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ORIGINAL ARTICLE

Durable fear memories require PSD-95

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Traumatic fear memories are highly durable but also dynamic, undergoing repeated reactivation and rehearsal over time. Although overly persistent fear memories underlie anxiety disorders, such as posttraumatic stress disorder, the key neural and molecular mechanisms underlying fear memory durability remain unclear. Postsynaptic density 95 (PSD-95) is a synaptic protein regulating glutamate receptor anchoring, synaptic stability and certain types of memory. Using a loss-of-function mutant mouse lacking the guanylate kinase domain of PSD-95 (PSD-95^{GK}), we analyzed the contribution of PSD-95 to fear memory formation and retrieval, and sought to identify the neural basis of PSD-95-mediated memory maintenance using *ex vivo* immediate-early gene mapping, *in vivo* neuronal recordings and viral-mediated knockdown (KD) approaches. We show that PSD-95 is dispensable for the formation and expression of recent fear memories, but essential for the formation of precise and flexible fear memories and for the maintenance of memories at remote time points. The failure of PSD-95^{GK} mice to retrieve remote cued fear memory was associated with hypoactivation of the infralimbic (IL) cortex (but not the anterior cingulate cortex (ACC) or prelimbic cortex), reduced IL single-unit firing and bursting, and attenuated IL gamma and theta oscillations. Adeno-associated virus-mediated PSD-95 KD in the IL, but not the ACC, was sufficient to impair recent fear extinction and remote fear memory, and remodel IL dendritic spines. Collectively, these data identify PSD-95 in the IL as a critical mechanism supporting the durability of fear memories over time. These preclinical findings have implications for developing novel approaches to treating trauma-based anxiety disorders that target the weakening of overly persistent fear memories.

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INTRODUCTION

Once formed, fear memories can last a lifetime.[1] However, evidence is accumulating that fear memories are not rigid, but plastic over time and labile following reactivation. Dysregulation of the balance between memory retention and revision, whereby fear memories are long lasting and inflexible, may contribute to persistent anxiety in disorders such as posttraumatic stress disorder.[2,3] Indeed, rendering fear memories unstable, and thereby more liable to erasure or reconsolidation, has been proposed as a novel approach to treating anxiety disorders.[4] Currently, however, the critical neural and molecular mechanisms determining fear memory stability and persistence over time are not fully understood.[5–9]

Glutamate-mediated signaling and plasticity is critical to various forms of fear learning and memory. By orchestrating protein–protein interactions and the scaffolding of glutamate receptors, postsynaptic density 95 (PSD-95) has an integral functional role within the postsynaptic machinery mediating glutamatergic plasticity.[10–18] PSD-95 stabilizes α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) at the synapse to promote synaptic function and spine growth, and decreasing PSD-95 leads to loss of synaptic AMPAR content, synaptic weakening, deficient long-term depression and spine elimination (for example, Nelson *et al.*[12], Xu *et al.*[16], Opazo *et al.* [17], Colledge *et al.*[19], Sturgill *et al.*[20], Bhattacharyya *et al.*[21] (*cf.* Woods *et al.*[22])). Following fear learning, PSD-95 expression is increased in brain regions such as the amygdala, and these increases are rapidly reversed by the formation of extinction memories that lead to the inhibition of fear.[23,24] Recent work has also shown that PSD-95 is actively degraded by myocyte enhancer factor 2,[25] and that virally overexpressing myocyte enhancer factor 2 caused AMPAR endocytosis, reduced synaptic strength and spine density, and impaired fear memory stability.[26] Collectively, these observations implicate PSD-95 as a key contributor to the dynamic regulation of synaptic functions critical for fear memory.

The contribution of PSD-95 to memory has been demonstrated behaviorally by studies showing that PSD-95 deletion or knockdown (KD) impairs spatial learning, conditioned taste aversion and simple operant associative learning.[27–29] Conversely, manipulations that lead to an upregulation of PSD-95, including estrogen treatment and insulin substrate-2 deletion, produce improvements in spatial and fear memory.[30–32] Interestingly, there is also emerging evidence that PSD-95 may be crucial to maintaining the stability of certain forms of memories at time points more remote from acquisition. For example, although gene deletion of PSD-95 does not prevent the initial formation and recent expression of ethanol conditioned place preference, the absence of PSD-95 leads to a loss of conditioned place preference within 2 weeks.[33] Along similar lines, mutant mice with a ligand-binding-deficient knockin mutation of PSD-95 show deficient contextual fear memory expression within 1 week, but not 1 day, of conditioning.[34]

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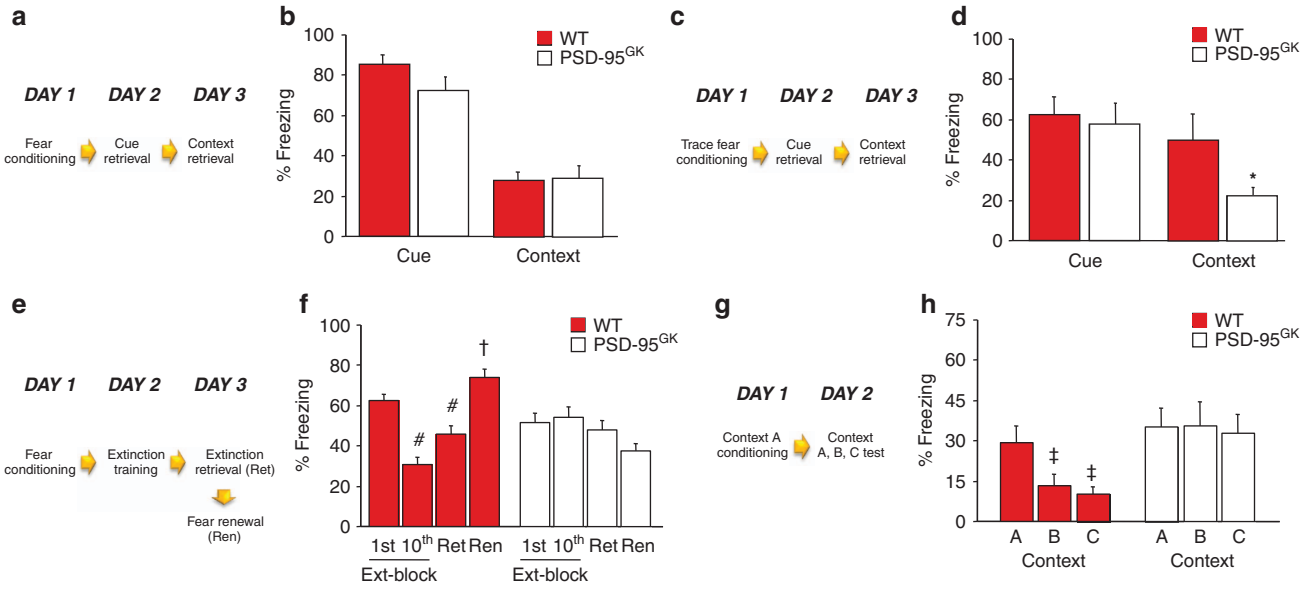

Figure 1. Postsynaptic density 95 (PSD-95) deletion does not affect retrieval of recent fear memories, but impairs their extinction and precision. (**a**) Schematic of experimental design for testing recent fear memory retrieval following delay conditioning. (**b**) Genotypes showed equivalent levels of freezing during cue and context retrieval ($n = 11$–17). (**c**) Schematic of experimental design for testing recent fear memory retrieval following trace conditioning. (**d**) Following trace fear conditioning, genotypes showed similar cue fear retrieval, but PSD-95^{GK} mice froze significantly less than wild-type controls during context retrieval ($n = 8$–10). (**e**) Schematic of experimental design for testing extinction of a recent fear memory. (**f**) Wild-type (WT) controls, but not PSD-95^{GK} mice, decreased freezing from the first to the last extinction trial block, and froze less during extinction retrieval as compared with the first trial block of extinction training ($n = 11$). (**g**) Schematic of experimental design for testing the precision of a recent contextual fear memory. (**h**) WT controls, but not PSD-95^{GK} mice, froze more to the conditioned context than either of two different contexts ($n = 13$). Data are means ± s.e.m. $^{*}P < 0.05$ vs WT/Context, $^{\#}P < 0.05$ vs 1st Ext-block/WT, $^{\dagger}P < 0.05$ vs Ret/WT, $^{\ddagger}P < 0.05$ vs Context A/WT.

Taken together, the existing evidence provides preliminary support for a role for PSD-95 in various forms of memory, but does not establish the precise role of PSD-95 in fear memory or establish the neural mechanisms that underlie such a role. This study sought to clarify these questions by integrating behavioral analysis of a loss-of-function PSD-95 mutant mouse with *ex vivo* immediate-early gene (IEG) mapping, *in vivo* neuronal recordings and viral-mediated KD in specific brain regions. Our results reveal that PSD-95 is dispensable for the formation and expression of recent fear memories, but is essential for the precision and flexibility of recent memories and for their maintenance of remote fear memories. Our data identifying PSD-95 as a critical mechanism in fear memory stability could have implications for developing novel anxiolytic treatments that work to enhance the lability of fear memories.

MATERIALS AND METHODS

Subjects

PSD-95^{GK} mutant mice were engineered with a disruption of the guanylate kinase (GK) domain of the PSD-95 (*Dlg4*) gene and have been previously reported.[33,35,36] In other models, short hairpin RNA KD or mutation of the PSD-95^{GK} domain disrupts N-methyl-D-aspartate receptor-mediated AMPAR endocytosis and long-term depression.[21] PSD-95^{GK} were repeatedly backcrossed onto a C57BL/6J background. Analysis of 150 single-nucleotide polymorphism markers at ~15–20 megabase intervals across all autosomal chromosomes confirmed 95% C57BL/6J congenicity (JRS Allele Typing Services, The Jackson Laboratory, Bar Harbor, ME, USA). To avoid potential phenotypic abnormalities resulting from genotypic differences in maternal behavior and early-life environment,[37] wild-type (WT) and PSD-95^{GK} were littermates generated from HET x HET matings. Mice were bred and maintained at the Jackson Laboratory and shipped to the NIH at 7–9 weeks of age.

Mice were housed with same-sex littermates in a temperature and humidity controlled vivarium under a 12-h light–dark cycle (lights on 0600 hours). Testing began at least 1 week after acclimation to the animal facility. Males and females were used. Test-naive mice were used in each experiment and experimenters remained blind to genotype during testing (subjects were identified by subcutaneously implanted microchips or ear notch). The number of mice tested is given in the figure legends. All procedures were approved by the NIAAA Animal Care and Use Committee and followed the NIH guidelines outlined in 'Using Animals in Intramural Research.'

General procedures for Pavlovian fear conditioning

Fear conditioning was conducted in a 27 × 27 × 11 cm chamber with transparent walls and a metal rod floor (Context A), as previously described.[38] To provide a distinctive olfactory cue, Context A was cleaned between subjects with a 79.5% water/19.5% ethanol/1% vanilla extract solution. After a 120- to180-s acclimation period, the mouse received three pairings (60- to 90-s inter-trial-interval) between a (30-s, 80 dB) white noise cue (conditioned stimulus, CS) and (0.6 mA scrambled) footshock (unconditioned stimulus, US) presented during the last 2 s of the tone. Mice remained in the chamber for 120 s after the final pairing.

Cued fear memory was tested in a Plexiglas cylinder with black/white-checkered walls and a solid-Plexiglas, opaque floor, cleaned between subjects with a 99% water/1% acetic acid solution and housed in a different room from training (Context B). After a 180-s acclimation period, the CS was presented continuously for either 180 s or via 3 × 30-s presentations (5-s inter-CS interval). Contextual fear memory was tested via exposure to Context A for 5 min. CS and US presentation was controlled by the Med Associates Freeze Monitor system (Med Associates Incorporated, Georgia, VT, USA). Freezing was defined as the absence of any visible movement except that required for respiration, and was scored at 5-s intervals by an observer blind to genotype. The number of observations scored as freezing were converted to a percentage ((number of freezing observations/total number of observations) × 100) for analysis.

Formation, extinction and precision of fear memory

Recent fear memory. Delay fear was tested using the same procedure described above. Cue fear retrieval was tested 1 day after conditioning, and context fear retrieval the following day (for schematic, see Figure 1a).

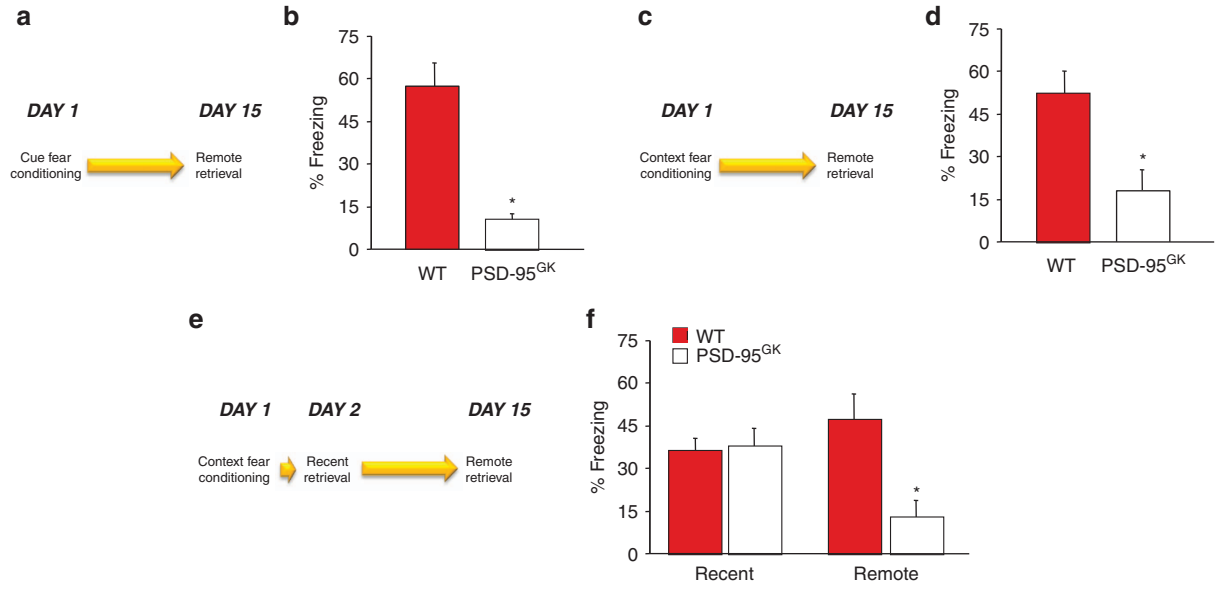

Figure 2. Postsynaptic density 95 (PSD-95) deletion impairs retrieval and stability of remote fear memories. (**a**) Schematic of experimental design for testing remote retrieval of a cued fear memory. (**b**) PSD-95^{GK} mice froze less than wild-type (WT) controls during remote fear memory retrieval ($n = 8$–9). (**c**) Schematic of experimental design for testing the remote retrieval of a contextual fear memory. (**d**) PSD-95^{GK} mice froze less than WT controls during remote fear memory retrieval ($n = 9$–10). (**e**) Schematic of experimental design for testing recent and remote retrieval of a contextual fear memory in the same mice. (**f**) PSD-95^{GK} mice froze less than WT controls during remote, but not recent, fear memory retrieval ($n = 8$). Data are means ± s.e.m. *$P < 0.05$ vs WT/same time point.

Trace fear was tested using the same procedure as for delay fear, except for the imposition of a 30-s no-stimulus interval between CS and US presentations during conditioning (interval based on previous studies[39–41]) (for schematic, see Figure 1c).

Fear extinction, retrieval and renewal. Conditioning involved the same delay fear procedure as above. The following day, extinction training was given via 50 × 30-s CS presentations (5-s inter-CS interval) in Context B after 180 s of context acclimation. The next day, extinction retrieval was tested in Context B via 3 × 30-s CS presentations (5-s inter-CS interval) after 180 s of context acclimation (for schematic, see Figure 1e). As extinction memories are context dependent, such that extinguished fear will renew when tested in the non-extinguished context,[42] fear renewal was also tested (~4 h after extinction retrieval), via (after 180 s of acclimation) 3 × 30-s CS presentations in Context A.

Precision of fear memory. Contextual fear memory discrimination was assessed using previously described procedures[43] (for schematic, see Figure 1g). Conditioning was conducted in Context A (specifications as above) via 3 x US presentations (60- to 90-s inter-US interval) after 180 s of context acclimation. The following day, fear was tested in Context A, Context B (specifications as above) and Context C *via* 120-s exposure to each context (~4-h interval between exposures, counter balancing for the order of context exposure). Context C had a metal grid floor and was the same size and shape as Context A, but had black/white-checkered walls and the same odor as Context B.

Stability of fear memories with time and reactivation

Remote fear memory. Remote cue fear was tested using the same delay fear procedure as above, with the exception that cue retrieval was tested 14 days after conditioning (and there was no contextual fear retrieval test) (for schematic, see Figure 2a). This remote interval was based on previous studies of remote fear memory in mice (for example, Frankland *et al.*[44]), and evidence of fear memory consolidation across brain systems within 1 week of fear acquisition.[45]

In a second experiment, remote contextual fear memory was tested (via 5-min exposure to Context A) 14 days after contextual conditioning (conducted in Context A via 3 x US presentations, 60- to 90-s inter-US interval, after 180 s of context acclimation) (for schematic, see Figure 2c).

A third experiment was conducted to provide a within-subjects comparison of fear retrieval on recent versus remote intervals. Following contextual conditioning, context fear was tested 1 day and again 14 days later using the same procedure as above, with the exception that the recent retrieval test was limited to 120 s to minimize extinction (for schematic, see Figure 2e).

Fear reactivation as a function of memory maturity. Mice were given cued fear conditioning and then fear memory was reactivated 1, 3 or 5 days later, in separate groups of mice, via 1 × 30-s CS presentation in Context B, after 180 s of context acclimation. Non-reactivated controls received Context B exposure for the equivalent duration (that is, 210 s). Cue retrieval was tested the following day via 3 × 30-s CS presentations (5-s inter-CS interval) after 180 s of context acclimation (for schematic, see Supplementary Figure S1a).

Fear-related medial prefrontal activation

Activation of cells positive for the plasticity-related IEG, Zif268, was examined in subregions of the prefrontal cortex (PFC) following memory retrieval, as previously described.[46,47] Cued fear conditioning and recent and remote retrieval was tested (for schematic, see Figure 3a). Two hours after retrieval, mice were killed via cervical dislocation and rapid decapitation, and brains flash frozen. Brains were sectioned in the coronal plane at 30 µm thickness on a cryostat (CM1850, Leica Microsystems, Buffalo Grove, IL, USA) and collected on gelatin-coated slides.

Sections were postfixed for 40 min in 4% paraformaldehyde and pre-incubated for 30 min in normal goat serum. Sections were then incubated with a rabbit anti-Zif268 polyclonal primary antibody (1:5000; sc-189; Santa Cruz Biotechnology, Dallas, TX, USA) and a biotinylated goat anti-rabbit secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA). An avidin–biotin–horseradish peroxidase procedure (Vectastatin ABC Kit, Vector Laboratories) with 3,3′-diaminobenzidine as a chromogen (DAB, Sigma, Munich, Germany) was used to visualize Zif268-positive cells. The anatomical localization of Zif268-positive cells was made with reference to a mouse stereotaxic atlas. Cells containing a nuclear brown-black reaction product were considered to be Zif268-positive cells and counted, bilaterally, in a representative tissue area of 0.01 mm^2 with the help of a light microscope (Olympus BX-40, Olympus, Center Valley, PA, USA) equipped with an ocular grid.

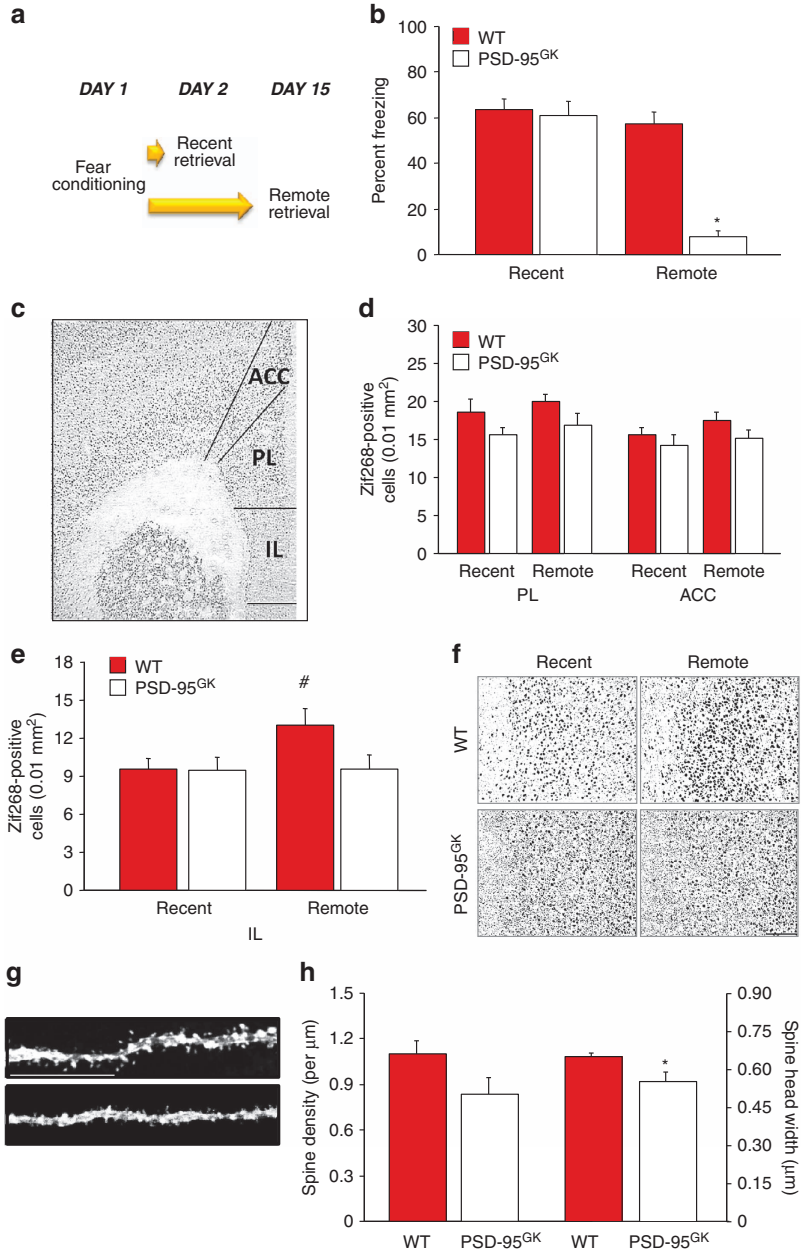

Figure 3. Postsynaptic density 95 (PSD-95) deletion disrupts activation of the infralimbic (IL) cortex during remote fear memory retrieval. (**a**) Schematic of experimental design for testing recent and remote retrieval of a cued fear memory. (**b**) PSD-95^{GK} mice froze less than wild-type (WT) controls during remote, but not recent, fear memory retrieval. (**c**) Representative Zif268-labeled coronal section showing the three prefrontal regions analyzed. (**d**) Immediate-early gene (IEG) analysis of prefrontal regions found no change in the number of Zif268-positive cells in either the anterior cingulate cortex (ACC, Cg1 subregion) or prelimbic (PL) cortex, irrespective of memory age or genotype. (**e**) WT mice exhibited an elevated number of Zif268-positive cells in the IL cortex after remote, as compared with recent retrieval, whereas the number of cells did not increase in the PSD-95^{GK} mice. (**f**) Representative coronal sections showing Zif268-positive cells in the IL (scale bar = 150 µm). (**g**) Example images of dendritic spines in IL pyramidal neurons from a PSD-95 knock-out (KO) (top) and WT controls (bottom) (scale bar = 15 µm). (**h**) PSD-95 KO mice had marginally lesser density and significantly lesser spine head width on IL pyramidal neurons, relative to WT controls ($n = 3$ mice per group, $n = 7$–12 cells per group). Data are means ± s.e.m. $^{*}P < 0.05$ vs WT remote or WT controls, $^{\#}P < 0.05$ vs WT recent.

Fear-related medial prefrontal neuronal recordings

Implantation of a microelectrode array (Innovative Neurophysiology, Durham, NC, USA) targeting the (right) medial prefrontal cortex (infralimbic (IL) cortex and rostral into medial orbital) was carried out in mice anesthetized with isoflurane and placed in a stereotaxic alignment system (Kopf Instruments, Tujunga, CA, USA). Each array contained 16×35-µm diameter tungsten microelectrodes arranged into two rows of eight (150-µm spacing between microelectrodes within a row, 200-µm spacing between rows). Rows were positioned lengthwise anterior to posterior (coordinates for center of array, relative to bregma: AP +1.75–1.80, ML +0.35–0.50, DV −2.90). Mice were given at least 1 week of recovery before testing.

Mice were given cued fear conditioning and a within-subjects design was used to give the same mice the recent and remote retrieval tests. In addition, to measure basal firing and single-unit responses to the CS before fear conditioning, mice were tested and recorded in a unique context 1 day before conditioning, using the same procedure as for conditioning,

but with no US presentation. Recordings were made using a Multichannel Acquisition Processor (Plexon Inc., Dallas, TX, USA) as previously described.[48–51] Extracellular waveforms exceeding a set voltage threshold were digitized at 40 kHz and stored in a personal computer. Waveforms were manually sorted using principal component analysis of spike clusters and visual inspection of waveforms and inter-spike intervals.

Neuronal activity was time stamped to CS onset, and spike and time stamp information integrated and analyzed using NeuroExplorer (NEX Technologies, Littleton, MA, USA). Activity 2 s after CS onset was Z-score normalized to activity during a 1-s pre-CS baseline and presented in 100-ms perievent histograms. CS-related single units were defined as a unit with an increase in firing during the first 100 ms of CS onset that was >3 s.d. from the unit's pre-CS baseline firing. CS-related burst firing was defined as three or more consecutive spikes with an inter spike interval of <25 ms between the first two spikes and <50 ms between subsequent spikes, and was expressed as the percentage of all CS-related firing.[48,51,52]

Local field potential (LFP) waveforms were sampled at 1 kHz, preamplified at 1000x and low-pass filtered at 250 Hz. The NeuroExplorer perievent spectrogram function was used to calculate power density values for each LFP, with PSD values expressed as power per frequency, binned into 0.02 Hz bins and smoothed using a postprocessing filter width of five bins to determine the average power density within the theta (4.5–8 Hz) and gamma (30–70 Hz) ranges. The NeuroExplorer perievent spectrogram function (waveforms high-pass filtered at 4 Hz, frequency binned into 0.30 Hz bins for theta activity and into 0.15 Hz bins for gamma activity) was used to plot representative spectrograms of the power density of LFP activity.

At the completion of testing, array placement was verified by passing 100 μA current through the electrodes for 20 s using a current stimulator (S48 Square Pulse Stimulator, Grass Technologies, West Warwick, RI, USA) to make electrolytic lesions. Brains were removed and 50 μm coronal sections cut with a vibratome (Classic 1000 model, Vibratome, Bannockburn, IL, USA) and stained with cresyl violet. Placements were estimated with the aid of an Olympus BX41 microscope (Olympus) and referenced to a mouse stereotaxic atlas (for placements, see Supplementary Figure S2a).

Medial prefrontal PSD-95 KD

Adeno-associated virus generation. The PSD-95 KD adeno-associated virus (pAAV-H1-sh95-CAG-GFP) was modified from the pAAV-CAG-GFP virus, which served as a control.[53] Briefly, the *Eco*RI site between enhanced green fluorescent protein and posttranscriptional regulatory element in pAAV-CAG-GFP was destroyed, and *Eco*RI and *Bst*BI sites were introduced upstream of the *Nde*I site (pAAV-EB-CAG-GFP). An H1 promoter-driven short hairpin RNA expression cassette targeting the mouse PSD-95 sequence (5'-TCACG ATCATCGCTCAGTATA-3')[15] was introduced at the *Eco*RI/*Bst*BI site. Recombinant adeno-associated viruses were purified using a 1-ml heparin column and concentrated using the Amicon 100 kDa cutoff columns.[54–56]

One Tx175 flask of 80–90% confluent HEK293 cells was transfected with the expression plasmid together with plasmids pDp1 (serotype 1) and pDp2 (serotype 2) at the amount of 20, 10 and 10 μg each. Sixty hours after transfection, HEK293 cells were washed once with phosphate-buffered saline (PBS), collected into 15-ml falcon tubes with 5-ml cold PBS supplemented with 5 mM EDTA, and pelleted by centrifugation at $800\times g$ for 5 min. The pellet was washed with PBS, and re-suspended in 0.75 ml of the lysis buffer (150 mM NaCl, 50 mM Tris; pH8.4). The lysate was frozen–thawed three times at −70 °C with dry ice/ethanol and 37 °C with water bath. Benzonase was supplemented to the lysate at a final concentration of 50 U ml^{-1}. The cell lysate was then incubated at 37 °C for 60 min and centrifuged at $3000\times g$ for 15 min at 4 °C. The supernatant was collected with a syringe and filtered through a 32 mm 0.45 μm Acrodisc syringe filter and loaded to a preequilibrated heparin column (preequilibrated with PBS-MK, PBS + 1 mM $MgCl_2$, 2.5 mM KCl, pH 7.2) at a rate of 1 ml per minute. The column was washed with PBS-MK, and the virus was eluted with five volumes of PBS-MK + 0.5 M NaCl. The virus solution was washed and concentrated with PBS at least twice in a 15 ml Amicon 100 kDa column with centrifugation of $5000\times g$ at 4 °C. The final viral suspension was aliquoted and stored at −80 °C. The genomic copy numbers of the two viruses were $\sim 1\times 10^9$ copies per ml, tested with quantitative PCR with green fluorescent protein (GFP) primers.

The efficacy of the virus was confirmed *in vitro* by quantifying PSD-95 protein loss using western blot, and by visualizing GFP expression in infected brain tissue, *ex vivo* (Figures 5a–c). For western blot, dissociated mouse cultures were prepared using standard methods.[57] Neurons were infected with recombinant adeno-associated viruses at DIV7 and cell lysates were collected at DIV17 for western blot analysis with PSD-95 (Thermo Fisher Scientific, Rockville, MD, USA, catalog #MA1-046) and β-actin (Sigma Aldrich, St Louis, MO, USA, catalog #A2228) antibodies.

Behavioral testing

For behavioral experiments, virus was infused into the brain via stereotaxic surgery (as described above for microelectrode implantation) using a Hamilton syringe with a 33 gauge needle (Hamilton, Reno, NV, USA). A total volume of 0.25 μmol of the virus was infused at a rate of 0.02 μmol per minute. Virus was bilaterally infused into either the IL (coordinates relative to bregma: AP +1.8, ML +0.25, DV −3.0) or the anterior cingulate cortex (ACC; coordinates relative to bregma: AP +2.1, ML +0.25, DV −2.0). The needle was left in place for 5 min to allow for diffusion and the scalp was cleaned and sutured. Mice were left undisturbed for 2 weeks to allow for virus expression. Mice were tested for cued fear conditioning and subsequent recent and remote retrieval (for schematic, see Figure 5d). In a separate experiment, mice were tested for fear extinction (for schematic, see Figure 5f).

At the completion of testing, mice were terminally overdosed with ketamine/xylazine and transcardially perfused with PBS, then 4% paraformaldehyde. After suspension in 4% paraformaldehyde overnight and then 4 °C 0.1 M phosphate buffer for 1–2 days, 50-μm coronal sections were cut with a vibratome (Classic 1000 model, Vibratome) and coverslipped with Vectashield HardSet mounting medium and DAPI (Vector Laboratories). Virus location was estimated by visualizing fluorescence with the aid of an Olympus BX41 microscope (Olympus) (for estimates, see Supplementary Figure S2a). Four out of 33 mice were discarded because of poor or misplaced virus expression.

Dendritic spine analysis

Virally infected dendritic segments on IL pyramidal neurons were identified under a 10X/1.2 NA objective (Zeiss Model LSM 700, Carl Zeiss Microscopy, Thornwood, NY, USA). Segments that met all of the following criteria were selected for analyses: (1) were >150 μm from the soma, (2) showed clear spine protrusions, (3) were not obscured by other dendrites and (4) were parallel or near parallel with the coronal plane of the section. All confocal stacks comprised ±5 μm above and below the extent of the dendritic segment. Segments were imaged under a 63X/1.4 NA oil-immersion objective, using voxel dimensions $0.1\times 0.1\times 0.1$ μm^3 and 2.5x zoom at a resolution of 512×512 pixels. Z series were obtained by imaging serial confocal planes at 0.20-μm intervals. Settings for pinhole size (1 airy disc) and gain (600) were optimized initially and remained constant throughout imaging to ensure images were digitized under consistent illumination. These parameters resulted in dendritic segments ~30–80 μm in length. Data were obtained from three to eight segments per neuron.

Spines were designated manually from digital confocal image stacks using NeuronStudio software.[58] Spine density was calculated as the number of spines divided by dendritic segment length. Spine head width was defined as the maximum diameter of the spine head. For analysis of PSD-95 KD in IL neurons, data were additionally segregated into narrow and wide spines using a median split based on head width. As in previous analyses,[59] spines >4.0 μm in length were not included in the analysis.

Statistical analysis

Effects of genotype x conditioned-to-retrieval interval, genotype x recording time bin and genotype x CS onset were analyzed via two-factor analysis of variance followed by Fisher's least significant difference *post hoc* tests. Effects of genotype on CS-related units (as a percentage of total recorded per genotype and task phase) were analyzed using the chi-square test. Effects of extinction test phase and context type were analyzed via repeated-measures analysis of variance followed by Fisher's least significant difference *post hoc* tests. The effect of genotype and viral KD on levels of freezing, the number of Zif268-labeled cells and spine density/morphology was analyzed using Student's *t*-tests. The threshold for statistical significance was set at $P<0.05$.

RESULTS

Formation and retrieval of recent fear memories

We began by testing the contribution of PSD-95 to fear memory formation and the expression of these memories within the first few days of learning. To this end, mice were first tested for the

ability to form fear memories for cues and contexts, using delay and trace versions of conditioning. Following multi-trial fear conditioning, freezing was no different between genotypes during preconditioning baseline or the final conditioning CS presentation (Supplementary Table S1). Genotypes also showed equivalent levels of freezing during cue retrieval, measured 1 day after multi-trial delay cued conditioning, and similar freezing during context retrieval, tested 2 days after conditioning (Figure 1b). To confirm that a subtle memory deficit in PSD-95^{GK} was not being masked by overtraining in the multi-trial procedure, a separate cohort of mice underwent one-trial conditioning using a single (0.8 mA) US, as previously described.[40] Genotypes did not differ in freezing during cue (WT = 79.6 ± 4.0, MUT = 70.8 ± 7.7, $n = 6 = 9$) or context (WT = 32.8 ± 10.3%, MUT = 22.5 ± 6.6, $n = 6–9$) retrieval following one-trial conditioning. Finally, in a cohort given trace fear conditioning in which there was a longer interval between cue and shock, genotypes did not differ in freezing during preconditioning baseline, but PSD-95^{GK} mice froze more than WT controls to the final CS presentation ($t(16) = 3.58$, $P < 0.01$) (Supplementary Table S1). Genotypes showed similar freezing during cue fear retrieval, but the PSD-95^{GK} mice froze less than WT controls during context retrieval ($t = 2.20$, df = 16, $P < 0.05$) (Figure 1d). Thus, PSD-95^{GK} mice failed to fully form an association with a fear-associated context when the context was experienced as a background stimulus to temporally discontiguous cued fear learning.

Overall, the results of these initial experiments show that loss of PSD-95 has minimal effects on the ability to form and express most recently acquired fear memories. However, impaired trace context fear in the PSD-95^{GK} mice hints at certain fear memory deficits in the mutants, potentially reflecting abnormalities when structures mediating trace fear, such as the hippocampus and medial prefrontal cortex,[60] are more strongly taxed.

Extinction and precision of recent fear memories

To further probe the nature of the fear memories formed in the absence of PSD-95, we examined fear extinction and memory precision. We first asked whether, once a fear memory has been generated, loss of PSD-95 affects the ability to extinguish it. During multi-trial cued fear conditioning, PSD-95^{GK} mice froze slightly more than WT controls during baseline ($t(26) = 2.81$, $P < 0.01$) and the final CS presentation ($t(26) = 2.52$, $P < 0.05$) (Supplementary Table S1). However, genotypes did not differ in freezing during recent fear memory retrieval, as indicated from freezing on the first trial-block of extinction training. By contrast, although WT controls decreased freezing from the first to last extinction trial-block, PSD-95^{GK} mice showed no reduction in freezing across trial blocks (genotype x trial block interaction: $F1, 26 = 12.76$, $P < 0.01$) (Figure 1f). Moreover, WT controls froze less during extinction retrieval, relative to the first trial block of extinction training, indicating successful extinction memory retrieval ($t(13) = 4.56$, $P < 0.01$), whereas PSD-95^{GK} mice did not show a reduction (Figure 1f). Finally, WT controls froze more during the fear renewal test as compared with extinction retrieval ($t(13) = 6.68$, $P < 0.01$), but PSD-95^{GK} mice did not (Figure 1f).

These findings indicate that recent fear memories formed in the absence of PSD-95 are highly resistant to extinction. In light of the recent finding that the cell surface expression of PSD-95 and AMPARs is decreased with extinction,[23] these data suggest that dynamic changes in PSD-95 may be a molecular signal for extinction. Another, not necessarily exclusive, possibility is that the PSD-95^{GK} mice failed to retrieve a sufficiently accurate representation of the original CS–shock association to permit effective reappraisal of the new, CS = no-shock, association during extinction training.

To examine the precision of fear memory in the PSD-95^{GK} mice, we assessed the retrieval of a contextual fear memory by conditioning mice to Context A and then testing for fear to either that context or two dissimilar contexts (B and C). WT controls froze more to Context A than Context B or C ($F2, 24 = 8.91$, $P < 0.01$). By contrast, PSD-95^{GK} mice froze at the same level to all three contexts (Figure 1h). This indicates a failure of discriminative contextual fear memory in the mutants. Whether discrimination of cued fear memories is also impaired currently remains unclear.

Thus, although PSD-95 is not necessary for the formation and recent retrieval of fear memories, the fear memories that are generated without PSD-95 are imprecise, as well as resistant to extinction. In other words, when PSD-95 is deleted, alternate mechanisms can support the formation of fear memories, but the resultant memories are rigid and qualitatively inferior to those produced in the presence of PSD-95.

Retrieval of remote fear memories

Our next set of experiments asked whether the type of fear memories formed in the absence of PSD-95 remained robust over time. In our first experiment, genotypes froze at similar levels during preconditioning baseline and, as in earlier experiments, PSD-95^{GK} mice froze more than WT controls to the final CS presentation of multi-trial cued fear conditioning ($t(15) = 2.82$, $P < 0.01$) (Supplementary Table S1). On a remote retrieval test 2 weeks after conditioning, PSD-95^{GK} mice showed markedly less freezing than WT controls ($t(15) = 5.19$, $P < 0.01$) (Figure 2b). This finding of reduced remote freezing was replicated in a second cohort of PSD-95^{GK} mice tested under the same conditions ($t(11) = 6.02$, $P < 0.01$, $n = 6–7$, data not shown).

Deficient retrieval of remote memories in the PSD-95^{GK} mice extended to contextual fear memory. Following multi-trial context conditioning, PSD-95^{GK} mice froze less than WT controls during a remote retrieval test 2 weeks after conditioning ($t(17) = 3.21$, $P < 0.01$) (Figure 2d). Another experiment using a within-subjects design demonstrated the time-dependent loss of memory in the same PSD-95^{GK} mice tested at both the recent and remote time points (genotype x day interaction: $F1, 14 = 10.21$, $P < 0.01$) (Figure 2f).

These observations confirm and extend earlier evidence that PSD-95 deficiency impairs remote conditioned place preference and contextual fear [33,34] by showing that PSD-95 is essential for the retention of fear memories at remote time points.

Stability of reactivated fear memories within 1 week of acquisition

If fear memories formed in the absence of PSD-95 are quicker to decay with time, they may also become increasingly fragile as they age. We tested for this by reactivating memories at progressively longer intervals (1, 3 or 5 days) after conditioning, in separate groups of mice, and then probing memory the following day (Supplementary Figure S1a). During multi-trial cued conditioning, genotypes froze at similar levels during baseline and PSD-95^{GK} mice froze more than WT controls to the final CS presentation ($t(92) = 2.38$, $P < 0.05$) (Supplementary Table S1). On the retrieval tests, freezing was affected by genotype and memory reactivation in a time-dependent manner (genotype x day interaction: $F2, 82 = 3.72$, $P < 0.05$). Specifically, genotypes froze at similar levels during retrieval 2 days after conditioning, irrespective of whether memory had been reactivated the day before. Conversely, PSD-95^{GK} mice froze less than WT controls when tested 4 days after conditioning, but only if memory had been reactivated the day before (Supplementary Figure S1b). Moreover, PSD-95^{GK} mice tested 6 days after conditioning froze less than WT controls regardless of prior reactivation, although freezing in the mutants was reduced to a greater extent if

memory had been reactivated the previous day (Supplementary Figure S1b).

These preliminary data reveal that by the end of the first week after acquisition, loss of PSD-95 renders fear memories vulnerable to disruption by reactivation. This effect is particularly striking given fear memories typically become more, not less, resistant to destabilization as they age.[61,62]

IL recruitment by remote fear memories

Our next objective was to localize the neural basis of the age-dependent fear memory impairment caused by loss of PSD-95. Earlier work has shown that retrieval of multiple forms of remote memory, including spatial learning, cocaine reinstatement, conditioned taste aversion, trace-eye blink conditioning and contextual fear, becomes dependent on sensory[63] and prefrontal cortical regions.[61,64–76] Protein synthesis in the PFC is also necessary for remote contextual fear memory reconsolidation.[61] Moreover, a phenotype of impaired remote fear memory retrieval (for example, in αCaMKII-deficient mice) analogous to that currently observed in the PSD-95^{GK} mice, is associated with deficient activation of areas of the PFC.[77] On the basis of these various observations, we examined recruitment of the PFC following retrieval of recent and remote fear memories by quantifying expression of the plasticity-related IEG, Zif268, in the IL, prelimbic (PL) cortex and Cg1 subregion of the ACC.[77]

Genotypes froze at similar levels during preconditioning baseline and PSD-95^{GK} mice froze more than WT controls to the final CS presentation ($t(34) = 2.32$, $P < 0.05$) (Supplementary Table S1). As expected, PSD-95^{GK} mice froze less than WT controls during remote, but not recent, cue fear retrieval (genotype x day interaction: $F1, 32 = 25.18$, $P < 0.01$) (Figure 3b). IEG analysis of prefrontal regions (Figure 3c) found no change in the number of Zif268-positive cells in either the PL or ACC (Figure 3d), irrespective of memory age or genotype. However, WT mice exhibited an elevated number of Zif268-positive cells in the IL after remote, as compared with recent retrieval ($t(14) = 2.30$, $P < 0.05$), whereas the number of cells did not increase in the PSD-95^{GK} mice (Figures 3e and f). Thus, consistent with a model whereby cortical regions become critical to the expression of fear memories as they age,[64,78] these data identify a failure of IL recruitment as one possible neural locus for the impaired remote fear memory in the PSD-95^{GK} mice. The IL is particularly well positioned to mediate fear via rich, PSD-95-positive projections to the amygdala[79] although interestingly, the IL-specific involvement found here reflects a more restricted pattern of PFC activation with remote fear retrieval of a discrete cue than that typically seen with remote fear retrieval of contextual fear, which encompasses IL, PL and ACC.[77]

Dendritic spines are posited to be physical sites of memory storage and the enlargement of spines is coupled to the functional potentiation of synapses.[80] In turn, spine morphology is tightly regulated by PSD-95; for example, PSD-95 increases and persists in enlarged spines and those spines that are rich in PSD-95 are highly stable over time, whereas KD of the GK domain of PSD-95 disrupts spine growth.[81–83] Moreover, previous studies have shown that successful remote context fear memory associates with, and may require, spine growth in subregions of the PFC (in this case, the ACC).[84,85] We therefore asked whether memory-associated IL hypofunction was associated with dendritic spine abnormalities by quantifying the spine density and morphology of GFP-labeled IL pyramidal neuronal dendrites in behaviorally naive PSD-95^{GK} mice and WT controls (Figure 3g). This analysis found that PSD-95^{GK} mice had marginally lower IL dendritic spine density ($t(17 = 1.88$, $P < 0.077$) and significantly narrower head width ($t(17 = 2.99$, $P < 0.01$), as compared with WT controls (Figure 3h). These data reveal a spine morphology correlate of the fear memories caused by loss of PSD-95. The absence of PSD-95 in the IL may disrupt the spine remodeling and associated synaptic strengthening necessary to support the maintenance of a remote memory and other IL-dependent behaviors, including extinction memory formation.

IL encoding of recent and remote fear memories

Although our *ex vivo* IEG and spine morphology results implicate the IL in deficient remote fear retrieval in the PSD-95^{GK} mice, these approaches have limited scope to establish a close functional and temporal link between IL neuronal activity and memory retrieval. Therefore, we next performed *in vivo* recordings of CS-evoked IL single-unit firing as mice retrieved recent and remote fear memories.

Genotypes froze at similar levels during preconditioning baseline and PSD-95^{GK} mice froze more than WT controls to the final CS presentation ($t(10) = 2.24$, $P < 0.05$) (Supplementary Table S1). PSD-95^{GK} mice also froze more than WT controls during recent retrieval in this experiment (WT = 59 ± 2%, PSD-95^{GK} = 72 ± 5, $t(10) = 2.36$, $P < 0.05$), possibly due to sensitivity to the more stressful recording test conditions. Nonetheless, PSD-95^{GK} mice showed lower freezing than WT controls during the remote test (WT = 64 ± 4%, PSD-95^{GK} = 43 ± 4, $t(10) = 3.66$, $P < 0.01$). Our recording data showed that genotypes did not differ in baseline unit firing (that is, pre-CS data, averaged across test phases) (Supplementary Figure S2b). In addition, recordings made during preconditioning showed that the CS produced a slight but significant increase in Z-scored unit firing (time: $F29, 4350 = 2.93$, $P < 0.01$) that did not differ between genotypes (Supplementary Figure S2c).

Following conditioning, unit firing changed after CS onset in a genotype-dependent manner during recent (time x genotype interaction: $F29, 4466 = 1.84$, $P = 0.05$) and remote (time x genotype interaction: $F29, 4650 = 1.45$, $P = 0.055$) retrieval (Figure 4a). Specifically, IL neurons increased firing during the 100-ms post-CS interval in WT controls, but not PSD-95^{GK} mice, on both retrieval tests (Figure 4a, insets). Further confirming genotype differences in CS-related IL firing, the number of 'phasic IL units' that fired to the CS at >3 s.d. over pre-CS baseline were higher in WT mice than PSD-95^{GK} mice during recent ($\chi(1) = 9.30$, $P < 0.01$) and remote ($\chi(1) = 14.89$, $P < 0.01$) retrieval (Figure 4b). In addition, correlating firing and behavior during remote retrieval revealed that high IL unit firing was associated with higher freezing ($r = +0.67$, $P < 0.05$) (Figure 4c). Finally, we found that the bursting of IL unit firing was increased over pre-CS baseline in both genotypes during recent retrieval (CS: $F1, 156 = 20.94$, $P < 0.01$), but was increased in WT mice and not in PSD-95^{GK} mice during remote retrieval (CS x genotype interaction: $F1, 163 = 5.88$, $P < 0.05$) (Figure 4d). Attenuated IL burst firing in the mutants is consistent with earlier work showing that low bursting predicts deficient retrieval of other IL-dependent memory fear memories (for example, extinction).[48,52,86,87]

In addition to these marked differences in single-unit activity, analysis of LFPs revealed lower gamma (genotype: $F1, 20 = 81.96$, $P < 0.01$) and theta ($F1, 20 = 17.74$, $P < 0.01$, test: $F1, 20 = 7.45$, $P < 0.05$) oscillations in the PSD-95^{GK} mice relative to WT controls, during recent and remote retrieval (Figures 4e and f). The loss of gamma and theta power in the mutant IL is generally consistent with prior studies showing that low power at these frequencies predicts loss of memory retrieval. For example, the strength of gamma and theta oscillations in the human frontal cortex during learning correlates with the strength of subsequent episodic memory retrieval.[88] Similarly, increased gamma power in the rodent auditory cortex, PL or basolateral amygdala is associated with relatively strong fear learning and the maintenance of high fear after extinction.[51,89,90] Low, extinction-induced, fear also correlates with reduced theta oscillations between the mPFC and basolateral

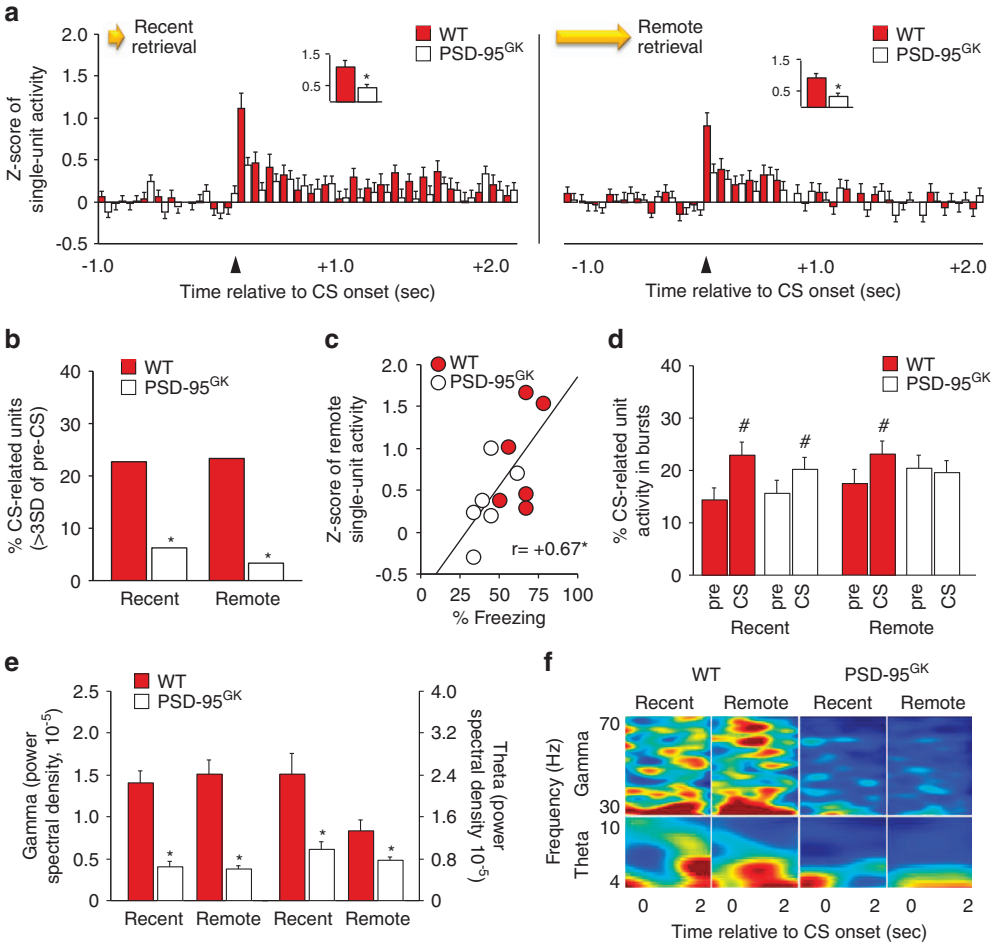

Figure 4. Postsynaptic density 95 (PSD-95) deletion disrupts conditioned stimulus (CS)-related single-unit activity in the infralimbic (IL) cortex during fear memory retrieval. (**a**) PSD-95^{GK} mice showed less CS-related IL single-unit activity than wild-type (WT) controls during recent (left panel) and remote (right panel) fear memory retrieval. Insets show first 100-ms timebins. (**b**) PSD-95^{GK} mice had fewer phasic CS-related IL single units than WT controls during recent and remote retrieval. (**c**) IL single-unit activity correlated positively with freezing during remote retrieval. (**d**) IL single-unit burst activity increased during the CS in both genotypes during recent retrieval, but only in WT mice during remote retrieval. (**e**) PSD-95^{GK} mice showed lower gamma and theta oscillations than WT controls, on recent and remote retrievals. (**f**) Representative perievent spectrograms showing the gamma and theta frequency spectra around CS onset. For gamma panels, darkest blue = 0 mV^2*s and darkest red = $2e^{-5}$. For theta panels, darkest blue = 0 and darkest red = $4e^{-4}$ mV^2*s. Data are means ± s.e.m. $n = 6$ mice per genotype, $n = 74–87$ single units per genotype. *$P < 0.05$ vs WT/same time point, #$P < 0.05$ vs pre-CS/same genotype.

amygdala,[91] although a weak or inverse relationship between mPFC (primarily PL) theta power and freezing is seen in some studies.[51,92]

Together, these electrophysiological findings provide compelling, *in vivo* evidence of deficient IL recruitment during fear memory retrieval in the PSD-95^{GK} mice. Notably, the loss of IL neuronal firing and LFP power was evident during the recent and remote memory tests although only remote retrieval was impaired. Indeed, WT controls showed strong firing during both tests despite our IEG data showing greater region-wide IL recruitment with remote retrieval. Together, these data suggest that while lesion studies show PFC regions are dispensable for recent memory retrieval,[64] the retrieval of recent cued memories does actively engage IL neurons. This is consistent with models in which PFC neurons are recruited early and 'tagged' during memory formation, but only acquire a demonstrable functional role with time, as memories are replayed and cortico-hippocampal networks are reorganized.[78,93] Of note, this early tagging process is known to be dependent on PSD-95-related mechanisms including AMPAR and N-methyl-D-aspartate receptor signaling.[93] These data raise the possibility that impaired memory persistence caused by loss of PSD-95 may partly originate in an inability to effectively tag fear memories in the IL during formation.

IL PSD-95 mediation of recent fear extinction and remote fear memory

Our findings thus far provide convergent, correlative evidence that loss of PSD-95 in the IL destabilizes remote cued fear memories, but do not constitute causal support for this. Therefore, our final step was to test whether PSD-95 loss specifically within the IL affected fear memory retrieval by engineering a PSD-95 KD virus and infusing it into the IL of C57BL/6J mice (Figures 5a and b).

We found that in mice infused 2 weeks before multi-trial cued conditioning (Figure 5c), freezing did not differ between PSD-95 KD mice and GFP virus controls during preconditioning baseline or the final CS presentation (Supplementary Table S1). The groups also showed similar freezing during recent fear memory retrieval. By contrast, PSD-95 KD mice froze less than GFP controls during remote fear memory retrieval ($t(27) = 2.22$, $P < 0.05$) (Figure 5d). In

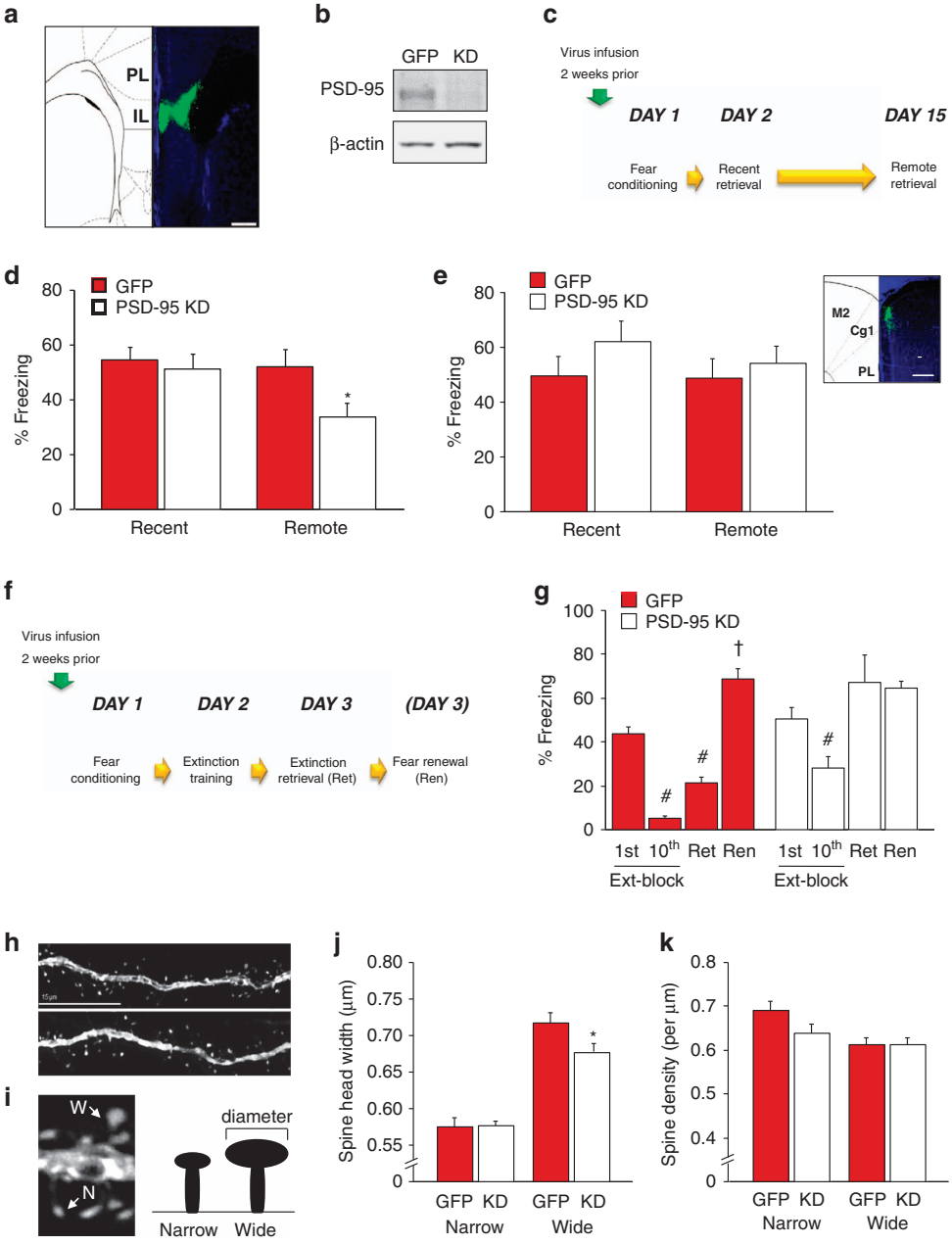

Figure 5. Postsynaptic density 95 (PSD-95) knockdown (KD) in infralimbic (IL) cortex impairs remote fear memory retrieval. (**a**) Example of green fluorescent protein (GFP) localization of the PSD-95 KD adeno-associated virus in the IL (scale bar = 200 μm). (**b**) Example western blots showing viral-induced KD of PSD-95 protein. (**c**) Schematic of experimental design for testing recent and remote retrieval of a cued fear memory. (**d**) IL PSD-95 KD mice showed less freezing than GFP virus controls during remote, but not recent, retrieval ($n = 12–17$). (**e**) Anterior cingulate cortex (ACC) PSD-95 KD mice showed similar freezing levels to GFP controls during recent and remote retrieval ($n = 9–11$). Inset: example of GFP localization of the PSD-95 KD adeno-associated virus in the Cg1 region of the ACC (scale bar = 200 μm). (**f**) Schematic of experimental design for testing fear extinction. (**g**) IL PSD-95 KD mice decreased freezing from the first to the last extinction trial block, but less than GFP virus controls. GFP virus controls, but not IL PSD-95 KD mice, froze less during extinction retrieval as compared with the first trial block of extinction training ($n = 7–9$). (**h**) Example images of dendritic spines in IL pyramidal neurons from a PSD-95 KD (top) and GFP control (bottom) (scale bar = 15 μm). (**i**) Examples and cartoon showing examples of narrow (N) and wide (W) sub-populations of dendritic spines. (**j** , **k**) PSD-95 KD decreased the spine head width, but not density, of relatively wide spines on IL pyramidal neurons, relative to GFP controls ($n = 7$ mice per group, $n = 15–23$ cells per group). Data are means ± s.e.m. $^{*}P < 0.05$ vs GFP/same time point or spine sub-population; $^{\#}P < 0.05$ vs 1st Ext-block in same group; $^{\dagger}P < 0.01$ vs Ret/GFP.

a separate cohort of mice tested for fear extinction after multi-trial cued conditioning, groups did not differ in freezing during baseline or the final CS presentation of conditioning (Supplementary Table S1), or the first trial block of extinction training (Figure 5g). However, PSD-95 KD mice showed a significant, but lesser reduction in freezing across trial-blocks than GFP virus controls (virus: $F1, 14 = 15.80$, $P < 0.01$; trial-block: $F1, 14 = 63.98$, $P < 0.01$; genotype x trial-block interaction: $F1, 14 = 4.87$, $P = 0.053$) (Figure 5g). In addition, although GFP virus controls froze less during extinction retrieval than in the first trial block of extinction training ($t(8) = 5.82$, $P < 0.01$), PSD-95 KD mice failed to reduce freezing (Figure 5g). GFP virus controls froze more during the fear

renewal test as compared with extinction retrieval ($t(8) = 9.81$, $P < 0.01$) (Figure 5g). These data confirm that loss of PSD-95 within the IL is sufficient to impair retrieval of remote but not recent fear memories, as well as recent fear extinction, and thereby establish a causal link between IL-PSD-95 and these behaviors.

We also found that, analogous to our findings in PSD-95^{GK} mice, the behavioral impairments in IL PSD-95 KD mice were associated with abnormal morphology of dendritic spines in virally infected IL pyramidal neurons (Figure 5h). Overall spine density and head width was not significantly affected by PSD-95 KD, indicating a more subtle effect of the viral KD than the constitutive gene mutation—which caused an overall reduction in spine head width in the IL. However, a median split into relatively wide and narrow spines (Figure 5i), revealed that the head width of wider spines was significantly less in PSD-95 KD mice, relative to GFP controls ($t(33) = 2.23$, $P < 0.05$) (Figure 5j). Consistent with a selective narrowing of head size rather than an overall loss of wide spines, the density of wide or narrow spines was not different between groups (Figure 5k). These data provide further evidence of a functional link between PSD-95 regulation of IL dendritic spine morphology and IL-mediated extinction and remote cued memory.

Although the current findings clearly implicate PSD-95 in IL neurons in the behaviors found to be disrupted by loss of PSD-95, they do not fully exclude a role for PSD-95 in other brain regions. Of note, the dorsal hippocampus, basolateral amygdala and ACC are all known to be important for remote context fear under certain conditions.[77,94,95] In this context, the magnitude of recent extinction and remote memory impairments produced by IL KD was less than that caused by the constitutive loss-of-function in PSD-95^{GK} mice. This could either reflect incomplete viral-induced deletion of PSD-95 in IL, or the extant capacity for PSD-95 in other regions, such as the ACC and hippocampus, to partially support behavior. We did find, however, that when the PSD-95 KD virus was infused into the ACC before conditioning, freezing was unaltered on the remote (or recent) test (Figure 5e). Therefore, in agreement with our negative ACC IEG data, this region, unlike the IL, does not appear to be critical to the retrieval of remote cued fear memories. Notwithstanding, it would be premature to rule out a contribution of PSD-95 in other brain regions to this behavior, and further work will be needed to explore this more comprehensively.

DISCUSSION

Despite its known role as a key postsynaptic molecule, essential to various forms of learning, this study found that various types of Pavlovian fear memory could be acquired and expressed in the absence of functional PSD-95. However, the fear memories that were formed were imprecise and inflexible at recent time points and highly unstable at more remote time points. Loss of remote fear memory in PSD-95^{GK} mice was associated with decreased IL recruitment, as well as reduced IL neuronal firing and bursting. Establishing a causal relationship between IL PSD-95 and fear durability, virus-mediated KD of PSD-95 in the IL was sufficient to alter IL dendritic spine morphology and disrupt recent fear extinction and remote fear memory. Collectively, these data reveal PSD-95 expressed on neurons in the IL as a critical mechanism underlying the maintenance of fear memories over time. These findings further our understanding of the neural and molecular basis of disorders in which fear memories are overly persistent.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)