dHPC subfields after social isolation.

(*A*) Protocol for novel object recognition test (NORT). In initial habituation session, mice were allowed to freely explore in an open field with two identical objects. During the recognition session, a novel object randomly replaced one of the familiar objects, the investigating duration toward novel and familiar objects were respectively recorded. **(*B*)** The hippocampal subfields of interest delineation. Blue, dCA1; red, dCA2; orange, dCA3; green, dDG. **(*C*)** The distribution of the proportion of PV$^+$ cells in distinct subfields of dHPC. **(*D*—*G*)** Comparisons of PV$^+$ counts in dCA1 **(*D*)**, dCA2 **(*E*)**, dCA3 **(*F*)** and dDG **(*G*)**. GH: n = 10; IH: n = 11. All data are expressed as mean ± SEM. ***p* < 0.05; ns, not significant. **(*H* and *I*)** The scatter plot describing the correlation between mice's performance in SDT **(*H*)** or NORT **(*I*)** and the number of PV$^+$ cells in the dHPC (n = 21). **(*J*—*M*)** The scatter plot describing the correlation between mice's performance in SDT and the number of PV$^+$ cells in the dCA1 **(*J*)**, dCA2 **(*K*)** , dCA2 **(*L*)** or dDG **(*M*)**. **(*N*—*Q*)** The scatter plot describing the correlation between mice's performance in NORT and the number of PV$^+$ cells in the dCA1 **(*N*)**, dCA2 **(*O*)**, dCA2 **(*P*)** or dDG **(*Q*)**. Red and black dots represent individual-housed (IH) and group-housed (GH) mice, respectively. SDI: social discrimination index, ODI: object discrimination index.

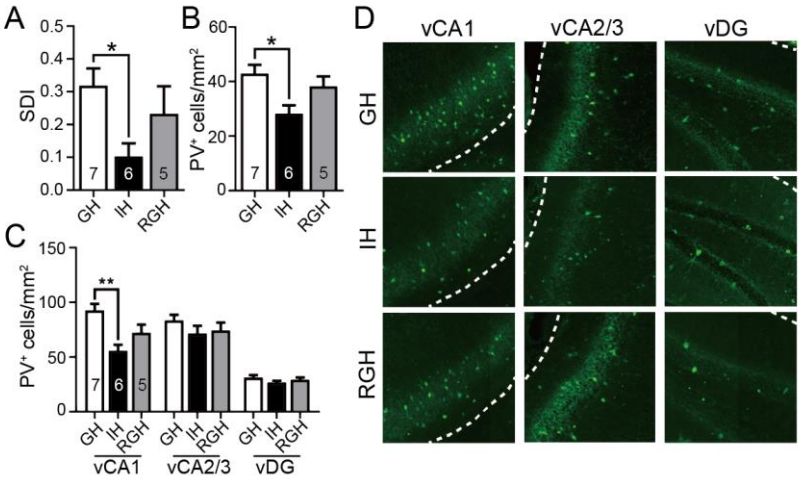

Fig. S3. Measurement of PV$^+$ counts in vCA1 and social recognition after two weeks of social re-group housing in social-isolated mice.

(*A*) To determine the effect of social re-exposure for individual-housed mice, some were housed in a social group (2–5 mice per cage) for 2 weeks (re-group housed). The SDT was conducted after social isolation and social re-exposure treatments. ***(B-D)*** The number of PV$^+$ counts in the vHPC **(*B*)** and its subfields **(*C*)**. One week after the completion of all tests, the mice were sacrificed and fixed with 4% paraformaldehyde in phosphate buffer saline (PBS) solution. The PV immunostaining was performed. **(*D*)** Representative images of PV expression in different groups. GH: group-housed, n = 7; IH: individual-housed, n = 6; RGH: re-group housed, n = 5; SDI: social discrimination index. All data are expressed as mean ± SEM. *****$p < 0.05$; ******$p < 0.01$.

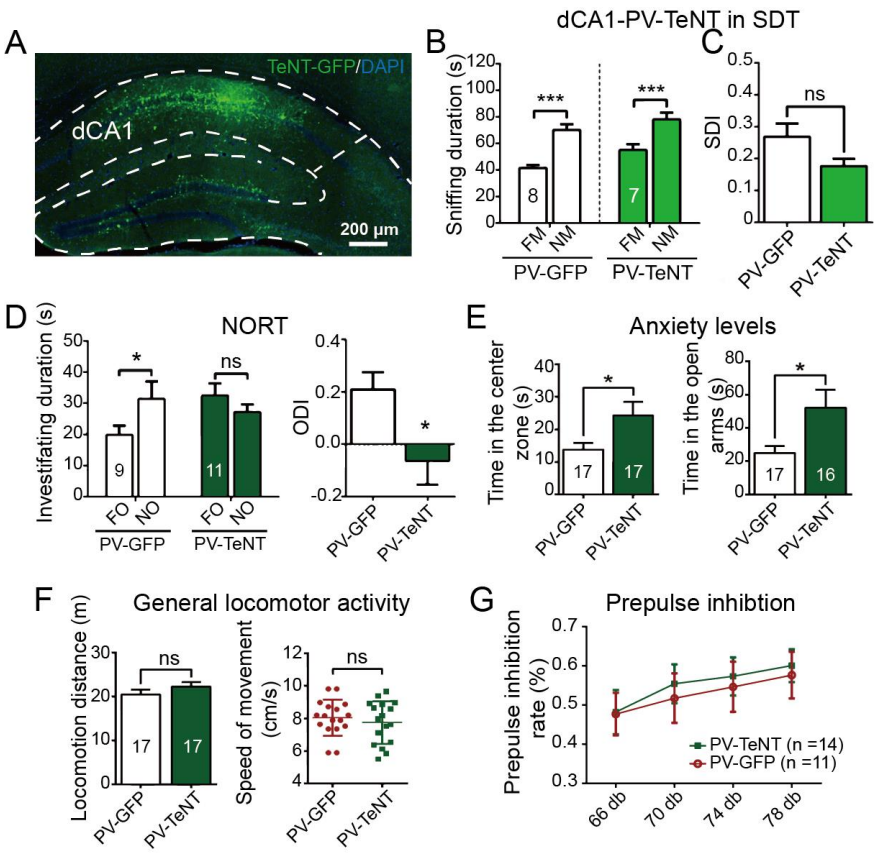

Fig. S4. Other behavioral tests in PVIs-inactivation experiment.

(*A*) Coronal sections of a PV-Cre mouse injected with AAV9-CMV-DIO-TeNT-GFP into dCA1, which were stained with anti-GFP (green) and DAPI (blue). **(*B* and *C*)** Chronic inactivation of dCA1-PVIs by TeNT (n = 8) did not affect mice performance in SDT **(*B*)** or comparison of SDI with control groups (n = 7) **(*C*)**. **(*D*)** Investigation duration toward novel or familiar object in NORT (left) and comparison of object discrimination index (right) (GFP: n = 9; TeNT: n = 11). **(*E*)** Total duration that the subject mice spent in the open arm of EPM (*right*) or in the center zone of open field (*left*) showing a reduced anxiety in vCA1-PV-TeNT mice (EPM: n = 17 for each group; open field: n = 17 for GFP group, n = 16 for TeNT group). **(*F*)** Travel distance (*left*) and average speed (*right*) in the open field (n = 17 for each group). **(*G*)** Measurement of prepulse inhibition at different prepulse intensities (66, 70, 74 or 78 dB) (GFP: n = 11; TeNT: n = 14). All data are expressed as mean ± SEM. ***p* < 0.05; ****p* < 0.001; ns, not significant.

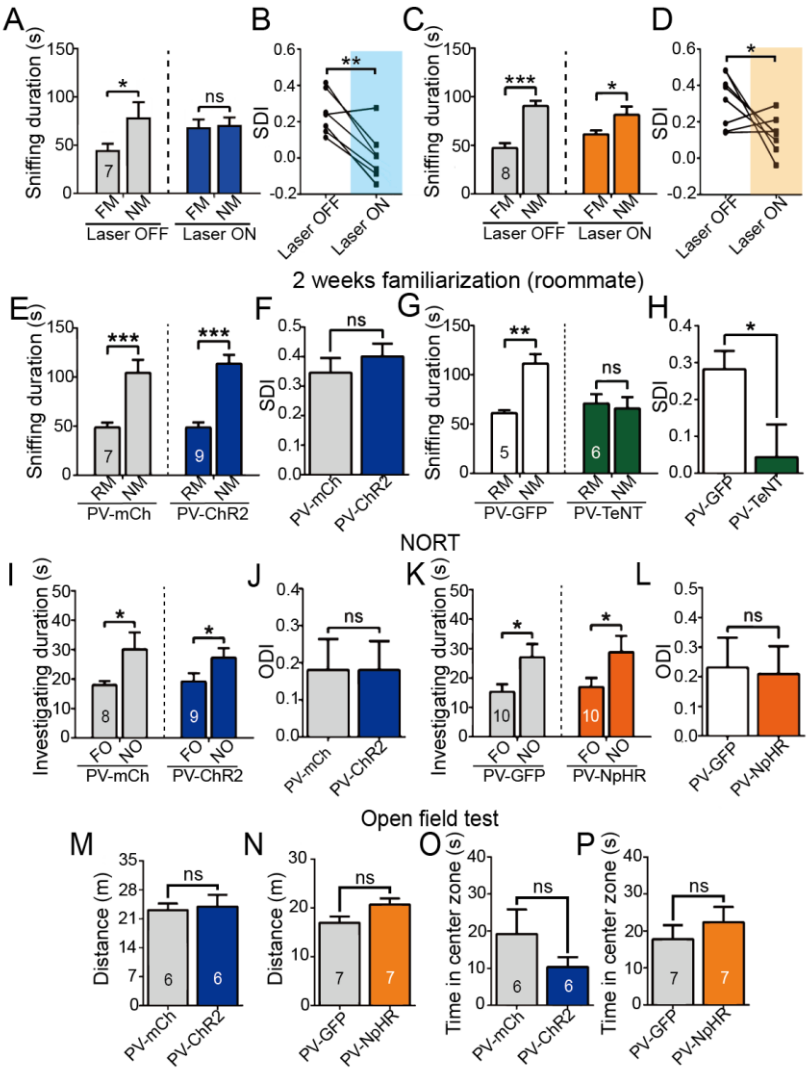

Fig. S5. Other behavioral tests in optogenetic experiment.

(*A* and *C*) Sniffing duration toward a novel and familiar mouse in SDT observed in PV-ChR2 (n = 7) **(*A*)** and PV-NpHR mice (n = 8) **(*C*)** when the optogenetic laser was turned on or off. **(*B* and *D*)** Comparisons of SDI under laser on and off condition. **(*E* and *F*)** PV-ChR2 mice (n = 9) spent more time in the sniffing area of novel mouse compared to their own roommate **(E)**; no difference in SDI was found between PV-mCh (n = 7) and PV-ChR2 group **(*F*)**. **(*G* and *H*)** PV-Cre mice treated by TeNT (n = 6) in the vCA1 spent similar time in investigating the novel mouse or their own roommate **(*G*)**; PV-TeNT mice exhibited significant lower SDI compared with PV-GFP mice (n = 5) **(*H*)**. **(*I*—*L*).** Both PV-ChR2 (n = 9) or PV-NpHR (n = 10) mice and their own controls (mCherry: n = 8; GFP: n = 10) showed a preference for novel object in NORT **(*I* and *K*,** respectively**)**. No difference was found in ODI between ChR2 or NpHR mice and their own controls **(*J* and *L*,** respectively**)**. **(*M*—*P*)** In open field test, total travel distance **(*M* and *N*)** and time spent in center zone **(*O* and *P*)** were compared between PV-ChR2 (n = 6) or PV-NpHR (n = 7) mice and their own controls (mCherry: n = 6; GFP: n = 7). All data are expressed as mean ± SEM. *****$p < 0.05$; ******$p < 0.01$; *******$p < 0.001$; ns, not significant.

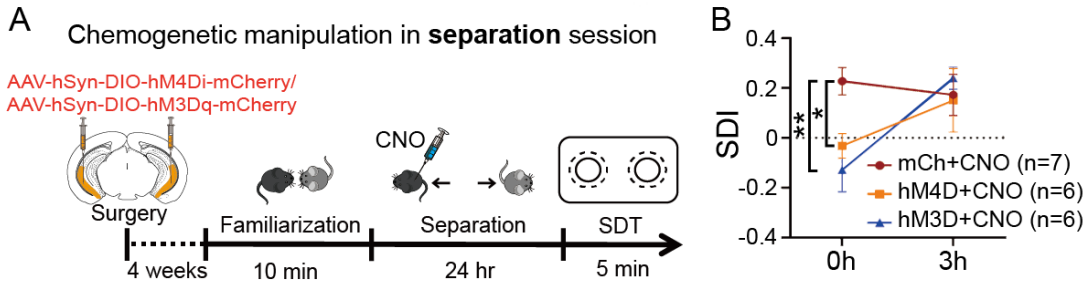

Fig. S6. Effects of chemogenetic manipulation of vCA1-PVIs during consolidation stage on social memory.

(*A*) Protocol for chemogenetic manipulation of vCA1-PVIs during separation session. **(*B*)** Changes of SDI in the AAV-DIO-hM3Dq-mCherry or AAV-DIO-hM4Di-mCherry infected PV-Cre mice, which were subjected CNO injections immediately or 3 hours after social familiarization. All data are expressed as mean ± SEM. *****$p < 0.05$; ******$p < 0.01$.

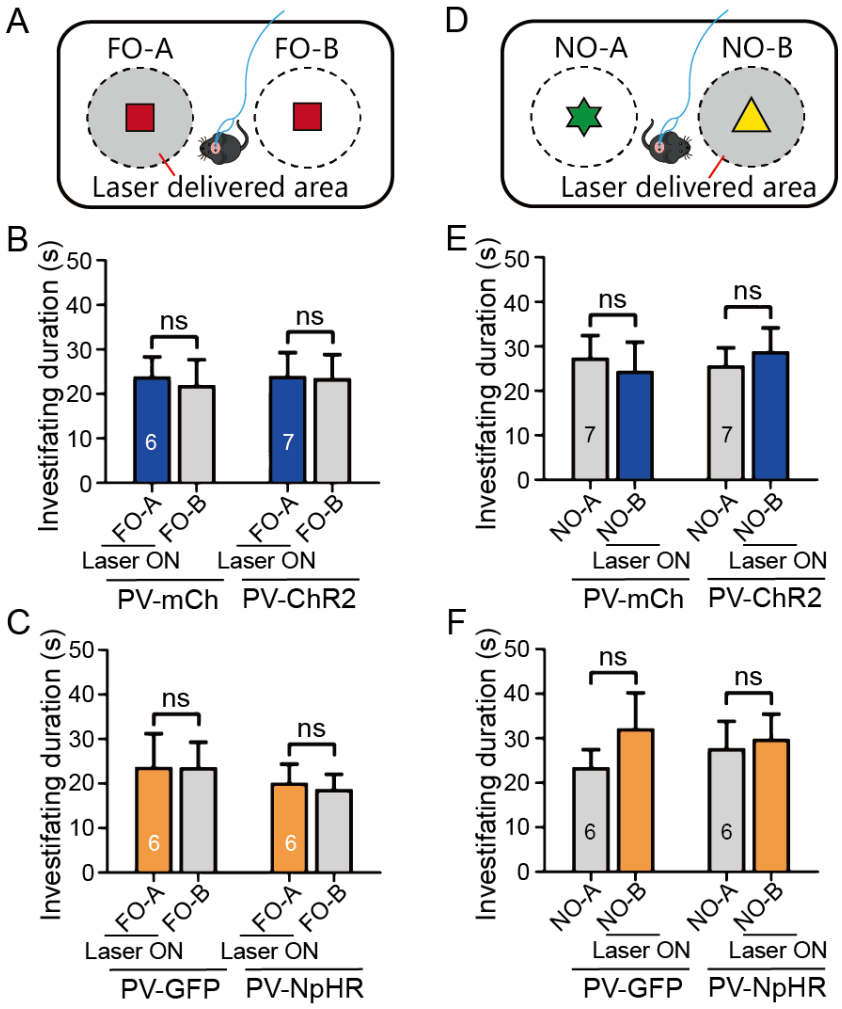

Fig. S7. Effects of optogenetic excitation or inhibition of vCA1-PVIs coupling with investigating one of a pair of familiar/novel objects on object discrimination. (*A* and *D*) A pair of familiar objects **(*A*)** or novel objects **(*D*)** was used as the targets in ODT and laser stimulation was delivered only when the subject mouse was in the sniffing area of one of the target objects. **(*B* and *C*)** No significant preference or avoidance exploration was observed in either ChR2 (n = 7; ***B***) or NpHR (n = 7; ***C***) mice, when a pair of familiar objects was used as the targets. **(*E* and *F*)** Similarly, no changed exploration was found in either ChR2 (n = 6; ***E***) or NpHR (n = 6; ***F***) mice, when a pair of novel objects was used as the targets. FO: familiar object; NO: novel object. All data are expressed as mean ± SEM. ns, not significant.

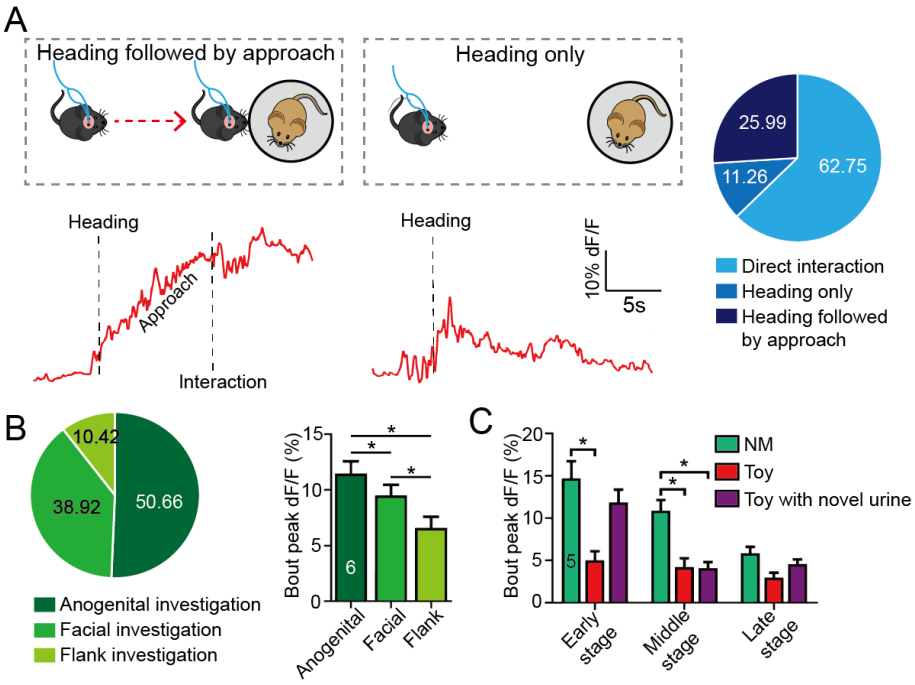

Fig. S8. Ca^{2+} dynamics of vCA1-PVIs in freely moving mice during heading to social mice and investigating different body parts of social mice. (*A*) A representative sample showing Ca^{2+} transient relative to heading behavior followed by approach or heading action only (*left*). Proportion of interaction-associated behaviors, including direct interaction, heading only and heading followed by approach (*right*). **(*B*)** Proportion of anogenital, facial and flank investigation during social interaction (*left*). Quantitative analysis of peak dF/F of Ca^{2+} signals in different part investigation (*right*). **(*C*)** Quantitative analyses of peak dF/F of Ca^{2+} signals in different investigation stages. In this test, novel mouse, toy mouse and toy mouse with novel urine were individually used as the investigation targets. All data are expressed as mean ± SEM. ***p* < 0.05.

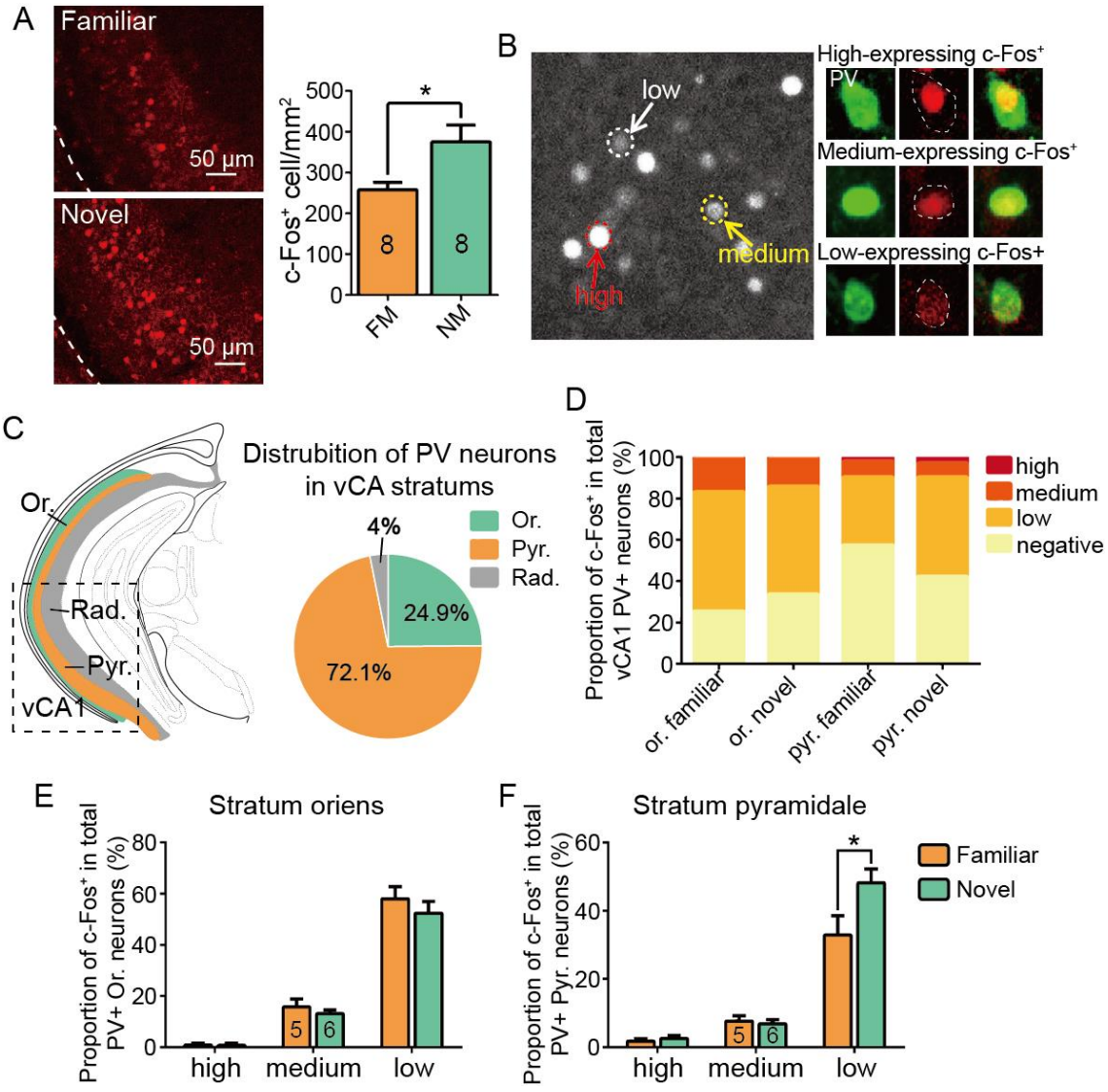

Fig. S9. Evaluation of c-Fos expression in the vCA1 after socially interacting with novel or familiar mouse.

(*A*) Expression of c-Fos proteins in the vCA1 was unregulated in novel-interaction mice (n = 8) compared with familiar-interaction ones (n = 8). The red puncta indicate c-Fos$^+$ neurons. **(*B*)** c-Fos nuclei were classified into three levels of expression (low, medium and high) according to its relative fluorescence intensity in brain section (left). Example of overlap between PV and distinct levels of c-Fos (right). **(*C*)** The distribution of the approximate proportion of neurons labeled by PV in distinct stratums of the vCA1. Green, stratum oriens (Or); orange, stratum pyramidale (Pyr); grey, stratum radiatum (Rad). **(*D*)** Proportion of PV colocalized with three distinct levels of c-Fos nuclei occupied within total number of PV$^+$ neurons in stratum Pyr or Or. **(*E* and *F*)** Comparison of activation level of PVIs in stratum Or **(*E*)** or Pyr **(*F*)** by examining their overlap with distinct levels of c-Fos under novel (n = 6) or familiar (n = 5) interaction condition. All data are expressed as mean ± SEM. *****$p < 0.05$.

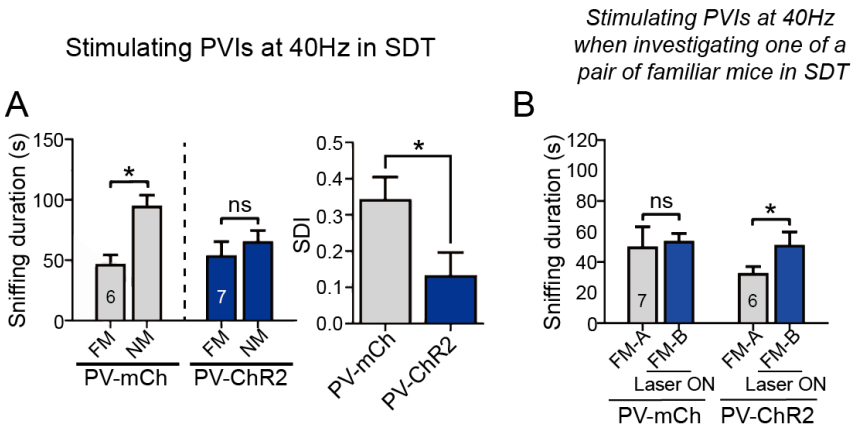

Fig. S10. Effect of 40 Hz optogenetic stimulation of vCA1-PVIs on the social memory retrieval.

(*A*) Total duration in sniffing area during SDT observed in ChR2 (n = 7) and mCherry (n = 6) mice (*left*)**.** Comparison of SDI between optogenetic mice and their control (*right*). **(*B*)** A pair of familiar mice was used as the social targets in SDT and laser stimulation was delivered only when the subject mouse was in the sniffing area of one of the target mice. The time that PV-ChR2 mice (n = 6) spent in investigating the familiar mouse (coupling with 473-nm laser stimulation) was increased, compared to the control (n = 7). All data are expressed as mean ± SEM. *$p < 0.05$; ns, not significant.

Table S1. Statistics

Impaired social memory parallel occurred with decreased PV^+ counts in the vHPC (related to Fig. 1)				
SDT	GH: familiar vs novel (C1)	Paired *t* test	*t* (10) = 5.866	*p* = 0.0002***
	IH: familiar vs novel (C2)	Paired *t* test	*t* (12) = 0.887	*p* = 0.3927
	SDI: GH vs IH (D)	Unpaired *t* test	*t* (22) = 3.352	*p* = 0.0029**
NORT	GH: familiar vs novel (E1)	Paired *t* test	*t* (9) = 4.038	*p* = 0.0029**
	IH: familiar vs novel (E2)	Paired *t* test	*t* (10) = 2.368	*p* = 0.0394*
	ODI: GH vs IH (F)	Unpaired *t* test	*t* (19) = 0.753	*p* = 0.4605
Number of PV^+ cells	dHPC: GH vs IH (H)	Unpaired *t* test	*t* (19) = 1.995	*p* = 0.0606
	vHPC: GH vs IH (I)	Unpaired *t* test	*t* (22) = 4.062	*p* = 0.0005***
	vCA1: GH vs IH (M)	Unpaired *t* test	*t* (20) = 4.785	*p* = 0.0001***
	vCA2/3: GH vs IH (N)	Unpaired *t* test	*t* (20) = 2.215	*p* = 0.0386*
	vDG: GH vs IH (O)	Unpaired *t* test	*t* (20) = 1.096	*p* = 0.2856
Functional absence of vCA1-PVIs by TeNT impaired social memory (related to Fig. 2)				
SDT: vCA1-PVIs	GFP: familiar vs novel (C1)	Paired *t* test	t (11) = 9.602	p < 0.0001***
	TeNT: familiar vs novel (C2)	Paired *t* test	t (10) = 1.505	p = 0.1631
	SDI: GFP vs TeNT (D)	Unpaired *t* test	t (21) = 4.037	p = 0.0006***
SDT: vCA1-SOMIs	GFP: familiar vs novel (E1)	Paired *t* test	*t* (5) = 5.802	*p* = 0.0021**
	TeNT: familiar vs novel (E2)	Paired *t* test	*t* (6) = 4.664	*p* = 0.0035**
	SDI: GFP vs TeNT (F)	Unpaired *t* test	*t* (11) = 0.139	*p* = 0.8918
Three chambers test: social interaction session (H)	Main effect of groups	Mixed two-way ANOVA	F (1, 29) = 0.930	*p* = 0.3427
	Main effect of chambers		F (2, 58) = 161.0	*p* < 0.0001***
	group × chambers interaction		F (2, 58) = 0.156	*p* = 0.8557
	Subjects		F(29,58)=5.561e-005	*p* > 0.9999
	Social interaction index: GFP vs TeNT (I)	Unpaired t test	*t* (29) = 0.393	*p* = 0.6973
Three chambers test: social novelty session (J)	Main effect of groups	Mixed two-way ANOVA	F (1, 29) = 0.172	*p* = 0.6811
	Main effect of chambers		F (2, 58) = 49.44	*p* < 0.0001***
	group × chambers interaction		F (2, 58) = 5.785	*p* = 0.0051**
	Subjects		F(29,58)=1.022e-007	*p* > 0.9999
	GFP: familiar vs novel (J1)	Bonferroni post hoc analysis	*t* (15) = 5.714	*p* < 0.0001***
	TeNT: familiar vs novel (J2)		*t* (16) = 1.069	*p* = 0.8691
	Social novelty index: GFP vs TeNT (K)	Unpaired *t* test	*t* (29) = 2.737	*p* = 0.0105*
Selective manipulation of PVIs in the vCA1 during encoding, consolidation or retrieval stage of social memory (related to Fig. 3)				
Laser delivered during recognition session of SDT	mCh: familiar vs novel (C1)	Paired *t* test	*t* (9) = 5.019	*p* = 0.0007***
	ChR2: familiar vs novel (C2)	Paired *t* test	*t* (9) = 2.044	*p* = 0.0713
	SDI: mCh vs ChR2 (D)	Unpaired *t* test	*t* (18) = 4.332	*p* = 0.0004***
	GFP: familiar vs novel (E1)	Paired *t* test	*t* (7) = 6.998	*p* = 0.0002***
	NpHR: familiar vs novel (E2)	Paired *t* test	*t* (8) = 3.713	*p* = 0.0059**
	SDI: GFP vs NpHR (F)	Unpaired *t* test	*t* (15) = 3.935	*p* = 0.0013**
Laser delivered	mCh: familiar vs novel (H1)	Paired *t* test	*t* (7) = 3.153	*p* = 0.0161*

during familiarization session of SDT	ChR2: familiar vs novel (H2)		Paired *t* test	*t* (7) = 5.504	*p* = 0.0009***
	SDI: mCh vs ChR2 (I)		Unpaired *t* test	*t* (14) = 0.691	*p* = 0.5009
	GFP: familiar vs novel (J1)		Paired *t* test	*t* (8) = 3.231	*p* = 0.0120*
	NpHR: familiar vs novel (J2)		Paired *t* test	*t* (10) = 3.556	*p* = 0.0052**
	SDI: GFP vs NpHR (K)		Unpaired *t* test	*t* (18) = 0.1417	*p* = 0.8889
Laser delivered during separation session of SDT	ChR2 (M)	Interaction	Mixed two-way ANOVA	F (3, 45) = 0.262	*p* = 0.8526
		SDI in time points		F (3, 45) = 0.531	*p* = 0.6633
		Groups		F (1, 15) = 0.756	*p* = 0.3984
		Subjects		F (15, 45) = 0.954	*p* = 0.5158
	NpHR (N)	Interaction	Mixed two-way ANOVA	F (3, 39) = 0.068	*p* = 0.9766
		SDI in time points		F (3, 39) = 0.339	*p* = 0.7975
		Groups		F (1, 13) = 0.046	*p* = 0.8330
		Subjects		F (13, 39) = 1.877	*p* = 0.0646
Optogenetic manipulation of PVIs during investigating one of a pair of novel/familiar mice (related to Fig. 4)					
Approaching one of a pair of familiar mice triggered 473nm laser in SDT	mCh: familiar (with laser) vs familiar (without laser) (A1)		Paired *t* test	*t* (7) = 0.008	*p* = 0.9938
	ChR2: familiar (with laser) vs familiar (without laser) (A2)		Paired *t* test	*t* (7) = 2.810	*p* = 0.0261*
Approaching one of a pair of familiar mice triggered 589nm laser in SDT	GFP: familiar (without laser) vs familiar (with laser) (B1)		Paired *t* test	*t* (6) = 0.832	*p* = 0.4525
	NpHR: familiar (without laser) vs familiar (with laser) (B2)		Paired *t* test	*t* (6) = 0.6527	*p* = 0.5382
Approaching one of a pair of novel mice triggered 473nm laser in SDT	mCh: novel (with laser) vs novel (without laser) (C1)		Paired *t* test	*t* (6) = 0.7110	*p* = 0.5038
	ChR2: novel (with laser) vs novel (without laser) (C2)		Paired *t* test	*t* (6) = 0.4858	*p* = 0.6443
Approaching one of a pair of novel mice triggered 589nm laser in SDT	GFP: novel (without laser) vs novel (with laser) (D1)		Paired *t* test	*t* (5) = 0.241	*p* = 0.8193
	NpHR: novel (without laser) vs novel (with laser) (D2)		Paired *t* test	*t* (5) = 0.751	*p* = 0.4867
In vivo measurement of Ca^{2+} dynamics of vCA1-PVIs in SDT (related to Fig. 5)					
SDT: Interaction	Bout peak: familiar vs novel (E)		Paired t test	*t* (6) = 4.4330	*p* = 0.0044**
	1st 1/3 bouts peak (F1)		Paired t test	*t* (6) = 3.565	*p* = 0.0119*
	2nd 1/3 bouts peak (F2)		Paired t test	*t* (6) = 4.263	*p* = 0.0053**

	3rd 1/3 bouts peak (F3)	Paired t test	t (6) = 1.197	p = 0.2765
SDT: heading toward (J)	Bout peak: familiar vs novel	Paired t test	t (5) = 4.126	p = 0.0091**
SDT: withdrawal (L)	Bout peak: familiar vs novel	Paired t test	t (6) = 7.836	p = 0.0002***
In vivo measurement of Ca^{2+} dynamics of vCA1-PVIs when investigating different targets (related to Fig. 6)				
Investigating novel/familiar mouse, object, mouse toy (C)	Novel mouse vs familiar mouse	Paired t test	t (5) = 3.593	p = 0.0157*
	Novel mouse vs object	Paired t test	t (5) = 7.897	p = 0.0005***
	Novel mouse vs mouse toy	Paired t test	t (5) = 4.310	p = 0.0078**
	Familiar mouse vs novel object	Paired t test	t (5) = 1.865	p = 0.1212
	Familiar mouse vs mouse toy	Paired t test	t (5) = 0.9467	p = 0.3873
	Novel object vs mouse toy	Paired t test	t (5) = 0.8573	p = 0.4304
Latency to peak (D)	Novel mouse vs familiar mouse	Paired t test	t (5) = 3.288	p = 0.0218*
	Novel mouse vs object	Paired t test	t (5) = 4.578	p = 0.006**
	Novel mouse vs mouse toy	Paired t test	t (5) = 3.652	p = 0.0147*
	Familiar mouse vs novel object	Paired t test	t (5) = 1.151	p = 0.3017
	Familiar mouse vs mouse toy	Paired t test	t (5) = 0.9139	p = 0.4027
	Novel object vs mouse toy	Paired t test	t (5) = 0.5721	p = 0.5920
Heading to novel/familiar mouse, object, mouse toy (F)	Novel mouse vs familiar mouse	Paired t test	t (4) = 4.906	p = 0.008*
	Novel mouse vs object	Paired t test	t (4) = 3.682	p = 0.0212*
	Novel mouse vs mouse toy	Paired t test	t (4) = 2.702	p = 0.054
	Familiar mouse vs novel object	Paired t test	t (4) = 0.3164	p = 0.7675
	Familiar mouse vs mouse toy	Paired t test	t (4) = 4.185	p = 0.0139*
	Novel object vs mouse toy	Paired t test	t (4) = 3.265	p = 0.0309*
Correlation analysis between PV^+ counts in vHPC and behaviors (related to Fig. S1)				
Correlation between PV^+ counts in the vHPC (H)	With SDI (B)	Pearson correlation	r = 0.5045	p = 0.0119*
	With ODI (C)	Pearson correlation	r = 0.1177	p = 0.6112
Correlation between PV^+ counts in the vCA1	With SDI (D)	Pearson correlation	r = 0.7574	p < 0.0001***
	With ODI (G)	Pearson correlation	r = 0.0060	p = 0.9800
Correlation between PV^+ counts in the vCA2/3	With SDI (E)	Pearson correlation	r = 0.3273	p = 0.1475
	With ODI (H)	Pearson correlation	r = -0.2385	p = 0.3113
Correlation between PV^+ counts in the vDG	With SDI (F)	Pearson correlation	r = 0.1254	p = 0.5881
	With ODI (I)	Pearson correlation	r = -0.0342	p = 0.8861
Correlation analysis between PV^+ counts in distinct subfields of the hippocampus and behaviors (related to Fig. S2)				
Number of PV^+ cells in dHPC subfields (D-G)	dCA1: GH vs IH	Unpaired t test	t (19) = 1.696	p = 0.1062
	dCA2: GH vs IH	Unpaired t test	t (19) = 0.3396	p = 0.7379
	dCA3: GH vs IH	Unpaired t test	t (19) = 0.5709	p = 0.5748
	dDG: GH vs IH	Unpaired t test	t (19) = 2.532	p = 0.0203
Correlation between PV^+ counts in the dHPC (I)	With SDI (H)	Pearson correlation	r = 0.1673	p = 0.4686
	With ODI (I)	Pearson correlation	r = 0.3593	p = 0.1097
Correlation between PV^+ counts in the dCA1	With SDI (J)	Pearson correlation	r = 0.02215	p = 0.9241
	With ODI (N)	Pearson correlation	r = 0.2897	p = 0.2027

Correlation between PV^+ counts in the dCA2	With SDI (K)	Pearson correlation	r = 0.3724	p = 0.0964
	With ODI (O)	Pearson correlation	r = -0.2045	p = 0.3738
Correlation between PV^+ counts in the dCA3	With SDI (L)	Pearson correlation	r = 0.3268	p = 0.1482
	With ODI (P)	Pearson correlation	r = -0.01471	p = 0.9495
Correlation between PV^+ counts in the dDG	With SDI (M)	Pearson correlation	r = 0.2794	p = 0.2200
	With ODI (Q)	Pearson correlation	r = 0.1521	p = 0.5105
Effect of re-group manipulation in PV^+ counts in vHPC subfields and social recognition ability (related to Fig. S3)				
SDI (A)	GH vs IH vs RGH	One-way ANOVA	F (2, 15) = 3.696	p = 0.0495
	GH vs IH	Bonferroni post hoc analysis	t (15) = 2.713	p < 0.05*****
	GH vs RGH		t (15) = 1.025	p > 0.05
	IH vs RGH		t (15) = 1.502	p > 0.05
The PV^+ counts in vHPC (B)	GH vs IH vs RGH	One-way ANOVA	F (2, 15) = 5.316	p = 0.0180
	GH vs IH	Bonferroni post hoc analysis	t (15) = 3.222	p < 0.05*****
	GH vs RGH		t (15) = 0.9596	p > 0.05
	IH vs RGH		t (15) = 2.032	p > 0.05
The PV^+ counts in vHPC subfields (C)	Groups	Mixed two-way ANOVA	F (2, 15) = 7.436	*p = 0.0057*
	Subfields		F (1.681, 25.21) = 81.83	*p < 0.0001*
	Interaction		F (4, 30) = 3.127	*p = 0.0291*
	vCA1: GH vs IH	Bonferroni post hoc analysis	t (10.99) = 4.542	p = 0.0025******
	All other comparisons		p > 0.05	
Other behavioral tests in PVIs-inactivation experiment (related to Fig. S4)				
SDT: dCA1-PVIs	GFP: familiar vs novel (B1)	Paired t test	t (7) = 6.338	p = 0.0004*******
	TeNT: familiar vs novel (B2)	Paired t test	t (6) = 7.853	p = 0.0002*******
	SDI: GFP vs TeNT (C)	Unpaired t test	t (13) = 1.841	p = 0.0886
NORT (D)	GFP: familiar vs novel (D1)	Paired t test	t (8) = 2.748	p = 0.0252*****
	TeNT: familiar vs novel (D2)	Paired t test	t (10) = 0.980	p = 0.3501
	ODI: GFP vs TeNT (D3)	Unpaired t test	t (18) = 2.306	p = 0.0332*****
EPM	Time in open arms: GFP vs TeNT (E2)	Unpaired t test	t (30) = 2.399	p = 0.023*****
Open field	Time in center zone: GFP vs TeNT (E1)	Unpaired t test	t (31) = 2.229	p = 0.033*****
	Total travel distance (F1)	Unpaired t test	t (31) = 1.181	p = 0.247
	Mean speed (F2)	Unpaired t test	t (31) = 0.784	p = 0.439
Prepulse inhibition (G)	groups	Mixed two-way ANOVA	F (1, 23) = 0.096	p = 0.7599
	prepulse intensity		F (3, 69) = 12.72	p < 0.0001*******
	Interaction		F (3, 69) = 0.265	p = 0.8503
	Subjects		F (23, 69) = 32.47	p < 0.0001*******
Other behavioral tests in optogenetic experiment (related to Fig. S5)				
ChR2: laser on vs laser off	Laser off: familiar vs novel (A1)	Unpaired t test	t (6) = 3.271	p = 0.0170*****
	Laser on: familiar vs novel (A2)	Unpaired t test	t (6) = 0.3032	p = 0.7719
	SDI: Laser off vs laser on (B)	Unpaired t test	t (6) = 4.465	p = 0.0043******

NpHR: laser on vs laser off	Laser off: familiar vs novel (C1)		Unpaired *t* test	*t* (7) = 6.160	*p* = 0.0005***
	Laser on: familiar vs novel (C2)		Unpaired *t* test	*t* (7) = 3.302	*p* = 0.0131*
	SDI: Laser off vs laser on (D)		Unpaired *t* test	*t* (7) = 2.370	*p* = 0.0496*
SDT (with roomate)	mCh: roommate vs novel (E1)		Paired *t* test	*t* (6) = 5.185	*p* = 0.0020**
	ChR2: roommate vs novel (E2)		Paired *t* test	*t* (8) = 7.135	*p* < 0.0001***
	SDI: mCh vs ChR2 (F)		Unpaired *t* test	*t* (14) = 0.827	*p* = 0.4221
	GFP: roommate vs novel (G1)		Paired *t* test	*t* (5) = 5.016	*p* = 0.0041**
	TeNT: roommate vs novel (G2)		Paired *t* test	*t* (5) = 0.4652	*p* = 0.6613
	SDI: GFP vs TeNT (H)		Unpaired *t* test	*t* (10) = *0.333*	*p* = 0.7458
NORT:	mCh: familiar vs novel (I1)		Paired *t* test	*t* (7) = *2.595*	*p* = 0.0357*
	ChR2: familiar vs novel (I2)		Paired *t* test	*t* (8) = 2.464	*p* = 0.0391*
	ODI: mCh vs ChR2 (J)		Unpaired *t* test	*t* (15) = 0.003	*p* = 0.9974
	GFP: familiar vs novel (K1)		Paired *t* test	*t* (9) = *2.635*	*p* = 0.0271*
	NpHR: familiar vs novel (K2)		Paired *t* test	*t* (9) = 2.392	*p* = 0.0404*
	ODI: GFP vs NpHR (L)		Unpaired *t* test	*t* (18) = 0.158	*p* = 0.8773
Open field	Total travel distance	mCh vs ChR2 (M)	Unpaired *t* test	*t* (10) = 0.262	*p* = 0.7989
		GFP vs NpHR (N)	Unpaired *t* test	*t* (12) = 2.043	*p* = 0.0637
	Time in center zone	mCh vs ChR2 (O)	Unpaired *t* test	*t* (10) = 1.241	*p* = 0.2431
		GFP vs NpHR (P)	Unpaired *t* test	*t* (12) =0.8058	*p* = 0.4360
Effects of chemogenetic manipulation of vCA1-PVIs during consolidation stage on social memory (related to Fig. S6)					
DREADDs manipulation during separation session of SDT (B)	Main effect of groups		Mixed two-way ANOVA	F (2, 16) = 2.968	*p* = 0.0801
	Main effect of times			F (1, 16) = 6.163	*p* = 0.0245*
	group × times interaction			F (2, 16) = 3.678	*p* = 0.0485*
	Subjects			F (16, 16) = 0.795	*p* = 0.6746
	0h: mCh vs hM3Dq		Bonferroni post hoc analysis	t (11) = 3.370	*p* = 0.0039
	0h: mCh vs hM4Di			t (11) = 2.599	*p* = 0.0280
	3h: mCh vs hM3Dq			t (11) = 0.6322	*p* > 0.9999
	3h: mCh vs hM4Di			t (11) = 0.2051	*p* > 0.9999
Optogenetic manipulation of PVIs during investigating one of a pair of novel/familiar object (related to Fig. S7)					
A pair of familiar objects	mCh: laser off vs laser on (B1)		Paired *t* test	*t* (5) = 0.2870	*p* = 0.7856
	ChR2: laser off vs laser on (B2)		Paired *t* test	*t* (6) = 0.0795	*p* = 0.9392
	GFP: laser off vs laser on (C1)		Paired *t* test	*t* (5) = 0.0072	*p* = 0.9945
	NpHR: laser off vs laser on (C2)		Paired *t* test	*t* (5) = 0.3131	*p* = 0.7668
A pair of novel objects	mCh: laser off vs laser on (E1)		Paired *t* test	*t* (5) = 0.5947	*p* = 0.5779
	ChR2: laser off vs laser on (E2)		Paired *t* test	*t* (6) = 1.098	*p* = 0.3142
	GFP: laser off vs laser on (F1)		Paired *t* test	*t* (5) = 1.298	*p* = 0.2509
	NpHR: laser off vs laser on (F2)		Paired *t* test	*t* (5) = 0.2836	*p* = 0.7881
Additional analysis of fiber-photometry results (related to Fig. S8)					
Investigating different body parts (B)	Main effect		Repeated One-way ANOVA	F (1.692, 8.462) = 45.04	*p* < 0.0001
	Anogenital vs. Facial		Bonferroni post	*t* (5) = 4.301	*p* < 0.05*

	Anogenital vs. Flank	hoc analysis	t (5) = 7.898	$p < 0.01$**
	Facial vs. Flank		t (5) = 6.322	$p < 0.01$**
Signal decay across bouts: Novel mouse vs mouse toy vs mouse toy with urine (C)	Bouts	Repeated Two-way ANOVA	F (1.267, 5.068) = 28.24	$p = 0.0025$**
	Investigation targets		F (1.712, 6.847) = 13.37	$p = 0.0050$**
	Interaction: bouts x investigation targets		F (1.729, 6.917) = 15.32	$p = 0.0033$**
	1st 1/3 bouts: Novel mouse vs. mouse toy	Bonferroni post hoc analysis	t (4) = 4.114	$p < 0.05$*
	2nd 1/3 bouts: Novel mouse vs. mouse toy		t (4) = 4.44	$p < 0.05$*
	2nd 1/3 bouts: Novel mouse vs. mouse toy with urine		t (4) = 6.16	$p < 0.05$*
	All other comparisons		$p > 0.05$	
The evaluation of c-Fos expression in the vCA1 after social interaction (related to Fig. S9)				
Number of c-Fos+ nuclei	Familiar vs novel (A1)	Mann-whitney test	$U = 11$	$p = 0.0281$*
Proportion that PV × c-Fos in Or.	PV × Low c-Fos: familiar vs novel (E1)	Mann-whitney test	$U = 10$	$p = 0.4004$
	PV × Medium c-Fos: familiar vs novel (E2)	Mann-whitney test	$U = 11$	$p = 0.5022$
	PV × High c-Fos: familiar vs novel (E3)	Mann-whitney test	$U = 15$	$p > 0.9999$
Proportion that PV × c-Fos in Pyr.	PV × Low c-Fos: familiar vs novel (F1)	Mann-whitney test	$U = 4$	$p = 0.0455$*
	PV × Medium c-Fos: familiar vs novel (F2)	Mann-whitney test	$U = 11$	$p = 0.5281$
	PV × High c-Fos: familiar vs novel (F3)	Mann-whitney test	$U = 10$	$p = 0.3853$
Stimulating vCA1-PVIs at 40Hz in SDT (related to Fig. S10)				
SDT at 40Hz	mCh: familiar vs novel (A1)	Paired t test	t (5) = 5.069	$p = 0.0039$**
	ChR2: familiar vs novel (A2)	Paired t test	t (6) = 1.790	$p = 0.1237$
	SDI: mCh vs ChR2 (B)	Unpaired t test	t (11) = 2.387	$p = 0.036$*
Approaching one of a pair of familiar mice triggered 473nm laser at 40Hz in SDT	mCh: familiar (with laser) vs familiar (without laser) (C1)	Paired t test	t (6) = 0.2953	$p = 0.7778$
	ChR2: familiar (with laser) vs familiar (without laser) (C2)	Paired t test	t (5) = 2.581	$p = 0.0494$*