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A Subpopulation of Prefrontal Cortical Neurons Is Required for Social Memory

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ABSTRACT

BACKGROUND: The medial prefrontal cortex (mPFC) is essential for social behaviors, yet whether and how it encodes social memory remains unclear.

METHODS: We combined whole-cell patch recording, morphological analysis, optogenetic/chemogenetic manipulation, and the TRAP (targeted recombination in active populations) transgenic mouse tool to study the social-associated neural populations in the mPFC.

RESULTS: Fos-TRAPed prefrontal social-associated neurons are excitatory pyramidal neurons with relatively small soma sizes and thin-tufted apical dendrite. These cells exhibit intrinsic firing features of dopamine D_1 receptor–like neurons, show persisting firing pattern after social investigation, and project dense axons to nucleus accumbens. In behaving TRAP mice, selective inhibition of prefrontal social-associated neurons does not affect social investigation but does impair subsequent social recognition, whereas optogenetic reactivation of their projections to the nucleus accumbens enables recall of a previously encountered but "forgotten" mouse. Moreover, chemogenetic activation of mPFC-to-nucleus accumbens projections ameliorates MK-801–induced social memory impairments.

CONCLUSIONS: Our results characterize the electrophysiological and morphological features of social-associated neurons in the mPFC and indicate that these Fos-labeled, social-activated prefrontal neurons are necessary and sufficient for social memory.

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Social memory is a fundamental social cognition process that is impaired in schizophrenia and autism (1,2). Previous studies have shown that the ventral CA1 and dorsal CA2 regions in the hippocampus are essential for social memory (3–5). However, it appears that the medial prefrontal cortex (mPFC) is also involved in social memory (6–8).

The mPFC is a key regulator in social cognition (2,7), optimizing social responses via its connectivity with diverse subcortical structures (9). It has been proposed that the mPFC plays a unique role in the encoding and retrieval of memories (10–12), but how a short-term memory including social memory might be maintained either by persistent activity in a subset of neurons or by dynamic (time-varying) activity across a neural population remains a debate (13–15). Accumulating evidence suggests that prefrontal engram cells (such as those labeled by immediate-early gene c-Fos) are produced at the onset of learning, gradually mature over time, and then become crucial for expression of memory (16,17). Indeed, immediate-early gene c-Fos expression correlates with social behaviors (18,19). However, these findings do not directly support whether prefrontal activation is causal for social behaviors. Surprisingly, nonspecific inhibition of the mPFC does not affect social investigation (20,21). Thus, the specific role of these Fos-labeled neurons in mPFC is not clear. A strong relationship between prefrontal c-Fos expression levels and fear memory engrams was demonstrated (16,17). Fos expression is often recognized as memory engram cell marker, and reactivated c-Fos–tagged neurons enable retrieval of stored memory information (22–25). Therefore, we hypothesize that c-Fos–expressing prefrontal neurons during social investigation serve a mnemonic function.

Here, we report the discovery of a subpopulation of thin-tufted D_1 receptor (D1R)–expressing and nucleus accumbens (NAc)–projecting pyramidal neurons in the mouse suggesting that the mPFC that is preferentially involved in social memory. Using a novel transgenic mouse tool, TRAP (targeted recombination in active populations) (26), in combination with physiological and morphological analysis, and optogenetic/chemogenetic manipulation, we found that the activity of these Fos-labeled, social-activated prefrontal neurons is necessary and sufficient for social memory encoding.

METHODS AND MATERIALS

Detailed methods are described in Supplement 1. Single-housed male mice were used (Discussion in Supplement 1). FosCreER mice with expression of Cre-dependent AAV (adeno-associated virus) constructs (opsin or DREADDs

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[designer receptors exclusively activated by designer drugs]) or FosTRAP (FosCreER;Ai14) mice were given 4-OHT (4-hydroxytamoxifen) (50 mg/kg intraperitoneal) 90 minutes after social exposure to TRAP social investigation–associated neurons (SINs). The social discrimination test (SDT) and three-chamber test were used to assess social preference and social memory. For chemogenetics, mice received a 5-mg/kg intraperitoneal injection of clozapine-N-oxide (CNO) 30 minutes before the tests. For optogenetic inhibition of SINs, 470-nm continuous light pulses were applied. For patch-clamp recordings, fluorescently labeled neurons were identified and recorded. After ex vivo recordings, the brain slices were fixed in 4% paraformaldehyde, then processed for morphological analysis with Neurolucida 9 (MBF Bioscience, Williston, VT). Immunofluorescence was performed as described previously (27).

RESULTS

A Subpopulation of Prefrontal Neurons Is Activated During Social Investigation

The mPFC has been implicated in various social behaviors in both humans (28,29) and rodents (21,30). We first characterized the prefrontal cortical neurons in young adult mice (8–12 weeks) during a simple social investigation task for 5 minutes in their home cages (Figure S1A in Supplement 1). We found that c-Fos was significantly increased in the prelimbic (PL) region after social investigation of a novel conspecific (Figure S1B, C in Supplement 1). Immunostaining revealed that 99% of activated cells were NeuN-positive cells (Figure S1D, E in Supplement 1), and 94% were colocalized with excitatory cell maker CaMKIIα (calcium/calmodulin-dependent protein kinase IIα) (Figure S1D, E in Supplement 1) (31). We also found little colocalization of GABAergic (gamma aminobutyric acid-ergic) interneuron subtypes with c-Fos–labeled neurons (Figure S2 in Supplement 1, Detailed Results 1 in Supplement 1). These results suggested that social exposure induces a robust activation of glutamatergic pyramidal neurons in the mPFC.

Inhibition of SINs in the mPFC Impairs Social Memory

We repeated the social investigation task with FosTRAP (FosCreER; Ai14) mice, which captured transiently active neurons with Cre-dependent expression of tdTomato by specific stimuli via administrating tamoxifen (26). The SINs in the FosTRAP mice were captured by administering the 4-OHT 90 minutes after social exposure in their home cage (Figure 1A). As 4-OHT is effective for about 6 hours, the animals were left in the cage undisturbed until the TRAPing window closed. This enabled permanent and stable tdTomato expression after 7 days (26). We found that compared with 4-OHT–treated home cage control animals that received no social exposure, social investigation activity resulted in robust labeling of cells in deep layers of the mPFC (Figure 1B, C), consistent with a previous report (26). The lack of labeling in home cage control animals suggests that the labeled cells are specific to activity induced by social exposure. Like their wild-type littermates, the FosTRAP mice showed normal behavior in the social investigation that occupied ∼50% of the total observation period (Figure 1D), implicating no influence of CreER transgene on social investigation behaviors. In contrast, without 4-OHT injection, few labeled cells were detected in the mPFC, indicating the specific labeling by 4-OHT pairing (Figure S3 in Supplement 1).

To further examine the roles of these TRAPed mPFC SINs in social behaviors, we injected AAV1-containing Cre-dependent novel soma-targeted *Guillardia theta* anion-conducting channelrhodopsins (stGtACR2) into the PL region of the FosCreER mice (32). This allowed us to inhibit the cell bodies of SINs optically and selectively in the PL region without interfering with the downstream axonal terminal regions of these neurons. After 1-week recovery from surgery, the mice were given a 4-OHT injection 90 minutes after exposure to a conspecific to TRAP SINs with stGtACR2 (Figure 1E). A subpopulation of social stimuli–responding PL neurons expressed stGtACR2-FusionRed (Figure 1F). Illumination of the PL region in slices with blue light (470 nm) resulted in remarked inhibition of action potential generation in these stGtACR2-expressing neurons (Figure 1G), as previously reported (32).

To determine whether the TRAPed SINs are functionally necessary for social-related behaviors, we tested whether inhibition of these neurons influenced social behaviors in two tasks. The nature of rodents determines that they prefer interacting with novel conspecifics, rather than familiar ones (Detailed Results 2 in Supplement 1). In the SDT, the subject mouse was exposed to a novel, juvenile stimulus mouse for 5 minutes on trial 1; after 1 hour, it was re-exposed to either the same (familiar) mouse or a novel stimulus mouse for 5 minutes on trial 2 (Figure 1H). Surprisingly, we found that optical inhibition of SIN cell bodies in the PL region did not affect social investigation time in trial 1 (Figure 1I), suggesting an intact social motivation. In contrast, in trial 2, continuous inhibition of SINs impaired social discrimination of familiar versus novel social stimuli. This discrimination requires an intact social memory, the ability to recognize a previously encountered familiar mouse. These data indicate that TRAPed SINs are necessary to support social memory but not social motivation.

To confirm these results, we manipulated TRAPed SINs during the three-chamber test (Figure 1J). We found that optical inhibition of SINs did not change social preference (Figure 1K, M) but impaired the preference for a novel mouse (Figure 1L, N), consistent with the social memory deficits observed in the SDT. We also confirmed that the deficits were not attributable to alterations in general recognition/novelty, as optically silencing SINs did not affect performance in a novel object recognition test (Figure S4 in Supplement 1). These data suggest that mPFC SINs do not regulate novelty in general, demonstrating a specific role for SINs in regulating social novelty specifically. A further question raised by these findings is whether these SINs represent a specific subpopulation of neurons in the mPFC.

Morphological and Electrophysiological Characterization of SINs in the mPFC

We then characterized the properties of SINs in layer 5 (L5) of the PL region by investigating the soma and proximal dendrite morphology. L5 pyramidal neurons in the mPFC are largely segregated into dopamine D1R–expressing (D1R+) and D_2 receptor–expressing (D2R+) neurons morphologically and physiologically (33–35). The L5 pyramidal neurons from

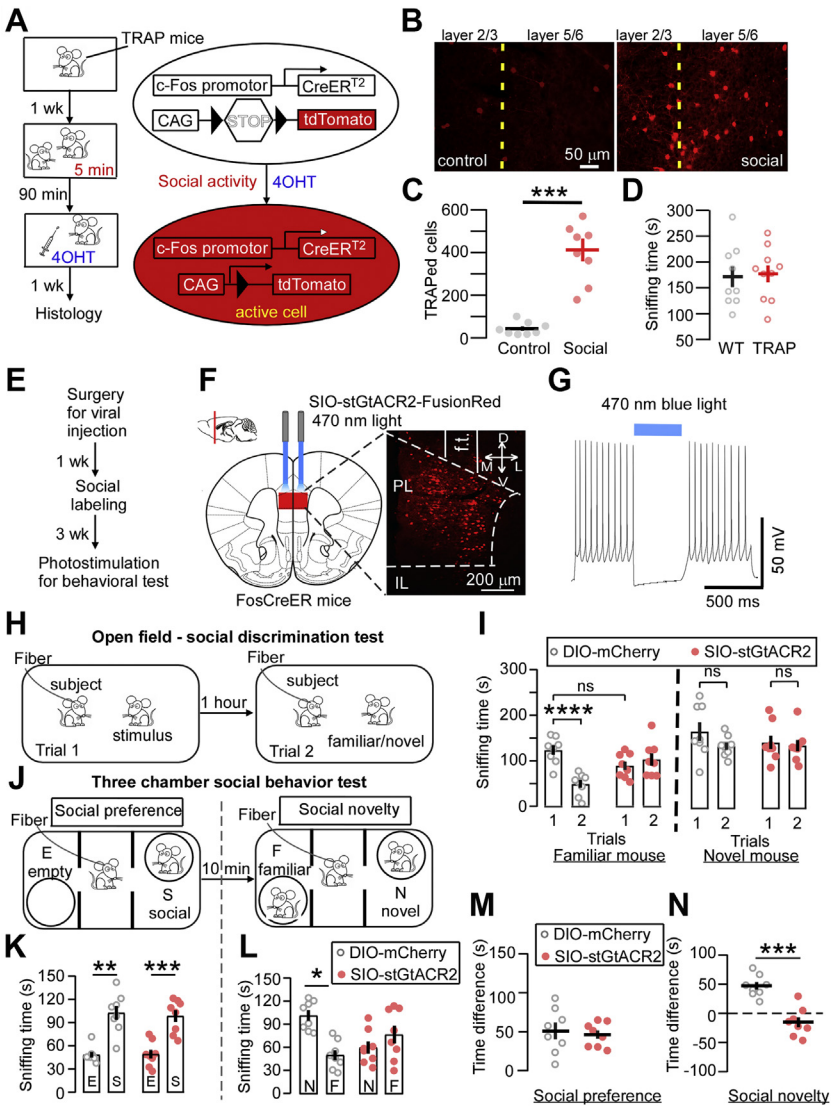

Figure 1. FosTRAP captures SINs that are implicated in social memory. **(A)** Strategy for labeling SINs in FosTRAP mice (left) and schematic of TRAPing of SINs by pairing social exposure with 4-OHT, resulting in permanent tdTomato expression (right). **(B)** Representative microscopic images of the PL region from social-exposed or home cage control TRAP mice, **(C)** showing increased TRAPed cells in a social group ($p < .0001$). **(D)** Quantification of social investigation behaviors of TRAP mice and their littermate WT control animals. **(E)** Experimental timeline for optogenetic inhibition of SINs. **(F)** Schematic showing AAV1-SIO-stGtACR2-FusionRed injection and representative confocal image of SIO-stGtACR2-FusionRed expression in the PL region. Scale bar = 200 μm. **(G)** Representative whole-cell current-clamp recording of a stGtACR2-expressing neuron silenced by blue light application (473 nm, 400 ms, 2 mW). **(H)** Schematic illustrating the 2-trial open-field social discrimination test with optical stimulation (continuous illumination, 6–8 mW at the fiber tip). **(I)** Total sniffing time by the resident toward intruder in familiar-intruder group (left) and novel-intruder group (right) (n = 8 mice, each group) in the social discrimination test. Mice with SIN inhibition showed impaired social memory. **(J)** Schematic illustrating the three-chamber social preference test (left) and social novelty test (right) with optical stimulation (continuous illumination, 6–8 mW at the fiber tip). Social preference levels based on **(K)** total sniffing time (left) and **(M)** time difference (sniffing the mouse enclosure minus sniffing the empty enclosure) in the social preference of three-chamber test. Mice with SIN inhibition showed normal sociability (n = 8 mice, each group). Social discrimination levels based on **(L)** total sniffing time (left) and **(N)** time difference (sniffing the familiar mouse enclosure minus sniffing the novel mouse enclosure) in the social novelty of three-chamber test. Mice with SIN inhibition showed impaired social novelty discrimination. Significance for multiple comparisons: **(C)** Mann-Whitney U test, **(D, M, N)** unpaired t test; **(I, L)** 2-way analysis of variance, post hoc, Bonferroni; **(K)** Kruskal-Wallis test, post hoc, Dunn. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < .0001$. Data were expressed as mean ± SEM. D, dorsal; E, empty; F, familiar; f.t., fiber tip; IL, infralimbic; L, lateral; M, medial; N, novel; PL, prelimbic; S, social; SIN, social investigation–associated neuron; TRAP, targeted recombination in active populations; V, ventral; WT, wild-type.

age-matched transgenic mice expressing tdTomato under D1R promoter (D1-Tom) or D2R promoter (D2-Tom) were used as comparison groups (Figure 2A), and SINs were TRAPed as described above. The somas of SINs were significantly smaller than D2-Tom neurons and slightly larger than D1-Tom neurons (Figure 2B; Detailed Results 3 in Supplement 1). The apical dendrite diameters of SINs were also significantly smaller than D2-Tom neurons but comparable to the D1-Tom neurons (Figure 2C).

Using ex vivo patch-clamp recording, in agreement with previous studies (34,35), we found that adult D1-Tom and D2-Tom neurons exhibited several distinct electrophysiological characteristics (Table S1 in Supplement 1). Notably, both SINs and D1R+ cells showed lower I_h (the hyperpolarization-activated nonspecific cation current) and little rebound depolarization versus D2R+ cells in L5 (Figure 2D–F, Table S1 in Supplement 1). Consistently, social-exposed mice showed a significantly higher percentage of colocalization of c-Fos/D1R+ neurons versus D2R+ cells (Figure 2G–K). These results indicated that social experience preferentially recruited D1R+ cells in the mPFC. Furthermore, similar to inhibiting SINs, inhibition of D1R+ cells in the mPFC using inhibitory hM4Di-DREADDs impaired social novelty without affecting social preference, whereas silencing D2R+ cells affected neither social preference nor social novelty (Figure S5 in Supplement 1), consistent with previous studies (30,36). Our results suggest that mPFC SINs that are required for social memory may represent a subpopulation of D1R-expressing neurons.

SINs That Project to the NAc Show Increased Intrinsic Burst Firing Induced by D1R-NMDA Coactivation

In the frontal cortex, some pyramidal neurons exhibit burst firing initiated by prior learning experience (37), a potential

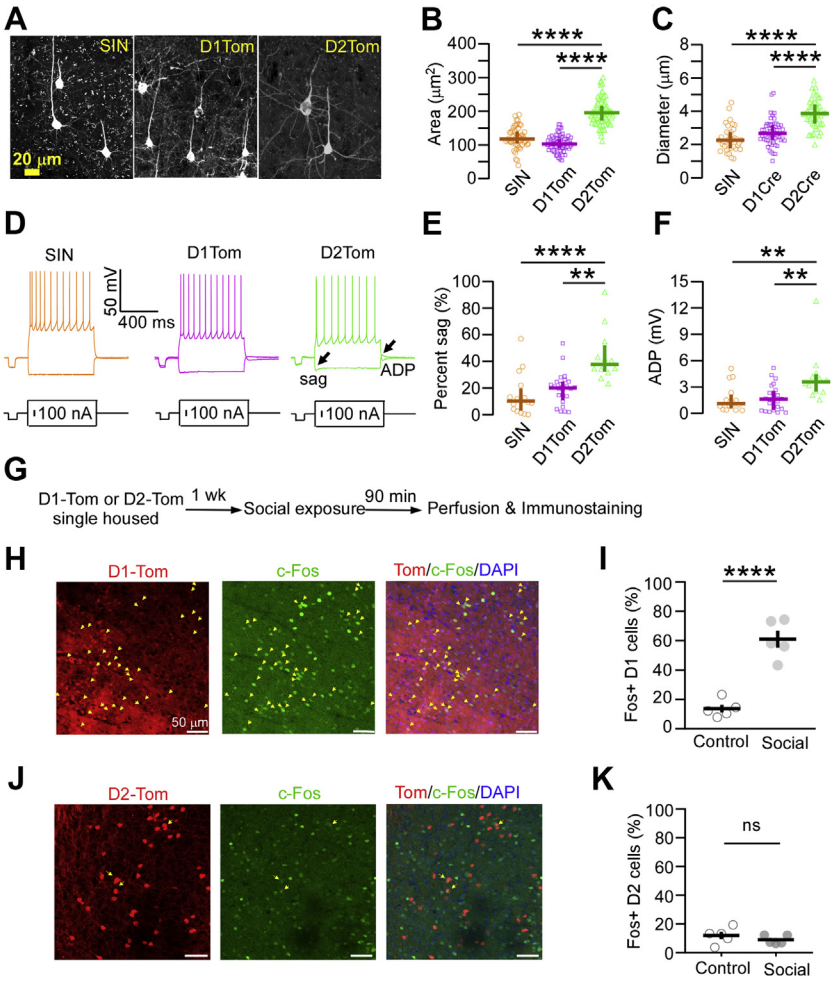

Figure 2. Morphological and electrophysiological properties of layer 5 SINs and characterization of PL neurons activated in social investigation. **(A)** Representative confocal images displaying soma and proximal dendrites of SINs and D1R+ and D2R+ neurons in layer 5 of the PL region. Comparison of **(B)** the neuronal soma size (μm^2) and **(C)** apical dendrite diameter (μm) among three groups. **(D)** Representative traces from SINs and D1R+ and D2R+ layer 5 pyramidal neurons in response to a hyperpolarizing current step and a depolarizing current injection. Black arrows highlight the prominent voltage sag and rebound ADP in D2R+ neurons. **(E, F)** Intrinsic electrical property differences in **(E)** percent sag and **(F)** ADP among groups. **(G)** Timeline of the experimental procedure for the behavioral test. **(H)** Representative single-channel and overlay confocal micrographs of a D1-Tom mouse section immunostained for the c-Fos from the social group. Yellow arrowheads indicate the overlap of D1R-containing cells and c-Fos–stained cells in social mice (scale bar = 50 μm). **(I)** Percent colocalization of c-Fos with D1-Tom (n = 5 animals per group, 2-tailed unpaired t test, $^{****}p < .0001$). **(J)** Representative single-channel and overlay confocal micrographs of D2-Tom (red) and c-Fos staining (green) in social mice. Yellow arrowheads indicate the overlap of D2R-containing cells and c-Fos–stained cells (scale bar = 50 μm). **(K)** Quantification of the percentage of D2-Tom cells that were also c-Fos-positive (n = 5 mice per group, $p > .05$, Mann-Whitney U test). Significance for multiple comparisons: **(B, C, E, F)** Kruskal-Wallis test, post hoc, Dunn, $^{**}p < .01$; $^{***}p < .0001$. Data are expressed as median ± interquartile range. **(I)** Two-tailed unpaired t test, $^{****}p < .0001$. Data are expressed as mean ± SEM. ADP, afterdepolarization; D1-Tom, tdTomato-labeled D_1 receptor–expressing cells; D1R, D_1 receptor; D1R+, D1R expressing; D2-Tom, tdTomato-labeled D_2 receptor–expressing cells; D2R, D_2 receptor; D2R+, D2R expressing; ns, not significant; PL, prelimbic; SIN, social investigation–associated neuron; TRAP, targeted recombination in active populations.

neural mechanism that may underlie information retention in the PFC (38,39). To investigate the effect of social experience on the neuronal firing patterns, we used FosTRAP mice to label the SINs. As predicted from their identification as D1R+ cells, SINs responded robustly to D1R agonist SKF81297 (Figure S6 in Supplement 1). The number of action potentials was significantly increased during SKF81297 application. In contrast, SKF81297 did not affect the action potential number in unTRAPed neurons in the same FosTRAP mice (Figure S6A–C in Supplement 1). These data further support that SINs express functional D1Rs.

Coactivation of NMDA receptor and D1R is necessary for in vivo persistent neuronal firing in the PFC (40,41). We thus coapplied NMDA and SKF81297 to initiate burst firing in the mPFC slices as a previous study reported (37). The recorded neurons exhibited 3 firing patterns: no firing, regular firing, or burst firing (Figure 3A). To test whether persistent firing pattern is correlated to social stimuli and is specific to SINs, we set up 4 groups: 2 groups of nonselective neurons from wild-type mice that underwent social encounter or home cage control and another 2 groups of SINs from FosTRAP mice with or without social exposure. We found that only SINs exhibited a significant higher rate of bursting and regular firing after social investigation, similar to that seen in the frontal cortex for motor learning (37) (Figure 3B, Detailed Results 4 in Supplement 1). These results suggest that SINs show an increased possibility of persistent firing.

To explore the possibility that distinct projection-defined subpopulations of SINs may undergo differential firing patterns, we tested NAc- and basolateral amygdala (BLA)–projecting prefrontal neurons, as these connections are essential for social recognition (42,43), and they are anatomically and molecularly distinct despite their similar passive and firing properties like D1R+ cells (20,31,44). We first determined whether SINs directly project to the NAc and BLA. A Cre-dependent mCherry reporter was pre-expressed in the PL region of FosCreER mice, and SINs were labeled by pairing social investigation with 4-OHT injection, as shown in Figure 1E. After 3 weeks, TRAPed SINs were observed in the PL region, and their strong mCherry-labeled terminals were detected in both the NAc and BLA (Figure 3C).

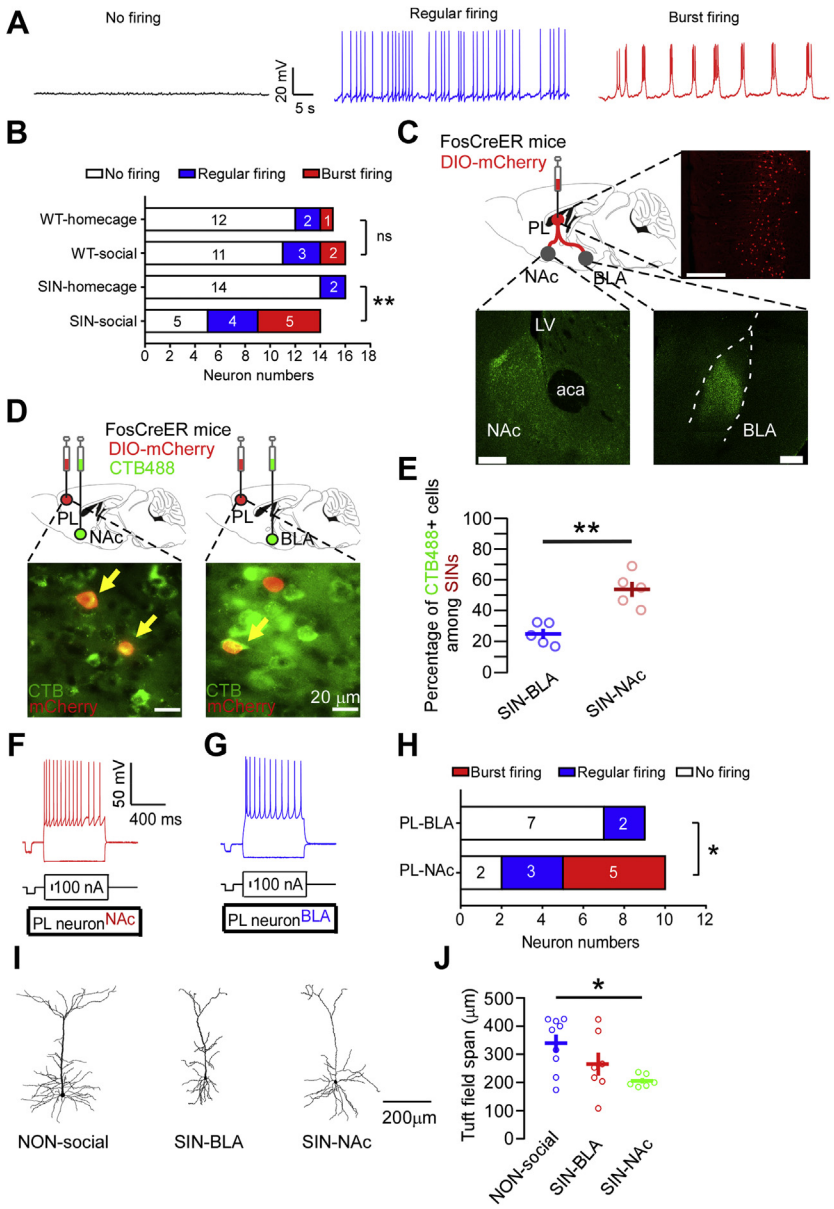

Figure 3. SINs exhibited increased burst firing induced by coactivation of NMDA and D1R. **(A)** Representative traces of SINs activity in response to a combination of NMDA (8 μM) and dopamine D1R agonist SKF81297 (10 μM) including no firing (black), regular firing (blue), and burst firing (red) patterns. **(B)** SINs exhibited increased burst firing patterns after social re-exposure. Social re-exposure did not affect the composition ratio of 3 cell types with distinct firing patterns in WT mice ($\chi^2_2 = 0.5451$, $p > .05$). The number of neurons exhibiting burst firing was increased in SINs after social re-exposure compared with the neurons of home cage control animals ($\chi^2_2 = 9.84$, $^{**}p < .01$). **(C)** Cre-dependent mCherry AAVs were injected into the PL region of FosCreER mice. Three weeks after TRAPing, SINs were detected in the PL region (upper right) while terminals were detected in NAc (lower left) and BLA (lower right) (mCherry signals in terminals were enhanced by anti-RFP antibody). **(D)** AAV8-DIO-mCherry was injected into the PL region, and CTB-488 was injected into the NAc or BLA of FosCreER mice, to quantify NAc-projecting (lower left) and BLA-projecting SINs (lower right) in the PL region. Scale bar = 20 μm. **(E)** Quantification of the proportion of BLA-projecting and NAc-projecting neurons among SINs (n = 5 mice per group). $^{**}p < .01$, unpaired t test. Recording conducted from **(F)** NAc-projecting and **(G)** BLA-projecting neurons in slice and representative traces showing neurons activity upon −200-pA and +200-pA current pulses. **(H)** The number of burst firing patterns was significantly increased in NAc-projecting neurons compared with BLA-projecting neurons after social investigation (NAc-projecting n = 5 of 10 cells [50.0%] vs. BLA-projecting n = 0 of 9 cells [0%]) ($\chi^2_2 = 7.947$, $^{*}p = .0188$). **(I)** Reconstructive images of biocytin-labeled non-social, SIN-BLA, and SIN-NAc neurons in the PL region, **(J)** showing a small tuft field span in NAc-projecting SINs compared with non-social cells ($^{*}p < .05$). aca, anterior commissure; AAV, adeno-associated virus; BLA, basolateral amygdala; D1R, D_1 receptor; LV, lateral ventricle; NAc, nucleus accumbens; ns, not significant; PL, prelimbic; RFP, red fluorescent protein; SIN, social investigation–associated neuron; TRAP, targeted recombination in active populations; WT, wild-type.

Next, we injected a retrograde tracer CTB-Alexa 488 into the NAc or BLA of FosCreER mice, in which SINs were TRAPed by mCherry. After 1 week, we detected rich retrogradely labeled neurons in the PL region from the NAc and BLA (Figure 3D), and significantly higher proportion of NAc- versus BLA-projecting SINs (Figure 3E), indicating that SINs preferentially project to the NAc. Further, we determined whether NAc- and BLA-projecting SINs show different firing patterns in response to coactivation of NMDA receptor and D1R after social investigation. We found that these neurons in both groups lacked voltage sag and rebound depolarization in response to hyperpolarizing current pulses, consistent with previous description (Figure 3F, G). Interestingly, the proportion of burst firing was markedly higher in NAc- versus BLA- projecting cells (Figure 3H, Detailed Results 4 in Supplement 1).

With biocytin reconstruction and morphological quantification, we found that the NAc-projecting neurons had a smaller apical dendrite tuft field span compared with the BLA-projecting cells and the unTRAPed cells (non-social neurons) (Figure 3I, J). There was no difference in apical dendrite nodes and branch orders among these three neuron groups (Figure S7A, B). However, further analysis revealed a significantly smaller number of apical dendritic intersections by Sholl analysis and lower spine densities in NAc-projecting SINs compared with either non-social neurons or BLA-projecting

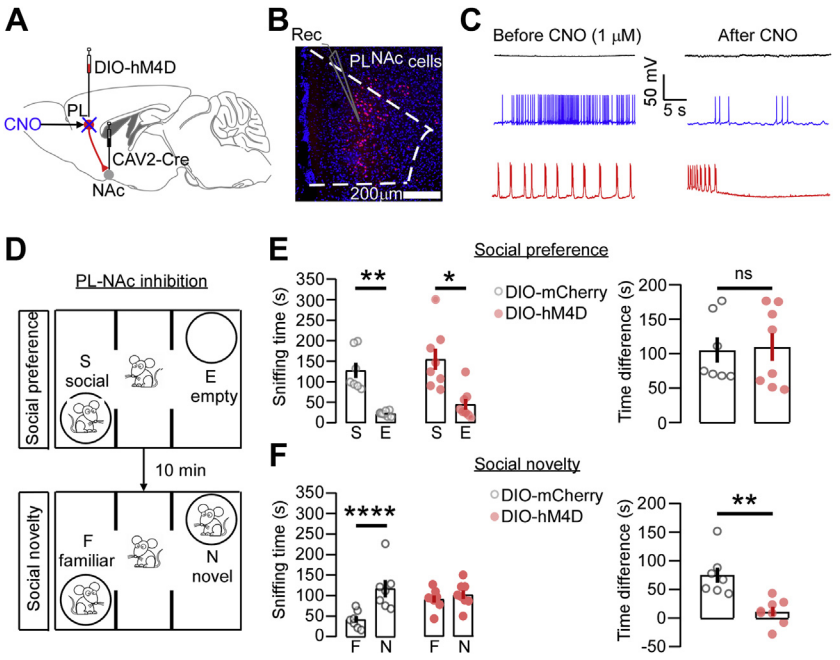

Figure 4. Activity of NAc-projecting PL neurons is required for social memory. **(A)** Schematic of viral strategy for inactivation of NAc-projecting PL neurons. Current clamp recording from the hM4D-infected NAc-projecting PL neurons indicated decreases of **(B)** regular firing (n = 3 all decreased) and **(C)** burst firing (n = 5 all decreased) after CNO application (1 μM). **(D)** Three-chamber test with CNO (5 mg/kg, intraperitoneal) 30 minutes prior to testing. **(E)** In social preference, both groups showed an apparent preference for sniffing the enclosure with mice over an empty one. No change in time difference (social minus empty) was observed. **(F)** In social novelty, inactivation of NAc-projecting PL neurons reduced preference for exploring a novel mouse. A significant decrease in time difference (novel minus familiar) was observed in hM4D-expressing mice. Significance for multiple comparisons: **(E,** right; **I,** right**)** Mann-Whitney U test; **(F,** right; **J,** right**)** unpaired t test; **(F,** left; **J,** left**)** 2-way analysis of variance, post hoc, Bonferroni; **(E,** left; **I,** left**)** Kruskal-Wallis test, post hoc, Dunn. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < .0001$. Data were expressed as mean ± SEM. CNO, clozapine-N-oxide; E, empty; F, familiar; N, novel; NAc, nucleus accumbens; ns, not significant; PL, prelimbic; S, social; WT, wild-type.

SINs (Figure S7C, D in Supplement 1). Together, these results suggest that prefrontal NAc-projecting SINs are morphologically distinct from those projecting to the BLA and functionally display more burst firing in response to the social investigation.

Chemogenetic Inactivation of NAc-Projecting PL Neurons Impairs Social Novelty but Not Social Preference in the Three-Chamber Test

We next asked whether inhibiting NAc-projecting PL neurons would produce a similar impairment to inhibition of PL SINs. We expressed AAV8-DIO-hM4Di in the PL region and injected NAc with retrograde CAV2-Cre. This virus is retrogradely transported from the site of injection to the cell bodies in the PL region, where it can inactivate the NAc-projecting neurons only when CNO is present (Figure 4A). In prefrontal slices, bath application of CNO significantly inhibited regular and burst firing induced by NMDA and SKF81297 in NAc-projecting PL neurons (Figure 4B, C). Using this combinatorial AAV8-DIO-hM4D and CAV2-Cre viral strategy, we found that inactivation of NAc-projecting neurons did not alter sociability (Figure 4D, E). In the social novelty test, however, although mCherry-expressing mice showed normal social novelty preference, mice with PL-NAc inactivation exhibited no preference to the enclosure containing novel mice (Figure 4F). Our results thus further suggested that inactivation of NAc-projecting PL neurons selectively impaired social novelty but not social preference, which could be ascribed to a "social memory" deficit, consistent with deficits seen when inhibiting TRAPed SINs. Using the same viral strategy, we also examined the influence of PL-BLA inactivation and found an effect on neither social preference nor social novelty (Figure S8A–D in Supplement 1). Altogether, these results suggest a critical role of a NAc- but not BLA-projecting PL subpopulation in social memory.

Optogenetic Reactivation of SIN Terminals in the NAc Enables a Social Memory Retrieval

A specific neuronal population could work as "engram cells" to store the information of a conspecific. The reactivation of the memory "engram cells" is thought to underlie memory retrieval (16,23,45,46). We thus proposed that the same social stimulus leads to the reactivation of a population of SINs in the PL region. To test this hypothesis, SINs were labeled with tdTomato in FosTRAP mice. One week later, these mice were exposed to the same (familiar) mouse or to a novel mouse for 5 minutes, and their brain tissues were collected and fixed 90 minutes after the social investigation for c-Fos immunostaining (Figure 5A). We found that the percentage of colocalized TRAPed/c-Fos+ cells in the PL region from the mice exposed to the same mouse was significantly higher than that exposed to a novel mouse (Figure 5B, C, Table S3 in Supplement 1). As a control, no difference in TRAPed/c-Fos+ colocalization was found in the insular cortex, a brain region also implicated in social behaviors (47) and taste memory processes (48), after social exposure to a familiar or novel conspecific (Figure S9 in Supplement 1). These results suggest that SINs in the PL region may serve as social memory–related "engram cells," which are capable of being reactivated by the same social stimuli.

Next, we sought to investigate whether reactivation of NAc-projecting SINs in the PL region could enable social memory retrieval. A combined approach of FosTRAP mouse tool and optogenetic stimulation was used to reactivate the terminals within NAc from prefrontal SINs, which were TRAPed during

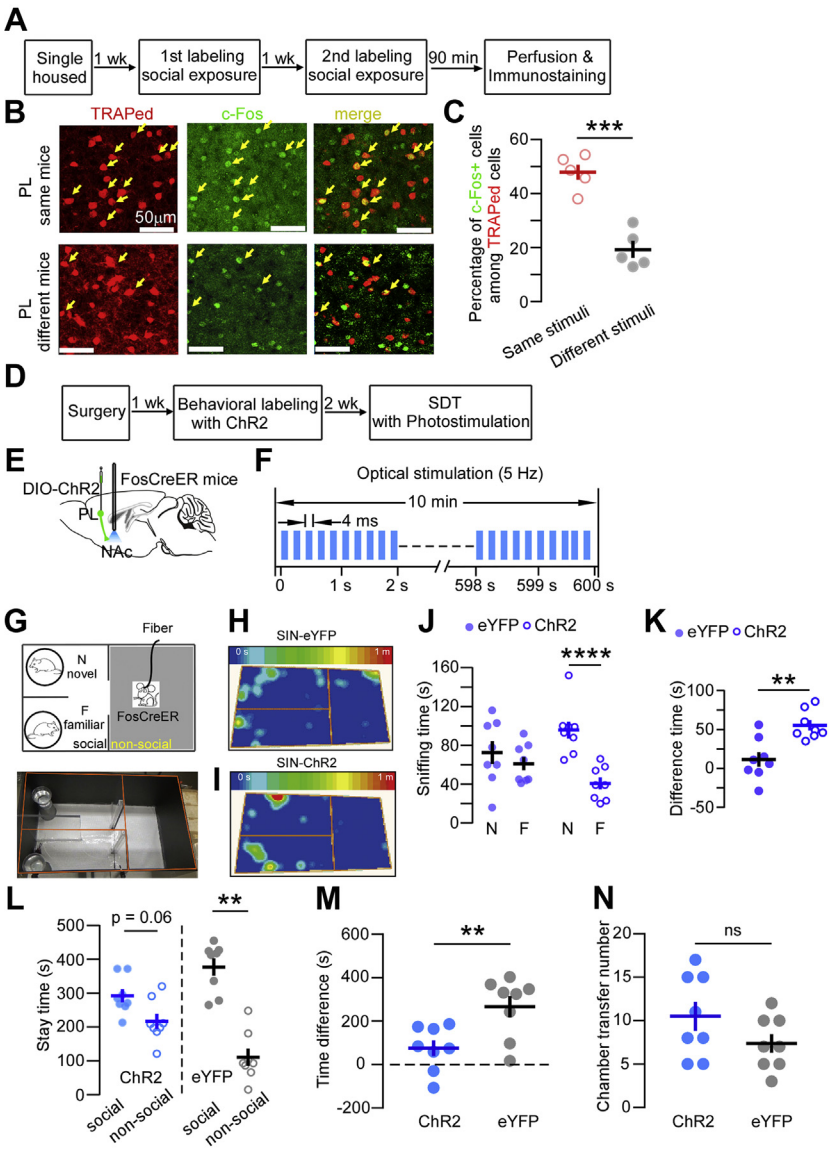

Figure 5. Reactivation of SINs projections in the NAc enables social memory retrieval. **(A)** Behavioral labeling procedure for discriminating the same or different social stimuli induced c-Fos expression. One week after TRAPing (first social exposure), the FosTRAP mice were exposed to the same or a different mouse for 5 minutes (second social exposure), then sacrificed for histology after 90 minutes. **(B)** Confocal images of the PL region with twice social stimuli exposure of same mice (top) and different mice (bottom). The first social stimuli–induced c-Fos expression was TRAPed with tdTomato (red), and the second social stimuli–induced c-Fos expression was labeled with immunostaining (green). Colocalization of two social stimuli–induced c-Fos was marked with yellow arrowheads. **(C)** Quantification of the proportion of second social stimuli–activated neurons among all TRAPed neurons during first social exposure. ***$p < .001$, 2-tailed Student's t test. **(D)** Experimental timeline for reactivation of SIN-NAc projection. **(E)** Schematic drawing of SIN viral transduction with ChR2 (AAV5-Ef1a-DIO-hChR2(H134R)-EYFP) and NAc optical fibers implantation in FosCreER mice. **(F)** Optical stimulation parameters: 473-nm light was delivered in 5-Hz, 4-ms pulse bursts for 10 minutes during the behavioral test. **(G)** Schematic illustrating the modified SDT. Representative heatmap images during the test session for **(H)** eYFP-expressing and **(I)** ChR2-expressing mice. **(J)** The ChR2-expressing, but not the eYFP-expressing, animals showed a significant preference to the novel mouse. **(K)** The time difference (novel minus familiar) was significantly higher in the ChR2 group. **(L)** The eYFP-expressing FosCreER mice showed a significant preference to the social chamber, which is only a trend in ChR2 mice ($p = .06$, 5 Hz, 4-ms duration, 6–8 mW at fiber tip, 10 min). **(M)** A decrease of the time difference (social chamber minus non-social chamber) in the ChR2-expressing group was observed (2-tailed Student's t test, $p < .01$). **(N)** No difference in chamber transfer numbers was found. Significance for multiple comparisons: **(C, M)** unpaired t test; **(J, L)** 2-way analysis of variance, post hoc, Bonferroni; **(K)** Mann-Whitney U test. **$p < .01$; ***$p < .001$; ****$p < .0001$. Data were expressed as mean ± SEM. ChR2, channelrhodopsin-2; eYFP, enhanced yellow fluorescent protein; F, familiar; N, novel; NAc, nucleus accumbens; PL, prelimbic; SDT, social discrimination test; SIN, social investigation–associated neuron; TRAP, targeted recombination in active populations.

the first social exposure. We injected AAV5-Ef1a-DIO-hChR2(H134R)-EYFP in the PL region of FosCreER mice and implanted optical fibers over the NAc (Figure 5D, E). The ChR2-eYFP (channelrhodopsin-2–enhanced yellow fluorescent protein) was selectively expressed in the SINs by pairing 4-OHT with a social investigation, which was validated by postmortem histology (Figure S10A, B in Supplement 1) and ex vivo recordings (Figure S10C, D in Supplement 1). A 5-Hz optogenetic stimulation (Figure 5F) was continuously delivered during a modified SDT (Figure 5G), and this protocol could efficiently activate mPFC neurons, as a recent study reported (49). Social memory in single-housed male mice lasts only hours (3,5). Thus, comparable sniffing times between chambers containing encountered familiar and novel mice were predicted for eYFP-expressing control mice because of the long interval of social stimuli re-exposure (2 weeks) (Figure 5H, J), suggesting no social memory retrieval after a 2-week interval. However, optogenetic activation of the axon terminals of SINs in the NAc in the ChR2-expressing group produced a significant preference for novel mice (Figure 5I–K), suggesting a social memory–guided novelty preference. Altogether, our results indicated that optogenetic reactivation of SIN terminals within the NAc enables retrieval of a previously forgotten social memory.

We also analyzed the influence of optogenetic stimulation of prefrontal SIN terminals on social preference, and notably, the time difference was significantly less in the ChR2 group (Figure 5L, M). These results are consistent with the

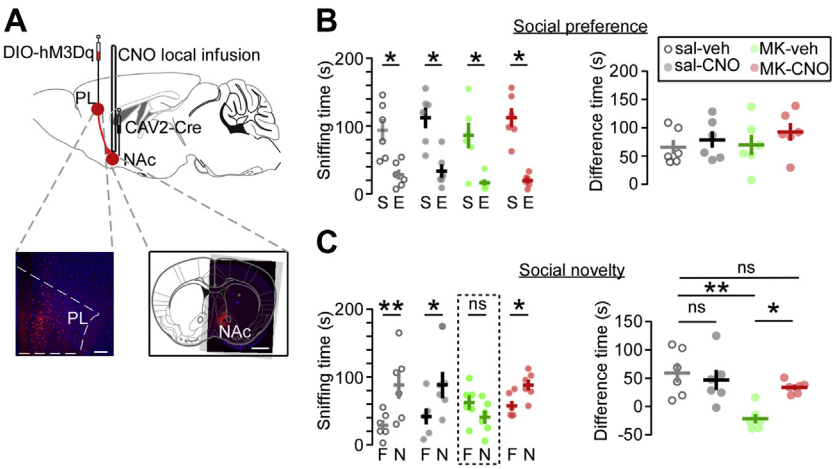

Figure 6. Local stimulation of PL-to-NAc projections prevents low-dose MK-801–induced social memory deficits. **(A)** Strategy for local activation of PL-NAc projection using excitatory DREADDs with CAV2-Cre. Lower panel showing Cre-dependent hM3D-mCherry labeling in PL (left) (scale bar = 100 μm) and NAc (right) (scale bar = 500 μm). **(B)** (Left) In social preference, all groups preferred to social stimuli over an empty enclosure. (Right) No time difference (mouse minus empty) was detected. **(C)** In social novelty, only the MK-veh group did not display a preference for novel mice. CNO application (5 mg/kg, intraperitoneal) 30 minutes prior to the test increased the time difference (novel minus familiar) in MK treatment mice but did not affect saline treatment groups. Significance for multiple comparisons: **(B,** left**)** Kruskal-Wallis test, post hoc, Wilcoxon matched-pairs signed-rank test; **(C,** left**)** 2-way analysis of variance, post hoc, Tukey; **(C,** right**)** 1-way analysis of variance, post hoc, Tukey. CNO, clozapine-N-oxide; DREADD, designer receptor exclusively activated by designer drugs; E, empty; F, familiar; MK, MK-801; N, novel; NAc, nucleus accumbens; ns, not significant; PL, prelimbic; S, social; sal, saline; veh, vehicle.

observations that photoactivation of the PL region or PL-NAc projection reduces social preference (20,21). We could not ascribe the reduced social preference to an altered locomotor activity in the ChR2-expressing group because no change in the number of chamber transfers during the test was detected (Figure 5N).

Chemogenetic Activation of PL-to-NAc Projections Ameliorates MK-801–Induced Social Memory Impairments

Previous studies have revealed that pretreatment with a low dose of NMDA receptor antagonists, such as MK-801, led to a deficit of social memory but not social preference (50–52). Given that the activity of PL-NAc projections is critical for social memory, we hypothesized that MK-801–induced social memory impairments could be prevented by enhancing PL-to-NAc projections. Using a combination of injecting CAV2-Cre into the NAc and expressing AAV8-hSyn-DIO-hM3D(Gq)-mCherry into the PL region, we selectively activated axon terminals of PL projection neurons within NAc by local CNO infusion (30 min before the behavioral test) (53,54) (Figure 6A, Detailed Results 5 in Supplement 1). We found that all groups displayed normal social preference (Figure 6B), but MK-801–treated mice showed impairment in social novelty, and this impairment was rescued by local infusion of CNO (Figure 6C). Altogether, our results indicated that enhancing PL-to-NAc projections prevented social memory deficits induced by MK-801 administration.

DISCUSSION

Here we discovered that a subpopulation of c-Fos–expressing PL neurons projecting to the NAc is essential for social memory. Using a combined approach (Discussion in Supplement 1), we found that these SINs display traits similar to D1R+ neurons and show persistent firing after social investigation. Inhibition of SINs or PL-to-NAc projections selectively impairs social memory but not social motivation (Discussion in Supplement 1). In contrast, reactivation of axon terminals of SINs in the NAc enables recall of previous social encounters. Moreover, stimulation of PL-to-NAc projections prevents social memory impairments induced by MK-801. Our studies revealed that SINs projecting to the NAc are a unique subpopulation that regulates the expression of social memory.

A recent study revealed a critical role of hippocampal input to the mPFC in social memory deficits in the MECP2 knockout mouse (6). However, whether and how the mPFC encodes social information to optimize behavior with previously encountered mice remain elusive. Here, we identified a subpopulation of PL neurons that were selectively activated by social investigation using a novel FosTRAP tool (26), consistent with a previous report with immunostaining (55). For the first time, we have characterized these functionally and morphologically defined neurons and their projections. We also revealed the necessity and sufficiency of these NAc-projecting neurons for social memory. Altogether, our data suggest that these neurons meet the criteria for social memory engram cells (45). First, c-Fos is not only a marker of neuronal activation, but also a key transcription factor that "tag" the engram cells, leading them to mature and eventually support a long-term memory (22,56–58) (Discussion in Supplement 1). Second, the mPFC is thought to control memory retrieval (10,11,59). It is conceivable that these engram cells in mPFC could be reactivated when the same stimuli reoccur, as we showed (Figure 5B, C). Further, we demonstrated that inhibition of targeted social neurons in the PL region specifically produced social memory deficits without affecting social preference. One interpretation of our findings is that social investigation TRAPed PL neurons do support general sociability, although other explanations are plausible (Discussion in Supplement 1).

Neural activity in the mPFC is strongly modulated by dopamine through differential activation of D1Rs and D2Rs (40,60–62). We found that the subset of social memory–associated prefrontal neurons exhibited D1R+-like electrophysiological and morphological properties (35). Prefrontal dopamine plays a critical role in social behaviors (30,63,64). Our findings indicate that "social memory" cells in the mPFC display the characteristics of D1R-expressing cells, while the

role of prefrontal D2R in social memory remains to be determined (Discussion in Supplement 1). Interestingly, SINs exhibited a significant increase in persistent firing (Discussion in Supplement 1). This firing pattern in the neocortex resembles the periodic depolarizations (up-states) in vivo (65,66) for working memory function (27,40,41,67–69) and is usually affected by prior experience in selective neuronal subpopulations (37,66). Interestingly, neuronal excitability of dentate gyrus engram cells is transiently increased following memory recall, and this excitability change is mediated by the internalization of the inward-rectifier potassium channel (Kir2.1) (46). Given that D1R activation increases Kir2 family–mediated rectifier K+ currents (70,71), D1R-like SINs may encode social information via D1R-Kir2 interaction (62).

NAc neurons integrate information from the interconnections among the ventral hippocampus, mPFC, and BLA in social recognition (42). A circuit from CA1 to NAc has been identified as a critical component for social memory expression (3). Still, the role of mPFC-to-NAc projections in social memory remains uncharacterized. Our data suggest that mPFC-NAc projection is implicated in "memory expression." The NAc seems not to determine the specificity of social memory, but serves as the output brain region during the social recognition to drive the mouse to investigate a novel instead of a familiar stimulus. The engram cells hypothesis suggests that initially generated memory gradually transmits information into remote memory access via a specific cell population (17,45), probably through synaptic facilitation (72,73). Consistently, when we reactivated the axon terminals of prefrontal TRAPed neurons in the NAc, the mice recalled the "forgotten" social memory by which to distinguish the novel from the familiar conspecific. Further, in agreement with recent studies that reported no influence in social preference (74) but enabled learning (75), we found that chemogenetic activation of PL-to-NAc projections prevented MK-801–induced social amnesia without affecting social motivation. Thus, prefrontal NAc-projecting SINs likely represent social memory traces (58), which may be supported by their combined social-spatial encoding (20). Further studies will be needed to investigate how prefrontal SINs transit to mature engram cells to enable a reliable social memory recall. Our study suggested that D1R signaling and c-Fos–mediated gene regulation might act synergistically in tagging engram cells and allowing them to mature later.

In summary, our findings provide novel insights into the cell-type heterogeneity of mPFC in the regulation of social behaviors and the role of a subset of NAc-projecting PL neurons in social memory. The identification of a novel PL-NAc circuit that mediates social memory without impacting social motivation may provide novel targets for the treatment of diseases with social memory deficits, such as schizophrenia and autism.

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BX, LL, and W-JG conceived the project, designed the experiments, and wrote the manuscript. BX conducted immunostaining, electrophysiological recording, surgery, and the behavioral test, and analyzed the related data; NRM did the surgery, behavioral tests, and viral injection confirmation; K-MG did biocytin immunostaining and Neurolucida reconstruction; Y-XZ helped BX breeding and genotyping transgenic mice; and BR performed genotyping, immunostaining, and behavioral test video analysis. S-SY conducted some surgery; Y-CL helped BX breeding transgenic mice and valuable manuscript discussion; and DVW commented on the manuscripts.

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REFERENCES

1. Green MF, Horan WP, Lee J (2015): Social cognition in schizophrenia. Nat Rev Neurosci 16:620–631.
2. Bicks LK, Koike H, Akbarian S, Morishita H (2015): Prefrontal cortex and social cognition in mouse and man. Front Psychol 6:1805.
3. Okuyama T, Kitamura T, Roy DS, Itohara S, Tonegawa S (2016): Ventral CA1 neurons store social memory. Science 353:1536–1541.
4. Hitti FL, Siegelbaum SA (2014): The hippocampal CA2 region is essential for social memory. Nature 508:88–92.
5. Smith AS, Williams Avram SK, Cymerblit-Sabba A, Song J, Young WS (2016): Targeted activation of the hippocampal CA2 area strongly enhances social memory. Mol Psychiatry 21:1137–1144.
6. Phillips ML, Robinson HA, Pozzo-Miller L (2019): Ventral hippocampal projections to the medial prefrontal cortex regulate social memory. Elife 8:e44182.
7. Blakemore SJ (2008): The social brain in adolescence—reviews. Nat Rev Neurosci 9:267–277.
8. Demolliens M, Isbaine F, Takerkart S, Huguet P, Boussaoud D (2017): Social and asocial prefrontal cortex neurons: A new look at social facilitation and the social brain. Soc Cogn Affect Neurosci 12:1241–1248.
9. Ko J (2017): Neuroanatomical substrates of rodent social behavior: The medial prefrontal cortex and its projection patterns. Front Neural Circuits 11:41.
10. Preston AR, Eichenbaum H (2013): Interplay of hippocampus and prefrontal cortex in memory. Curr Biol 23:R764–R773.
11. Eichenbaum H (2017): Prefrontal-hippocampal interactions in episodic memory. Nat Rev Neurosci 18:547–558.
12. Place R, Farovik A, Brockmann M, Eichenbaum H (2016): Bidirectional prefrontal-hippocampal interactions support context-guided memory. Nat Neurosci 19:992–994.
13. Cavanagh SE, Towers JP, Wallis JD, Hunt LT, Kennerley SW (2018): Reconciling persistent and dynamic hypotheses of working memory coding in prefrontal cortex. Nat Commun 9:3498.
14. Orhan AE, Ma WJ (2019): A diverse range of factors affect the nature of neural representations underlying short-term memory. Nat Neurosci 22:275–283.
15. Murray JD, Bernacchia A, Roy NA, Constantinidis C, Romo R, Wang XJ (2017): Stable population coding for working memory coexists with heterogeneous neural dynamics in prefrontal cortex. Proc Natl Acad Sci U S A 114:394–399.

16. Kitamura T, Ogawa SK, Roy DS, Okuyama T, Morrissey MD, Smith LM, *et al.* (2017): Engrams and circuits crucial for systems consolidation of a memory. Science 356:73–78.
17. DeNardo LA, Liu CD, Allen WE, Adams EL, Friedmann D, Fu L, *et al.* (2019): Temporal evolution of cortical ensembles promoting remote memory retrieval. Nat Neurosci 22:460–469.
18. Wall VL, Fischer EK, Bland ST (2012): Isolation rearing attenuates social interaction-induced expression of immediate early gene protein products in the medial prefrontal cortex of male and female rats. Physiol Behav 107:440–450.
19. Ago Y, Araki R, Tanaka T, Sasaga A, Nishiyama S, Takuma K, *et al.* (2013): Role of social encounter-induced activation of prefrontal serotonergic systems in the abnormal behaviors of isolation-reared mice. Neuropsychopharmacology 38:1535–1547.
20. Murugan M, Jang HJ, Park M, Miller EM, Cox J, Taliaferro JP, *et al.* (2017): Combined social and spatial coding in a descending projection from the prefrontal cortex. Cell 171:1663–1677.e16.
21. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, *et al.* (2011): Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477:171–178.
22. Miyashita T, Kikuchi E, Horiuchi J, Saitoe M (2018): Long-term memory engram cells are established by c-Fos/CREB transcriptional cycling. Cell Rep 25:2716–2728.e3.
23. Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, *et al.* (2012): Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature 484:381–385.
24. Tonegawa S, Pignatelli M, Roy DS, Ryan TJ (2015): Memory engram storage and retrieval. Curr Opin Neurobiol 35:101–109.
25. Roy DS, Arons A, Mitchell TI, Pignatelli M, Ryan TJ, Tonegawa S (2016): Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. Nature 531:508–512.
26. Guenthner CJ, Miyamichi K, Yang HH, Heller HC, Luo L (2013): Permanent genetic access to transiently active neurons via TRAP: Targeted recombination in active populations. Neuron 78:773–784.
27. Wang H, Stradtman GG 3rd, Wang XJ, Gao WJ (2008): A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. Proc Natl Acad Sci U S A 105:16791–16796.
28. Amodio DM, Frith CD (2006): Meeting of minds: The medial frontal cortex and social cognition. Nat Rev Neurosci 7:268–277.
29. Anderson SW, Bechara A, Damasio H, Tranel D, Damasio AR (1999): Impairment of social and moral behavior related to early damage in human prefrontal cortex. Nat Neurosci 2:1032–1037.
30. Brumback AC, Ellwood IT, Kjaerby C, Iafrati J, Robinson S, Lee AT, *et al.* (2018): Identifying specific prefrontal neurons that contribute to autism-associated abnormalities in physiology and social behavior. Mol Psychiatry 23:2078–2089.
31. Land BB, Narayanan NS, Liu RJ, Gianessi CA, Brayton CE, Grimaldi DM, *et al.* (2014): Medial prefrontal D1 dopamine neurons control food intake. Nat Neurosci 17:248–253.
32. Mahn M, Gibor L, Patil P, Cohen-Kashi Malina K, Oring S, Printz Y, *et al.* (2018): High-efficiency optogenetic silencing with soma-targeted anion-conducting channelrhodopsins. Nat Commun 9:4125.
33. Wei X, Ma T, Cheng Y, Huang CCY, Wang X, Lu J, *et al.* (2018): Dopamine D1 or D2 receptor-expressing neurons in the central nervous system. Addict Biol 23:569–584.
34. Gee S, Ellwood I, Patel T, Luongo F, Deisseroth K, Sohal VS (2012): Synaptic activity unmasks dopamine D2 receptor modulation of a specific class of layer V pyramidal neurons in prefrontal cortex. J Neurosci 32:4959–4971.
35. Seong HJ, Carter AG (2012): D1 receptor modulation of action potential firing in a subpopulation of layer 5 pyramidal neurons in the prefrontal cortex. J Neurosci 32:10516–10521.
36. Loiseau F, Millan MJ (2009): Blockade of dopamine D(3) receptors in frontal cortex, but not in sub-cortical structures, enhances social recognition in rats: Similar actions of D(1) receptor agonists, but not of D(2) antagonists. Eur Neuropsychopharmacol 19:23–33.
37. Ren M, Cao V, Ye Y, Manji HK, Wang KH (2014): Arc regulates experience-dependent persistent firing patterns in frontal cortex. J Neurosci 34:6583–6595.
38. Fuster JM (2001): The prefrontal cortex–an update: Time is of the essence. Neuron 30:319–333.
39. Miller EK, Cohen JD (2001): An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202.
40. Williams GV, Goldman-Rakic PS (1995): Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376:572–575.
41. Wang M, Yang Y, Wang CJ, Gamo NJ, Jin LE, Mazer JA, *et al.* (2013): NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. Neuron 77:736–749.
42. Tanimizu T, Kenney JW, Okano E, Kadoma K, Frankland PW, Kida S (2017): Functional connectivity of multiple brain regions required for the consolidation of social recognition memory. J Neurosci 37:4103–4116.
43. Tan Y, Singhal SM, Harden SW, Cahill KM, Nguyen DM, Colon-Perez LM, *et al.* (2019): Oxytocin receptors are expressed by glutamatergic prefrontal cortical neurons that selectively modulate social recognition. J Neurosci 39:3249–3263.
44. McGarry LM, Carter AG (2016): Inhibitory gating of basolateral amygdala inputs to the prefrontal cortex. J Neurosci 36:9391–9406.
45. Josselyn SA, Kohler S, Frankland PW (2015): Finding the engram. Nat Rev Neurosci 16:521–534.
46. Pignatelli M, Ryan TJ, Roy DS, Lovett C, Smith LM, Muralidhar S, *et al.* (2019): Engram cell excitability state determines the efficacy of memory retrieval. Neuron 101:274–284.e275.
47. Rogers-Carter MM, Varela JA, Gribbons KB, Pierce AF, McGoey MT, Ritchey M, *et al.* (2018): Insular cortex mediates approach and avoidance responses to social affective stimuli. Nat Neurosci 21:404–414.
48. Bermudez-Rattoni F (2014): The forgotten insular cortex: Its role on recognition memory formation. Neurobiol Learn Mem 109:207–216.
49. Zhou T, Zhu H, Fan Z, Wang F, Chen Y, Liang H, *et al.* (2017): History of winning remodels thalamo-PFC circuit to reinforce social dominance. Science 357:162–168.
50. Moy SS, Nonneman RJ, Shafer GO, Nikolova VD, Riddick NV, Agster KL, *et al.* (2013): Disruption of social approach by MK-801, amphetamine, and fluoxetine in adolescent C57BL/6J mice. Neurotoxicol Teratol 36:36–46.
51. Golden SA, Heshmati M, Flanigan M, Christoffel DJ, Guise K, Pfau ML, *et al.* (2016): Basal forebrain projections to the lateral habenula modulate aggression reward. Nature 534:688–692.
52. van der Staay FJ, Rutten K, Erb C, Blokland A (2011): Effects of the cognition impairer MK-801 on learning and memory in mice and rats. Behav Brain Res 220:215–229.
53. Yang Y, Liu DQ, Huang W, Deng J, Sun Y, Zuo Y, *et al.* (2016): Selective synaptic remodeling of amygdalocortical connections associated with fear memory. Nat Neurosci 19:1348–1355.
54. Beier KT, Kim CK, Hoerbelt P, Hung LW, Heifets BD, DeLoach KE, *et al.* (2017): Rabies screen reveals GPe control of cocaine-triggered plasticity. Nature 549:345–350.
55. Halasz J, Toth M, Kallo I, Liposits Z, Haller J (2006): The activation of prefrontal cortical neurons in aggression–a double labeling study. Behav Brain Res 175:166–175.
56. Kang H, Sun LD, Atkins CM, Soderling TR, Wilson MA, Tonegawa S (2001): An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. Cell 106:771–783.
57. Reijmers LG, Perkins BL, Matsuo N, Mayford M (2007): Localization of a stable neural correlate of associative memory. Science 317:1230–1233.
58. Lesburgueres E, Gobbo OL, Alaux-Cantin S, Hambucken A, Trifilieff P, Bontempi B (2011): Early tagging of cortical networks is required for the formation of enduring associative memory. Science 331:924–928.
59. Tomita H, Ohbayashi M, Nakahara K, Hasegawa I, Miyashita Y (1999): Top-down signal from prefrontal cortex in executive control of memory retrieval. Nature 401:699–703.
60. Wang M, Vijayraghavan S, Goldman-Rakic PS (2004): Selective D2 receptor actions on the functional circuitry of working memory. Science 303:853–856.

61. Xing B, Li YC, Gao WJ (2016): Norepinephrine versus dopamine and their interaction in modulating synaptic function in the prefrontal cortex. Brain Res 1641:217–233.
62. Tritsch NX, Sabatini BL (2012): Dopaminergic modulation of synaptic transmission in cortex and striatum. Neuron 76:33–50.
63. Gunaydin LA, Deisseroth K (2014): Dopaminergic dynamics contributing to social behavior. Cold Spring Harb Symp Quant Biol 79:221–227.
64. Lee YA, Lionnet S, Kato A, Goto Y (2018): Dopamine-dependent social information processing in non-human primates. Psychopharmacology (Berl) 235:1141–1149.
65. Haider B, McCormick DA (2009): Rapid neocortical dynamics: Cellular and network mechanisms. Neuron 62:171–189.
66. Tseng KY, O'Donnell P (2005): Post-pubertal emergence of prefrontal cortical up states induced by D1-NMDA co-activation. Cereb Cortex 15:49–57.
67. Cossart R, Aronov D, Yuste R (2003): Attractor dynamics of network UP states in the neocortex. Nature 423:283–288.
68. Wang XJ (2001): Synaptic reverberation underlying mnemonic persistent activity. Trends Neurosci 24:455–463.
69. Monaco SA, Gulchina Y, Gao WJ (2015): NR2B subunit in the prefrontal cortex: A double-edged sword for working memory function and psychiatric disorders. Neurosci Biobehav Rev 56:127–138.
70. Penit-Soria J, Audinat E, Crepel F (1987): Excitation of rat prefrontal cortical neurons by dopamine: An in vitro electrophysiological study. Brain Res 425:263–274.
71. Uchimura N, North RA (1990): Actions of cocaine on rat nucleus accumbens neurones in vitro. Br J Pharmacol 99:736–740.
72. Abdou K, Shehata M, Choko K, Nishizono H, Matsuo M, Muramatsu SI, *et al.* (2018): Synapse-specific representation of the identity of overlapping memory engrams. Science 360:1227–1231.
73. Moczulska KE, Tinter-Thiede J, Peter M, Ushakova L, Wernle T, Bathellier B, *et al.* (2013): Dynamics of dendritic spines in the mouse auditory cortex during memory formation and memory recall. Proc Natl Acad Sci U S A 110:18315–18320.
74. Qin L, Ma K, Yan Z (2019): Chemogenetic activation of prefrontal cortex in Shank3-deficient mice ameliorates social deficits, NMDAR hypofunction, and Sgk2 downregulation. iScience 17:24–35.
75. Volle J, Yu X, Sun H, Tanninen SE, Insel N, Takehara-Nishiuchi K (2016): Enhancing prefrontal neuron activity enables associative learning of temporally disparate events. Cell Rep 15:2400–2410.