

Visuosocial Preference Memory, but Not Avoidance Memory, Requires PLC γ 1 in the CA2 Hippocampus

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Visuosocial memory is defined as stored visual information containing social context. Primates have a powerful ability to associate visuosocial memory with episodic memory. However, the existence of visuosocial memory in mice remains unclear. Here, we design a novel vision-specific social memory test using a portrait picture or mirrored self-image and demonstrate that mice can distinguish conspecific from other species by forming a visuosocial memory. Because CA2 hippocampus has been reported as a critical brain region for social memory, we develop CA2-specific blockade of memory formation through deletion of phospholipase C gamma 1 (PLC γ 1), which is a key molecule in the brain-derived neurotrophic factor (BDNF) signaling pathway. Interestingly, these mice have intact sociability but impaired social memory in three chamber test and five-trial social memory test, which is highly dependent on visual information. Finally, PLC γ 1 deletion in CA2 impairs visuosocial preference memory, but not avoidance memory, whereas non-social object recognition is intact. Our study proposes that mice have visuosocial memory, just as primates and humans.

Key words: Visuosocial memory, CA2 hippocampus, Phospholipase C gamma 1, Social behavior

INTRODUCTION

Social animals can quickly and accurately discriminate conspecifics using various external sensory information such as visual, auditory, olfactory, and tactile cues [1, 2]. Humans are extremely efficient in integrating social information into episodic memory. In contrast, rodents, the most widely used animals for social behavior, have been thought to have poor vision compared to humans. Thus, most of the sociality-related studies with rodents focus on olfactory social cues, whereas very few studies have focused on “visuo-

social memory.” Visuosocial memory can be defined as one kind of social memory which contains visual information associated with social context. One potential brain region to store visuosocial memory is the hippocampus because; 1) Hippocampus has been considered as a key brain region to associate different types of information such as space, object, sound cues, and context into episodic memory [3-5], and 2) Recently the CA2 hippocampus was suggested as the critical brain region for social memory in mouse [6, 7]. Interestingly, receptors of oxytocin and vasopressin which are well-known for their function in social cognition are highly and specifically expressed in hippocampal CA2 [8, 9]. In addition, CA2 has been recently established as a distinct area with specific molecular markers such as Amigo2 and RGS14 with unique electrophysiological properties [10, 11]. CA2 has been revealed to play an essential role in social memory and exhibit long-term potentiation (LTP) at the entorhinal cortical input to CA2 synapses in mice

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[6, 12, 13]. However, up to date, no study has demonstrated the existence of visuosocial memory in rodents and the role of CA2 in this particular memory.

Memory can be formed by associating various types of stimuli with valence. For example, mice can associate a novel place with negative valences such as foot shock-induced fear [14] or empathic fear [15]. In contrast, mice can be conditioned to prefer a specific place by the treatment with DAMGO, a μ -opioid receptor (MOR) agonist [16]. Therefore, if visuosocial memory exists in mice, mice should associate a visuosocial stimulus with electric foot shock or DAMGO to establish visuosocial avoidance memory or visuosocial preference memory, respectively. During this association process, specific signaling pathways or molecules in CA2 must be involved.

Considerable lines of accumulating evidence demonstrate that brain-derived neurotrophic factor (BDNF) is required for synaptic plasticity and hippocampus-dependent memory [17, 18]. In animal studies, BDNF signaling in the hippocampus and mesolimbic circuit plays an important role in antidepressant action to relieve chronic social stress [19, 20]. Phospholipase C gamma 1 (PLC γ 1) is known to be direct downstream signaling molecule of BDNF and its high-affinity receptor, tyrosine receptor kinase B (TrkB) [21]. Also, a previous report suggested that PLC γ 1 is necessary for TrkB-mediated long-term potentiation in CA1 hippocampus [22]. However, little is known about whether PLC γ 1-mediated BDNF signaling in CA2 is linked with social cognition and memory. In this study, we investigated the role of CA2 PLC γ 1 in associating an episodic experience with visuosocial stimulus by utilizing a novel CA2-specific deletion of PLC γ 1 mouse model, RGS14-cre \times PLC γ 1^{fl/fl}.

MATERIALS AND METHODS

Animals

PLC γ 1^{fl/fl} mice were developed and genotyped as previously described [23]. They were maintained as heterozygotes on the C57BL/6J background and crossed to obtain homozygote mutants. RGS14-cre mouse (036535-UCD, MMRRC) was crossed with PLC γ 1^{fl/fl} mouse (PLC γ 1^{fl/fl} \times RGS14-cre) for CA2-specific knockout of PLC γ 1. To validate the CA2-specificity of RGS14-cre, tdTomato^{fl/fl} mouse (007914, Jackson Laboratory) was crossed with RGS14-cre mouse (RGS14-cre \times tdTomato^{fl/fl}). To validate PLC γ 1 expression level, triple transgenic mice were used (PLC γ 1^{fl/fl} \times RGS14-cre \times tdTomato^{fl/fl}). Wildtype C57BL/6J and C57BL/6N mice were used for visuosocial memory test. All experiments were performed with 8 to 16-week-old male mice. Mice were kept on a 12 hr light-dark cycle in a specific-patho-

gen-free facility with controlled temperature and humidity and had free access to food and water. All experimental procedures, animal care, and handling were performed according to the directives of the Animal Care and Use Committee and the institutional guidelines of IBS (Daejeon, Korea).

Behavioral tests

Mice were group-housed two to five in each cage and had free access to food and water. They were kept on a 12 hr (8:00 to 20:00) light-dark cycle in the mouse facility with tightly maintained temperature (18 to 22°C) and humidity (40 to 60%). All tests were performed at a similar time in the light cycle (14:00 to 20:00). Mice were gently handled daily for 3 days before the first day of experiments. Mice were given at least 1 hr to habituate after moving to the behavioral room before experiments. All behavior experiments were performed under 10 to 20 lux light intensity except the 'Darkroom shock' experiment (complete dark, 0 lux).

Visuosocial memory test

The overall experimental scheme of visuosocial avoidance and preference memory tests is described in Fig 1. For visuosocial avoidance test, one day before the experiment, the subject was allowed to explore the 2-chamber arena connected by a tunnel in the middle for 10 min to acclimate the apparatus (40 cm length \times 20 cm width \times 20 cm height for each chamber). Mice passed a tunnel at least one time in habituation session went to next session. On Day 1, one more habituation session (10 min) was done. Right after habituation, mice were placed into a fear conditioning chamber (H10-11M-TC, Coulbourn, see Fig. 1D). In this chamber, a visuosocial cue (mirror, pictures of C57BL/6, Balb/c, or chipmunk, see Fig. 1E) was attached at one side of the wall. The subject received the electric foot shock (0.3 mA, 2 sec) 2 times when the mouse interacted with the cue (head toward the cue). The subject returned to the home cage 1 min after the last shock. On Day 2, mice were freely moved in the arena same as on Day 1 for 10 min (Open tunnel session). After that, the cue was installed at the end of the tunnel and the number of entering the tunnel was recorded (Test session). For a control cue, white paper was used. To normalize the activity during the test session by basal activity, we used the discrimination index for comparison (Entering frequency in test session / Entering frequency in open tunnel session). We counted entering numbers only when a whole mouse body entered the tunnel. For the 'No shock' control experiment, mice were placed into the fear conditioning chamber with a mirror for 5 min without any electric shock. For the 'Darkroom shock' control experiment, there was no light in the chamber, and mouse behavior was recorded by the infrared camera. For visuosocial preference test, instead of

foot shock, DAMGO (1 mg/kg, i.p., 1171, Tocris) was injected right before transferring into the fear conditioning chamber and let mice stay for 30 min for 3 consecutive days (see Fig. 1B). We found that there is no significantly different entering frequency between before and after stimulation (foot shock or DAMGO) in both control and PLC γ 1 cKO groups by comparing open tunnel session of Day 1 with Day 2 (foot shock) or Day 5 (DAMGO).

Immunohistochemistry

Mice were deeply anesthetized with 2% avertin (i.p.) and perfused with 0.9% saline followed by ice-cold 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS). Excised brains were post-fixed overnight in 4% PFA at 4°C and immersed in 30% sucrose for over 24 hr for cryoprotection. Brain slices (30 μ m, coronal) containing the hippocampus were obtained. Sections were incubated for 1 hr in a blocking solution (0.3% Triton-X 100, 2% goat serum, and 2% donkey serum in 0.1M PBS) and then immunostained with a primary antibody (anti-RGS14, 1:50 dilution; 73-170; NeuroMab) in a blocking solution at 4°C on a shaker overnight. After washing in 0.1M PBS 3 times, sections were incubated with secondary antibody (anti-mouse 488, 1:500 dilution; 715-545-150; Jackson ImmunoResearch) for 1 hr and then washed with PBS 3 times. Finally, sections were mounted with a fluorescent mounting medium (S3023, Dako). All slice images were obtained by slide scanner (Axio scan.Z1, Zeiss).

Brain cell isolation

RGS14-cre \times tdTomato^{ff} and PLC γ 1 \times RGS14-cre \times tdTomato^{ff} mice were deeply anesthetized with 3% isoflurane and brains from these mice were quickly removed from the skull and submerged in ice-cold 0.1 M PBS. For brain cell isolation, the adult brain dissociation kit (130-107-677, Miltenyi Biotec) was used for brain cell isolation following the manufacturer's instructions. After dissociation, to collect tdTomato-positive cells specifically, fluorescence-activated cell sorting (FACS) experiment was performed using MoFlo Astrios Cell Sorter (Beckman Coulter). FACS-sorted tdTomato-positive cells were used for qRT-PCR and western blot to detect PLC γ 1 mRNA and protein level, respectively. We used 2 mice brains to make one sample. For qRT-PCR and western blot, we used 2 samples for each condition with duplicate or triplicate.

Quantitative real-time PCR (qRT-PCR)

Using FACS-sorted cells, RNA extraction (Qiagen RNeasy mini kit, 74104) and cDNA synthesis (SuperScriptTM III First-Strand Synthesis System, Invitrogen) were performed following the manufacturer's instructions. After cDNA synthesis, qRT-PCR (QuantStudio 1, Applied Biosystems) was performed in triplicates

in a total volume of 20 μ l containing 10 pM primers (forward and reverse), 4 μ l cDNA, and 5 μ l Power SYBR Green Master Mix (4367659, Applied Biosystems). The following sequences of primers were used for qRT-PCR.

PLC γ 1 forward: 5'-CCG GCC AGA TCA ATC ACA CT-3';
PLC γ 1 reverse: CCG GAG CCA CCT CTC AAT TT.

GAPDH forward: 5'- ACC CAG AAG ACT GTG GAT GG -3';
GAPDH reverse: 5'-CAC ATT GGG GGT AGG AAC AC-3'.

Western blot

For cell lysis, FACS-sorted cells were mixed with a lysis buffer containing 50mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 10 mM NaF, and protease and phosphatase inhibitor cocktail. The total protein concentration was determined with the BCA protein assay. Equal amounts of samples were loaded into 8% acrylamide gel and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After separation, gel was electrophoretically transferred to polyvinylidene fluoride (PVDF, Millipore) membrane by the gel transfer device (iBlot 2, Invitrogen). The membranes went through a blocking step using 5% skimmed milk in TBS-T buffer (Tris-buffered saline containing 0.05% Tween 20) for 1 hr. The membranes were incubated with primary antibody overnight at 4°C. After washing in TBS-T buffer, the membranes were incubated with horseradish peroxidase-labeled secondary antibody for 1 hr at room temperature. Protein bands were visualized by ECL reagents (GE Healthcare). Monoclonal anti-PLC γ 1 antibody was generated as previously described [24]. For loading control, anti- β -actin (691001, MP Biomedicals) antibody was used.

Three chamber test

The test was performed in three sessions within a three-chambered open arena (40 cm length \times 20 cm width \times 20 cm height for each chamber). In the habituation session (Day 1), the subject freely explored the apparatus for 10 min. In the sociability session (Day 2), the subject encountered a stranger mouse within a metal cup and an empty metal cup for 10 min. In the social novelty session (Day 3), the subject encountered the familiar one as well as a novel mouse in another metal cup in the social novelty session for 10 min. The duration for exploring each cup was analyzed.

Five-trial social memory test

This test was run as described previously with a minor modification [25]. In brief, animals were housed individually for 7~10 days before testing. On testing, a female C57BL/6 stimulus mouse was introduced into the home cage of each subject for 1 min and then returned to an individual holding cage. Four trials (1~4 trials) were

repeated with 10 min inter-trial intervals and the same stimulus to the resident in all four trials. In a fifth dishabituation trial, a novel stimulus female mouse was introduced. The time spent in the olfactory investigation for each trial was recorded and analyzed.

Novel object recognition test

Test was conducted in an open field arena (40 cm length \times 40 cm width \times 40 cm height) with two different kinds of objects (A, B). Initially there was no preference in both objects. During habituation, mouse was allowed to explore an empty arena for 10 min. 24 hr after habituation, the subject was exposed to the familiar arena with two identical objects (A, A') placed at an equal distance for 10 min (Acquisition). 1 hr later the subject explored the open field in the presence of the familiar object and a novel object (A, B) to test recognition memory for 10 min (Retention). The time spent exploring each object and the discrimination index was analyzed.

$$\text{Discrimination index (Acquisition)} = \frac{\text{time spent in A}}{\text{time spent in A} + \text{time spent in A'}}$$

$$\text{Discrimination index (Retention)} = \frac{\text{time spent in B}}{\text{time spent in B} + \text{time spent in A}}$$

Statistical analyses

All statistical analyses were performed using Prism 9 (GraphPad). Differences between two different groups were analyzed with a two-tailed Student's t-test. For comparison of multiple groups, one-way analysis of variance (ANOVA) with Tukey's multiple comparison test, or two-way ANOVA with Sidak's multiple comparison test was assessed. Animals were randomly and evenly allocated to each group for all experiments. All data were presented as mean \pm SEM. Asterisks indicate a significant difference as follow: ns, not significant, $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

RESULTS

To directly test whether mouse has an ability to form visuosocial memories, we designed a novel visuosocial memory test (Fig. 1, see detail in materials and methods session), in which mice learn to associate mirrored self-image or mouse-photo associated with electric shock-induced fear (visuosocial avoidance) or DAMGO-induced positive valence (visuosocial preference).

After pairing mirrored self-image or mouse-photo with electric shock, mice entered significantly less into the mirror- or photo-installed tunnel, compared to no-mirror control (white paper), no-shock control, or dark room condition (Fig. 2A), suggesting that mouse is capable of forming visuosocial avoidance memories

based on vision-specific fear-associated social cues. Unexpectedly, the C57BL/6-photo-shock paired mice showed a significant avoidance not only to C57BL/6-photo but also to Balb/c-photo and vice versa (Fig. 2B). This avoidance to the conspecific (Balb/c-photo) was not due to avoidance to the Balb/c-photo itself because the no-shock control showed no avoidance (Fig. 2B). Because C57BL/6 and Balb/c are conspecific, we hypothesized that mice can distinguish conspecific from heterospecific. To test this hypothesis, we used a photo of a chipmunk, a heterospecific animal. Surprisingly, mice did not avoid C57BL/6-photo when they received electric shock with chipmunk-photo or vice versa (Fig. 2C). In contrast, mice showed avoidance to chipmunk-photo when they received electric shock with chipmunk-photo (Fig. 2C), indicating that mice can distinguish conspecific from heterospecific and form visuosocial memories. Furthermore, mice can successfully associate not only visuosocial cues but also a neural cue (food pellet-photo) with electric shock-induced fear (Fig. 2D). Lastly, we test whether mice can associate positive valence with C57BL/6 photo using DAMGO (Fig. 2E). Interestingly, opposite to avoidance test, mice treated with DAMGO increased discrimination index, indicating that mice can associate positive valence with visuosocial cue (Fig. 2E). Taken together, using our novel visuosocial memory test, we discovered that mice can associate visuosocial cues with negative valence (fear) or positive valence (DAMGO).

To investigate the involvement of BDNF signaling in social memory in CA2, we utilized a previously reported CA2-specific cre-expressing mouse, RGS14-cre [26]. We validated cre expression in CA2 hippocampus using RGS14-cre crossed with tdTomato^{ff} mice (Fig. 3A). Then, to remove PLC γ 1 selectively in CA2 pyramidal neurons, we additionally crossed PLC γ 1^{ff} mice to make triple transgenic mice (PLC γ 1^{ff} \times RGS14-cre \times tdTomato^{ff}). Using qRT-PCR and western blot, we found a significant reduction of PLC γ 1 mRNA and protein expression in FACS-sorted cre-expressing cells (Fig. 3B–E). Taken together, we successfully developed CA2-specific PLC γ 1 KO using PLC γ 1^{ff} \times RGS14-cre mice.

To examine whether PLC γ 1 in CA2 is involved in social behavior, we performed three-chamber test with PLC γ 1^{ff} (Control) and PLC γ 1^{ff} \times RGS14-cre (PLC γ 1 cKO) mice to assess sociability and social novelty (Fig. 4A–F). We firstly found that there was no difference in locomotor activity between control and PLC γ 1 cKO (Data not shown). We also found that there was no impairment of sociability in both groups (Fig. 4B, C), but impaired social novelty in PLC γ 1 cKO group (Fig. 4E, F). This finding was consistent with the previous report of normal sociability but impaired social novelty in mice with CA2 pyramidal neuron inactivation by overexpression of tetanus-neurotoxin [6].

We performed another well-known social memory test, the five-

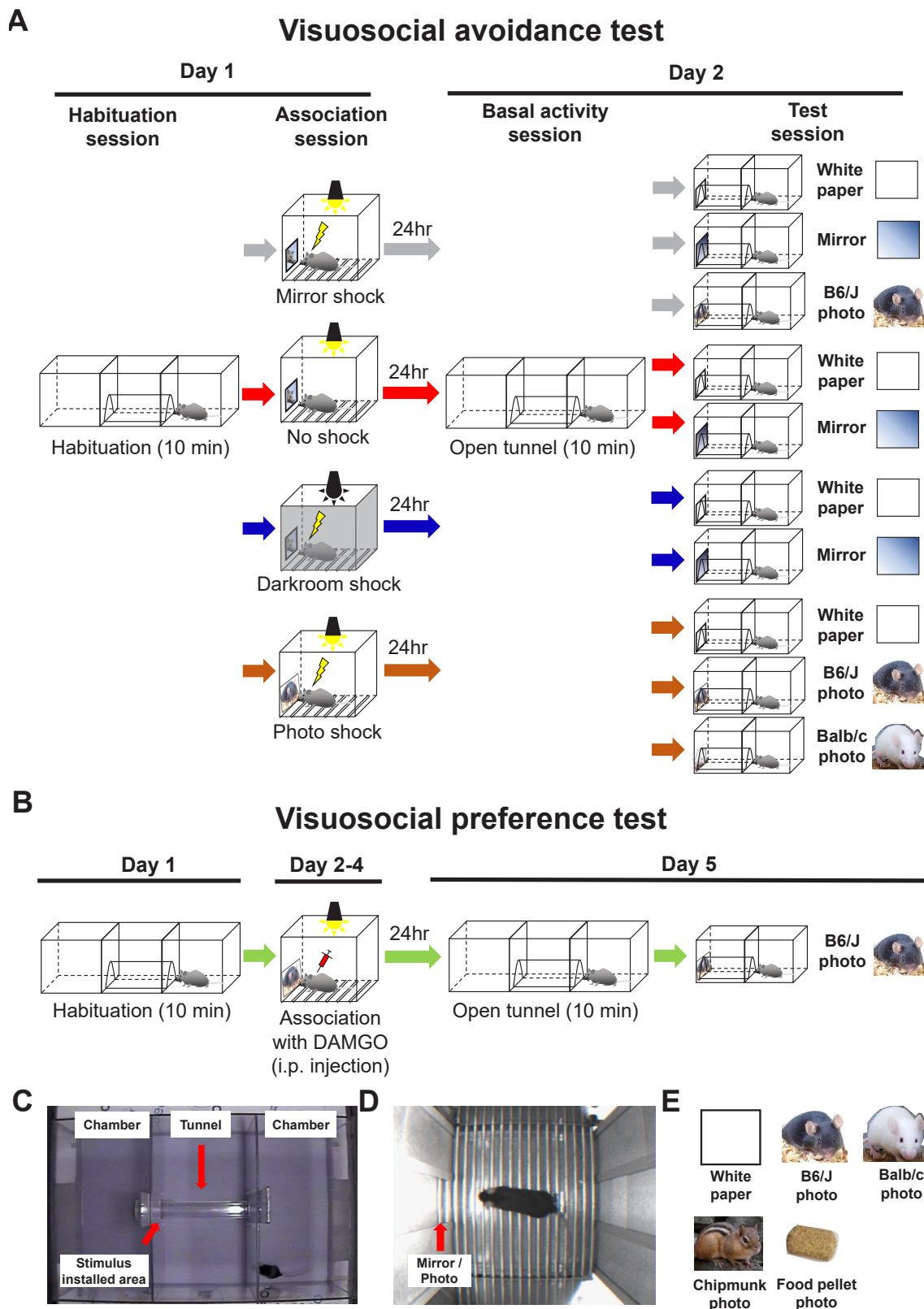


Fig. 1. Establishment of visuosocial memory test. (A, B) Overall schedule and experimental conditions of visuosocial avoidance (A) and preference (B) memory test. (C) An actual appearance of visuosocial memory test apparatus. A visuosocial cue (Mirror or photo) was placed at the end of the tunnel on the left side. (D) Visual cue-fear (or positive valence) conditioning chamber. A visuosocial cue was presented at the center of the left wall. (E) Actual images used in visuosocial memory test.

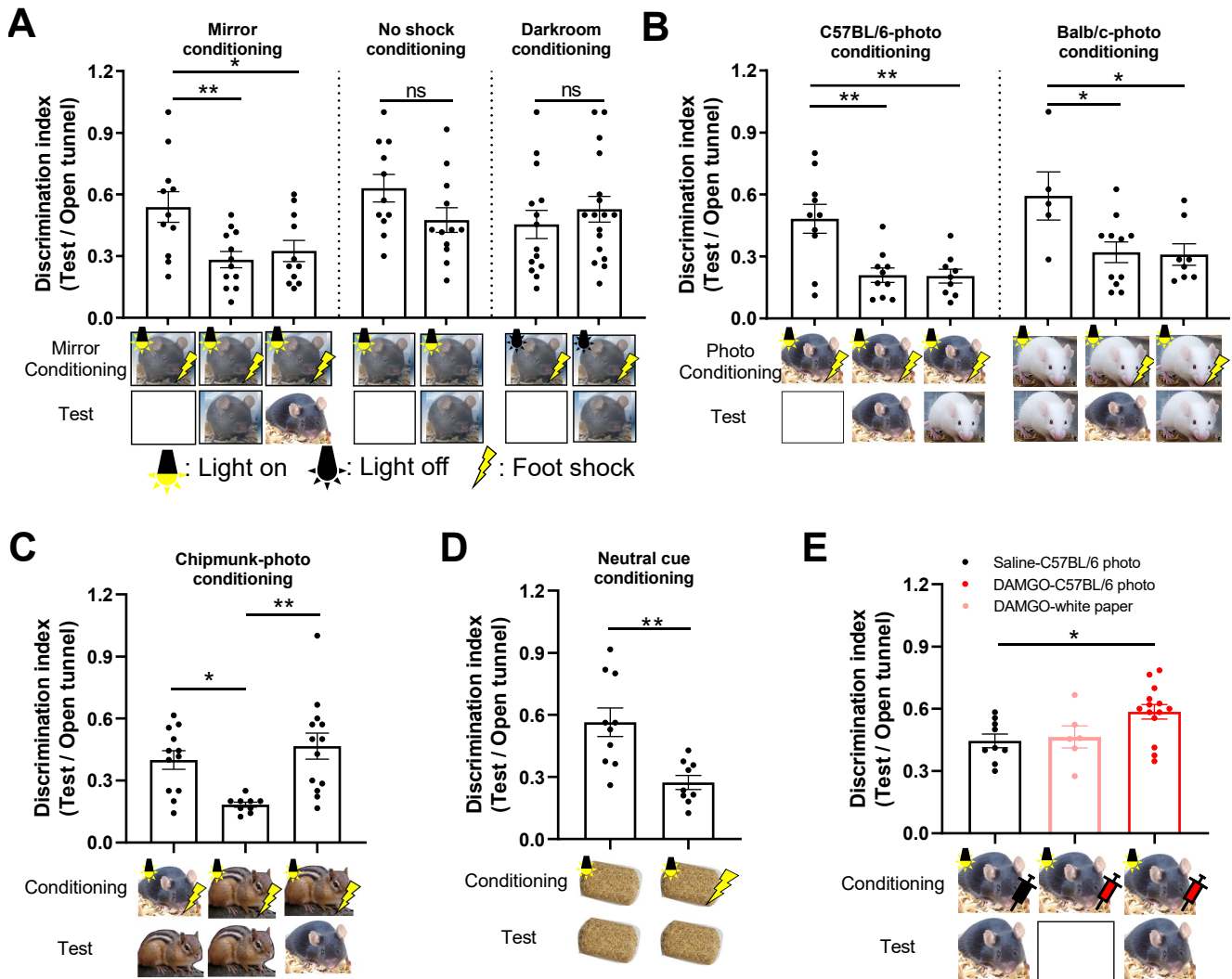


Fig. 2. Existence of visuosocial avoidance and preference memory in mice (A) Behavioral results from visuosocial avoidance memory test with mirror with various conditions (n=11, 12, 11, 11, 12, 14, and 17). Student's t-test, 1st vs 2nd lane, **p<0.01; 1st vs 3rd lane, *p<0.05; 4th vs 5th and 6th and 7th, p>0.05. (B) Behavioral results from visuosocial avoidance memory test with C57BL/6 and Balb/c photos (n=10, 10, 9, 5, 11, and 8). One-way ANOVA, 1st vs 2nd and 1st vs 3rd lane, **p<0.01; 4th vs 5th and 4th vs 6th lane, *p<0.05. (C) Behavioral results from visuosocial avoidance memory test with C57BL/6 and chipmunk photos (n=12, 9, and 13). One-way ANOVA, 1st vs 2nd lane, *p<0.05; 2nd vs 3rd lane, **p<0.01. (D) Visuosocial avoidance memory test result with neutral cue (food pellet) photo. Student's t-test, **p<0.01. (E) Visuosocial preference memory test result. One-way ANOVA, *p<0.05. Results are mean±SEM.

trial social memory test (Fig. 4G), in which the process of familiarization is assessed. We found that PLCγ1 cKO group showed a significantly impaired familiarization at the fourth trial, compared to the control group (Fig. 4H), suggesting that familiarization requires PLCγ1 in CA2. The impaired familiarization in PLCγ1 cKO group suggested that PLCγ1 is involved in a subset of social cues. To test if visuosocial cue is required for familiarization, we performed the same five-trial social memory test in the dark condition (Dark control) with naïve C57BL/6 mice. Surprisingly, interaction time in the entire trial was significantly reduced in the dark control condition (Fig. 4H), suggesting that the visuosocial cues

are required for social interaction but not for familiarization. Deletion of PLCγ1 in CA2 did not alter the ability to recognize non-social objects (Fig. 4I, J), suggesting that CA2 might be specific for visuosocial memory. Taken together, we concluded that PLCγ1 in CA2 is critical for both social novelty memory and familiarization which requires visuosocial information.

Finally, to evaluate which valence is associated with visuosocial cue by PLCγ1 in CA2, we tested both visuosocial avoidance memory and visuosocial preference memory with control and PLCγ1 cKO mice. We found that PLCγ1 cKO mice showed no significant difference in visuosocial avoidance memory with C57BL/6 photo

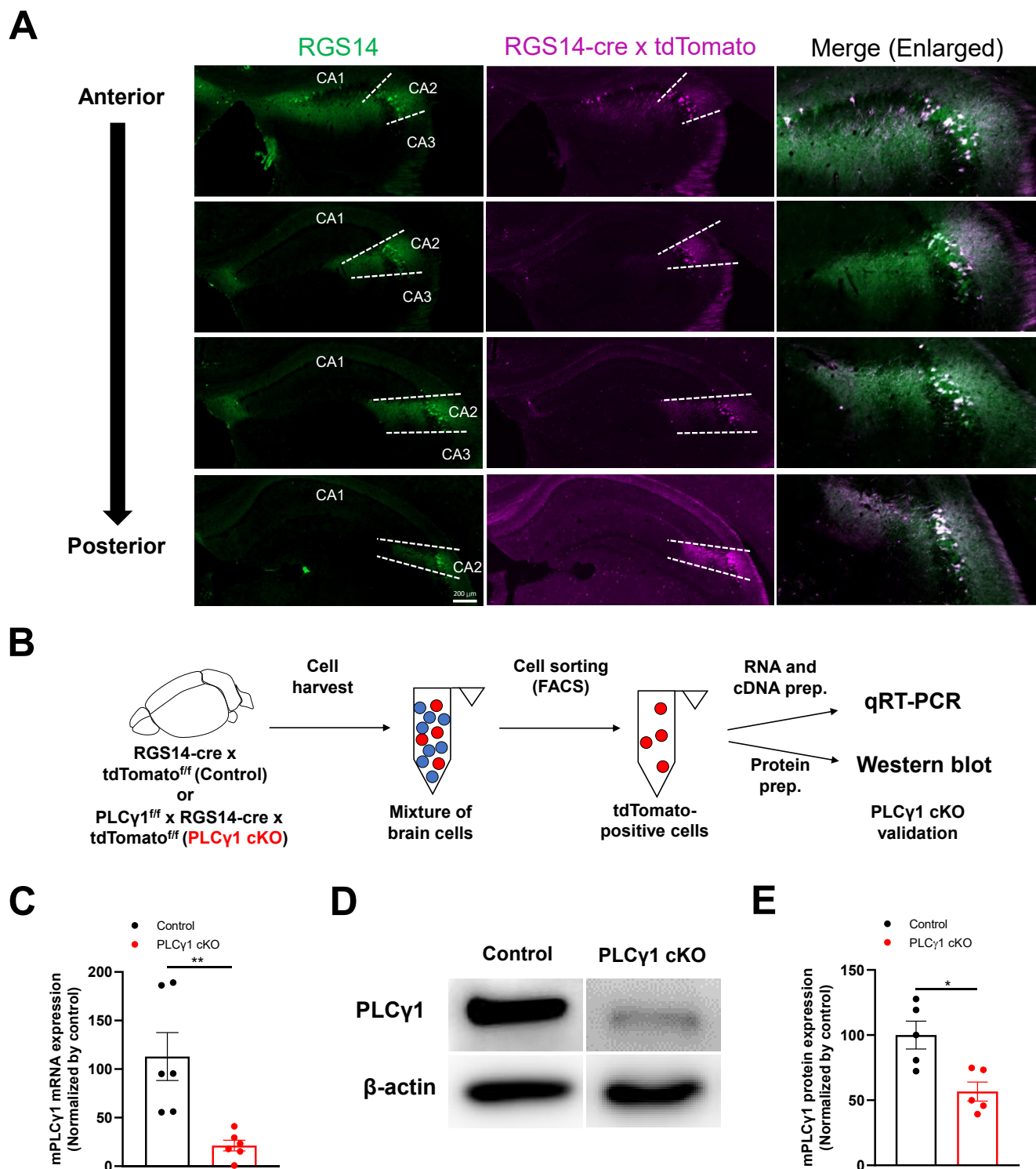


Fig. 3. Validation of CA2-specific expression with RGS14-cre \times tdTomato^{fl} mouse and PLC γ 1 knockout with PLC γ 1^{fl} \times RGS14-cre \times tdTomato^{fl} triple transgenic mice (A) Representative immunofluorescent images of hippocampus CA2 region of RGS14-cre \times tdTomato^{fl} mouse. Scale bar: 200 μ m. (B) Experimental procedure of PLC γ 1 KO (PLC γ 1 cKO) validation. (C) Summary bar graph of qRT-PCR results (n=6 and 6). Student's t-test, **p<0.01. (D) Representative western blot result of control and PLC γ 1 cKO. (E) Summary bar graph of western blot results from B (n=5 and 5). Student's t-test, *p<0.05. Results are mean \pm SEM.

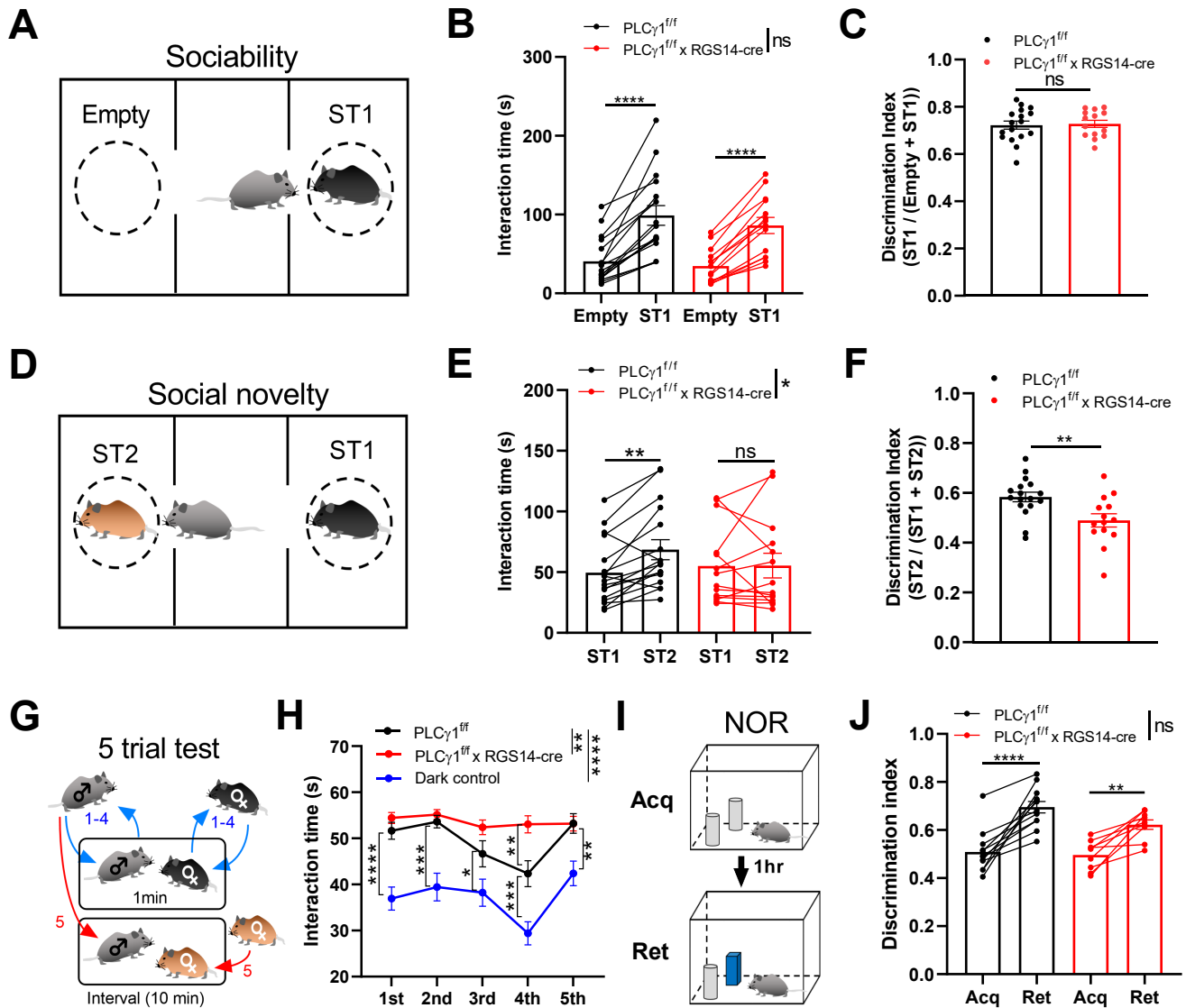


Fig. 4. Deletion of PLC γ 1 in CA2 shows intact sociability and object recognition memory but impaired social memory. (A) Schematic illustration of sociability test (ST1, stranger 1). (B, C) Summary bar graphs of interaction time (B) and discrimination index (C) of sociability (n=15 and 18). Two-way ANOVA (B), PLC γ 1^{fl/fl} vs PLC γ 1^{fl/fl} × RGS14-cre, $p > 0.05$; Student's t-test, Empty vs ST1 in PLC γ 1^{fl/fl} and PLC γ 1^{fl/fl} × RGS14-cre, **** $p < 0.0001$. Student's t-test (C), ns, not significant. (D) Schematic illustration of social novelty test (ST1, stranger 1; ST2, stranger 2). (E-F) Summary bar graphs of interaction time (E) and discrimination index (F) of social novelty test (n=15 and 18). Two-way ANOVA (E), PLC γ 1^{fl/fl} vs PLC γ 1^{fl/fl} × RGS14-cre, * $p < 0.05$; ST1 vs ST2 in PLC γ 1^{fl/fl}, ** $p < 0.01$; ST1 vs ST2 in PLC γ 1^{fl/fl} × RGS14-cre, ns, not significant. Student's t-test (F), ** $p < 0.01$. (G) Schematic diagram of five-trial social memory test. (H) Summary graph of five-trial social memory test with PLC γ 1^{fl/fl}, PLC γ 1^{fl/fl} × RGS14-cre, and dark control conditions (n=17, 14, and 14). Repeated two-way ANOVA, PLC γ 1^{fl/fl} vs PLC γ 1^{fl/fl} × RGS14-cre, ** $p < 0.01$; PLC γ 1^{fl/fl} vs dark control, **** $p < 0.0001$; PLC γ 1^{fl/fl} vs dark control in 3rd trial, * $p < 0.05$; PLC γ 1^{fl/fl} vs PLC γ 1^{fl/fl} × RGS14-cre in 4th trial and PLC γ 1^{fl/fl} vs dark control in 5th trial, ** $p < 0.01$; PLC γ 1^{fl/fl} vs dark control in 2nd and 4th trials, *** $p < 0.001$; PLC γ 1^{fl/fl} vs dark control in 1st trial, **** $p < 0.0001$. (I) Schematic illustration of novel object recognition test (NOR, Acq, acquisition; Ret, retention). (J) Summary bar graphs of discrimination index of NOR (n=8 and 11). Two-way ANOVA, PLC γ 1^{fl/fl} vs PLC γ 1^{fl/fl} × RGS14-cre, $p > 0.05$; Student's t-test, Acq vs Ret in PLC γ 1^{fl/fl}, ** $p < 0.01$; Acq vs Ret in PLC γ 1^{fl/fl} × RGS14-cre, **** $p < 0.0001$. Results are mean ± SEM.

(Fig. 5A), as well as with a neutral cue (Fig. 5B). On the other hand, PLC γ 1 cKO mice showed significantly decreased visuosocial preference memory (Fig. 5C), whereas neutral cue (food pellet photo)-induced preference memory was intact (Fig. 5D), indicating that PLC γ 1 in CA2 is crucial for visuosocial preference memory. Also,

these data suggested that mice recognize C57BL/6 photo as a social stimulus which is fundamentally different from a neutral object such as a food pellet. We additionally confirmed that these behavioral results were not affected by foot shock or DAMGO (Fig. 5E). Based on these results, we concluded that PLC γ 1 in CA2

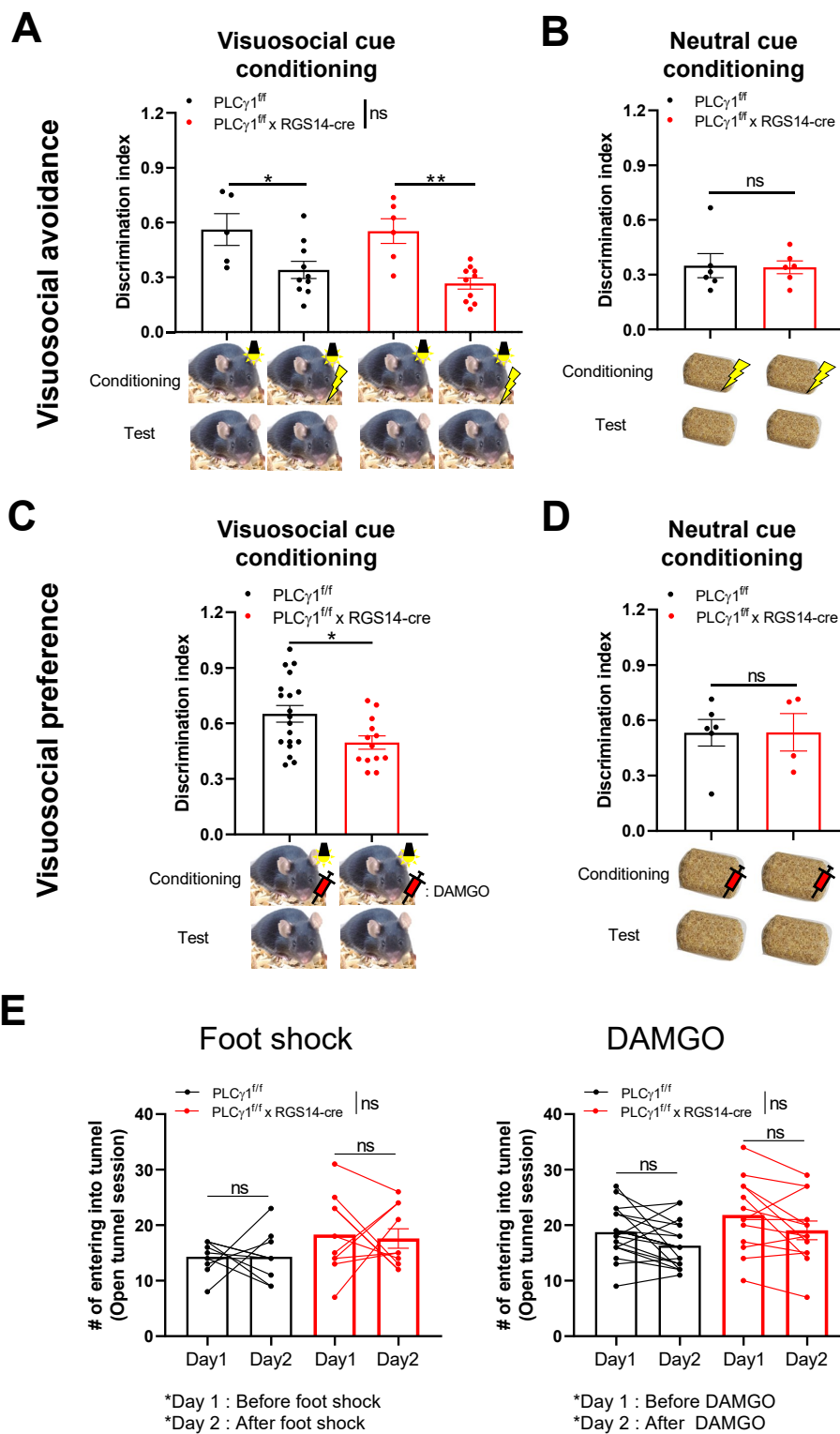


Fig. 5. PLCγ1 in CA2 hippocampus is critical for visuosocial preference, but not avoidance, memory. (A) Summary bar graph of visuosocial avoidance memory test with C57BL/6 photo (n=5, 10, 6, and 10). Two-way ANOVA, p>0.05. 1st vs 2nd lanes, *p<0.05; 3rd vs 4th lanes, **p<0.01. (B) Summary bar graph of visuosocial avoidance memory test with a neutral cue (n=6 and 6). Student's t-test, ns, not significant. (C) Summary bar graph of visuosocial preference memory test with PLCγ1^{fl/fl} (control) and PLCγ1^{fl/fl}xRGS14-cre (PLCγ1 cKO) mice (n=19 and 13). Student's t-test, *p<0.05. (D) Summary bar graph of visuosocial preference memory test with a neutral cue (n=6 and 4). Student's t-test, ns, not significant. (E) Comparison of entering frequency in open tunnel session between Day 1 (Before foot shock or DAMGO) and Day 2 (After foot shock or DAMGO) in control and PLCγ1 cKO mice. Two-way ANOVA, ns, not significant. Results are mean±SEM.

is critical for visuosocial preference memory, but not avoidance memory.

DISCUSSION

In this study, we have developed a novel animal model and behavioral test using visuosocial cue-fear (Foot shock) or positive valence (DAMGO) association to examine the neural network and molecular mechanisms of visuosocial memory. Our results propose that CA2 PLC γ 1, which is one of the key components of BDNF signaling, is critically involved in visuosocial preference memory, but not avoidance memory. DAMGO is known to activate MOR in CA1 hippocampal astrocyte to release glutamate which activates presynaptic mGluR1 to enhance synaptic plasticity and cause conditioned place preference [16]. Therefore, it is highly possible that visuosocial preference memory is formed through the similar mechanism by CA2 astrocytic MOR. Also, during social memory tests (three chamber test and five-trial social memory test) and visuosocial preference memory test, BDNF is released to CA2 neurons to induce PLC γ 1-mediated synaptic potentiation. These interesting hypotheses should be examined in the future.

In contrast to visuosocial preference memory, we found that visuosocial avoidance memory is unaltered in KO. This raises a possibility that visuosocial avoidance memory might be encoded in other hippocampal areas. One possible candidate can be ventral CA1, which has been suggested as a critical brain region for social memory [27] and fear memory encoding [28]. This interesting possibility should be investigated in the future.

There is one potential caveat of using RGS14-cre mouse. Even though RGS14-cre can target CA2 specifically in hippocampus, we observed cre expression in other brain areas such as piriform area and lateral part of cerebellum. Therefore, we cannot completely rule out the possible role of PLC γ 1 in the formation of visuosocial preference memory in those brain areas.

The molecular, genetic, and behavioral tools that we have developed will be useful in understanding the social memory processing in social animals including human, who is very well known for having a high capacity for visuosocial memories through the ability to recognize conspecifics by visual cues. Moreover, it would be the first step to addressing a critical question of how human remembers conspecific's face and associated memories. Finally, we propose that the BDNF-TrkB-PLC γ 1 axis in CA2 can be a novel therapeutic target for impaired facial information processing and related diseases such as prosopagnosia, dementia, and autism.

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