Drug-induced change in transmitter identity is a shared mechanism generating cognitive deficits

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Abstract: Cognitive deficits are a long-lasting consequence of drug use, yet the convergent mechanism by which different drugs cause similar deficits is unclear. We find that both phencyclidine and methamphetamine impair memory by causing the same glutamatergic neurons in the medial prefrontal cortex to gain a GABAergic phenotype and decrease their expression of the vesicular glutamate transporter. Drug-induced prefrontal hyperactivity drives this change in transmitter identity. Normalizing the activity of prefrontal glutamatergic neurons after drug-exposure reverses the change in transmitter phenotype and rescues the associated memory deficits. Increased activity of dopaminergic neurons in the ventral tegmental area is necessary and sufficient to produce the change in transmitter identity. The results reveal a shared and reversible mechanism by which exposure to different drugs causes cognitive deficits.

One-Sentence Summary: Activity- and dopamine-dependent change in the transmitter expressed by cortical neurons mediates drug-induced memory deficits.

Main Text:

Phencyclidine (PCP) and methamphetamine (METH) have been extensively studied for their ability to cause long-lasting cognitive deficits and mimic symptoms of schizophrenia (*1-3*). These drugs differ in their molecular targets in the brain (*4, 5*), and it has been unclear how they generate similar behavioral impairments. While studies have focused on the actions of single drugs, little attention has been given to investigating the mechanisms of action that different drugs of abuse have in common. Understanding shared neuronal mechanisms underlying drug-induced cognitive deficits could foster development of effective treatments and be beneficial for a spectrum of disorders (*6, 7*).

When neuronal activity is altered for a sustained period, neurons can change the neurotransmitter they express, often switching from an excitatory to an inhibitory transmitter or vice versa and causing changes in behavior (*8, 9*). Linkage of drug-induced behavioral alterations to increased expression of specific transmitters in subcortical regions (*10-12*) suggests that changes in transmitter identity could produce the behavioral effects of repeated exposure to drugs of abuse. Using a combination of genetic labeling strategies, RNA interference, chemogenetics and optogenetics, we investigated whether changes in cortical neuron transmitter phenotype are a shared mechanism underlying both PCP- and METH-induced cognitive deficits.

Phencyclidine induces cognitive deficits by changing the transmitter phenotype of neurons in the medial prefrontal cortex

To determine whether cognitive deficits result from changes in neurotransmitter phenotype, we tested the effect of a 10-day treatment with PCP (10 mg/kg/day), which induces long-lasting cognitive impairments and recapitulates deficits observed in schizophrenia (*3, 13*). We focused on the medial prefrontal cortex (mPFC), which is a major hub for cognitive control (*14, 15*). Because they represent the largest population in the mPFC, we examined the transmitter phenotype of glutamatergic neurons expressing vesicular glutamate transporter 1 (VGLUT1) (*15*). To identify these neurons following changes in transmitter profile, we labeled them permanently with a nuclear mCherry reporter using VGLUT1^{CRE}::mCherry mice (Fig. 1A).

In mice exposed to PCP, we identified 1198±59 mCherry^{+} neurons in the prelimbic subregion (PL) of the mPFC immunolabelled for GABA and 1096±81 labelled for its synthetic enzyme, glutamic acid decarboxylase 67 (GAD67) (Fig. 1, B to D, and fig. S1, A to C). In control mice, there were only 622±45 and 643±22 of these neurons, indicating that PCP increased the number mCherry^{+}/GABA^{+} and mCherry^{+}/GAD67^{+} cells by 1.9- and 1.7-fold. PCP did not alter the number of mCherry-labeled PL neurons (fig. S1D), and no sign of apoptosis or neurogenesis was detected (fig. S2). These results suggested that PCP induces the synthesis and expression of GABA in PL glutamatergic neurons not previously expressing this transmitter.

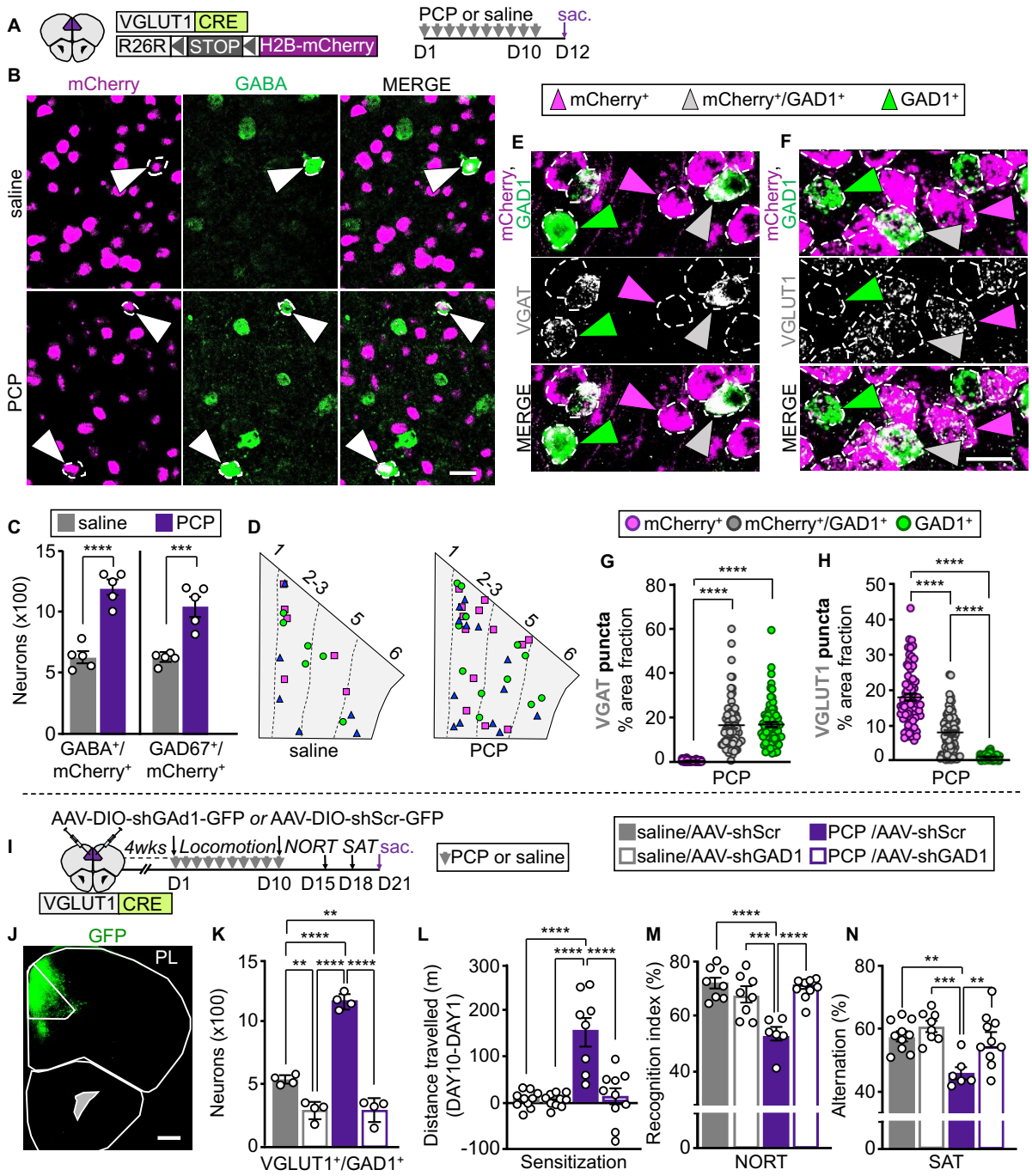

Fig. 1. PCP changes the transmitter identity of PL glutamatergic neurons that in turn causes locomotor sensitization and cognitive deficits. (**A**) Experimental protocol. (**B**) PL neurons co-expressing mCherry and GABA (arrowheads). (**C**) Quantification of mCherry$^+$/GABA$^+$ and mCherry$^+$/GAD67$^+$ neurons (n=5 mice per group). (**D**) PL locations of glutamatergic neurons that co-expressed GABA or changed transmitter identity upon treatment with PCP. Cartoons were produced by superimposing the locations of neurons in three PL sections, each from a different mouse, all positioned at Bregma 1.94. Different shapes represent cells from different mice. (**E** and **F**) Expression of VGAT and VGLUT1 mRNA puncta in PL neurons of PCP-treated mice. (**G** and **H**) Quantification of VGAT and VGLUT1 expression measured as percent of cell area occupied by mRNA puncta across PL neuronal subtypes (n=25 cells/type/mouse for 3 mice). (**I**) Experimental protocol. (**J**) Expression of shGAD1-GFP in the PL. (**K**) Quantification of neurons co-expressing VGLUT1 and GAD1 transcripts (n=4 mice per group). (**L** to **N**) shGAD1 prevents PCP-

induced deficits in locomotor sensitization (n=8-10 mice per group) and in the NORT (n=6 to 10 mice per group) and SAT (n=6 to 10 mice per group). Scale bars, (B) 25 μm, (E) and (F) 20 μm, (J) 500 μm. Statistical significance (**P<0.01, ***P<0.001, ****P<0.0001) was assessed using unpaired t-test (C), Kruskal-Wallis followed by Dunn's test (G and H), and two-way ANOVA followed by Tukey's test (K to N). Data are mean ± SEM. Further statistical details are presented in Table S1.

We then examined the expression of the GABA vesicular transporter (VGAT) and VGLUT1 in mCherry$^+$ neurons that co-expressed or gained GABA. We used fluorescent *in situ* hybridization (FISH) to detect transcripts for mCherry, for the GABA synthetic enzyme (GAD1), and for either VGAT or VGLUT1. To reveal changes in expression levels, we selectively decreased the amplification for VGAT and VGLUT1 to obtain punctate staining (Fig. 1, E and F). In the PL of PCP-treated mice, the density of neurons expressing both mCherry and GAD1 (mCherry$^+$/GAD1$^+$) was 1.6- fold higher than in controls (fig. S3F), mirroring the PCP-induced increase in number of mCherry$^+$/GAD67$^+$ neurons (Fig. 1C). Across treatments, mCherry$^+$/GAD1$^+$ neurons expressed VGAT at the level of GABAergic neurons (labeled with GAD1 and not mCherry) (Fig. 1G and fig. S3, A, B and D). At the same time, the expression level of VGLUT1 in mCherry$^+$/GAD1$^+$ neurons decreased by ~55% compared to that of glutamatergic cells expressing mCherry and not GAD1 in PCP-treated mice (Fig. 1H) and by 46% in saline controls (fig. S3, C and E). Thus, both the glutamatergic neurons that gained GABA after PCP-exposure, as well as those expressing GABA in drug-naïve conditions, are characterized by high expression levels of VGAT and lowered expression levels of VGLUT1.

We next asked whether PCP-treatment affects the transmitter phenotype of PL GABAergic neurons. No difference was observed in the number of neurons expressing GABA and not mCherry between PCP-treated animals and controls (8594±340 vs 8837±271) (fig. S1, E and F). However, PCP and other NMDA receptor antagonists have been shown to reduce expression of GAD67 and parvalbumin in prefrontal cortex parvalbumin-positive (PV$^+$) interneurons (*16, 17*). Because variability in the number of GABAergic neurons scored could have prevented the detection of loss of GABA from a small number of PV$^+$ neurons, we quantified the number of PV$^+$ neurons expressing GAD67 after PCP-treatment by permanently labeling them with a PV^{CRE}::TdTomato mouse line. PCP caused 223±45 TdTomato$^+$ neurons to stop expressing GAD67 (fig. S4). This result suggests that, except for its effect on PV$^+$ neurons, PCP does not affect the GABAergic phenotype of PL interneurons.

To investigate whether glutamatergic neurons that have gained GABA contribute to cognitive deficits, we selectively suppressed GABA expression in PL glutamatergic neurons by injecting a Cre-dependent adeno-associated virus (AAV) expressing shRNA for GAD1 (AAV-DIO-shGAD1-GFP or AAV-DIO-shScr-GFP as control) in the PL of VGLUT1^{CRE} mice before exposure to PCP (Fig. 1, I and J). shGAD1 suppressed GABA expression in transfected cells (fig. S5, A to C), and reduced the number of PL neurons co-expressing GAD1 and VGLUT1 transcripts in both PCP- and saline-treated mice to half of that in saline-ShScr controls (Fig. 1K). Having efficiently overridden PCP-induced gain of GABA, we examined the impact of shGAD1 on PCP-induced behavior. While shGAD1 did not affect PCP-induced hyperlocomotion on the first day of treatment, it prevented appearance of locomotor sensitization after a 10-day PCP-exposure (Fig.

1L and fig. S5, D and E), indicating that PCP-induced gain of GABA is required for sensitization to the acute locomotor effect of the drug. We next focused on deficits in recognition and working memory, since these behaviors are affected by repeated exposure to PCP (*13, 18*) and are regulated by the PL (*19, 20*). shGAD1 prevented PCP-induced impairments of recognition memory in the novel object recognition test (NORT) (Fig. 1M) and deficits in spatial working memory in the spontaneous alternation task (SAT) (Fig. 1N). Neither PCP-treatment nor shGAD1 changed exploratory behaviors in the NORT and in the SAT (fig. S5, G and H). shGAD1 did not affect the behavioral performances of saline-treated controls (Fig. 1, L to N), suggesting that glutamatergic neurons gaining GABA upon PCP-exposure, but not those co-expressing GABA before drug-exposure, mediated drug-induced locomotor sensitization and memory impairments. The number of PL VGLUT1^{+}/GAD1^{+} neurons was positively correlated with locomotor sensitization, and negatively correlated with object recognition and working memory performance (fig. S5, F, I and J). Overall, these data indicate that PCP-induced gain of GABA in PL glutamatergic neurons produces these behavioral alterations.

Methamphetamine changes the transmitter phenotype of the same prelimbic neurons affected by phencyclidine

Because METH causes memory deficits similar to those induced by PCP (*20-22*), we asked whether METH-treatment would also affect the transmitter identity of PL glutamatergic neurons. Resembling the effect of PCP, 10 days of METH-treatment (1 mg/kg/day) increased the number of mCherry^{+} PL neurons co-expressing GABA and GAD67 by 1.7- and 1.9-fold (Fig. 2, A to C, and fig. S6, A to C), without changing the number of mCherry^{+} cells (fig. S6D) or inducing apoptosis or neurogenesis (fig. S2). We next used FISH to examine the expression of the VGAT and VGLUT1 in mCherry^{+} neurons that co-expressed or gained GABA. Similar to the effects of PCP-treatment, mCherry^{+}/GAD1^{+} neurons in the PL of METH-treated mice expressed a high level of VGAT (equal to that of neurons expressing GAD1 but not mCherry) (Fig. 2D, and fig. S6E). The expression level of VGLUT1 decreased by ~76% compared to that of glutamatergic cells expressing mCherry and not GAD1 (Fig. 2E, and fig. S6F). The number of mCherry^{+} neurons gaining expression of GABA, GAD67 and VGAT and reducing their expression level of VGLUT1 after METH-treatment resembled the number observed in PCP-treated mice, indicating that both drugs affect PL glutamatergic neurons similarly.

Glutamatergic neurons that co-express GABA or gain it after treatment with either drug were most prevalent in layer 2/3 and layer 5 of the PL (Fig. 1D, Fig. 2C, and fig. S7). These PL layers innervate the nucleus accumbens (NAc) (*23*), which modulates behaviors that are affected by repeated intake of PCP or METH (*24-26*). To determine whether neurons that switch transmitter identity project to the NAc, we injected fluoro-gold (FG) into the NAc of VGLUT1^{CRE}::mCherry mice, treated them with PCP, METH or saline, and screened the PL for mCherry^{+}/GABA^{+} neurons expressing the retrograde tracer (fig. S8, A to C). In both PCP- and METH-treated mice, ~0.9% of FG^{+} neurons were mCherry^{+}/GABA^{+}. Such cells were less frequent

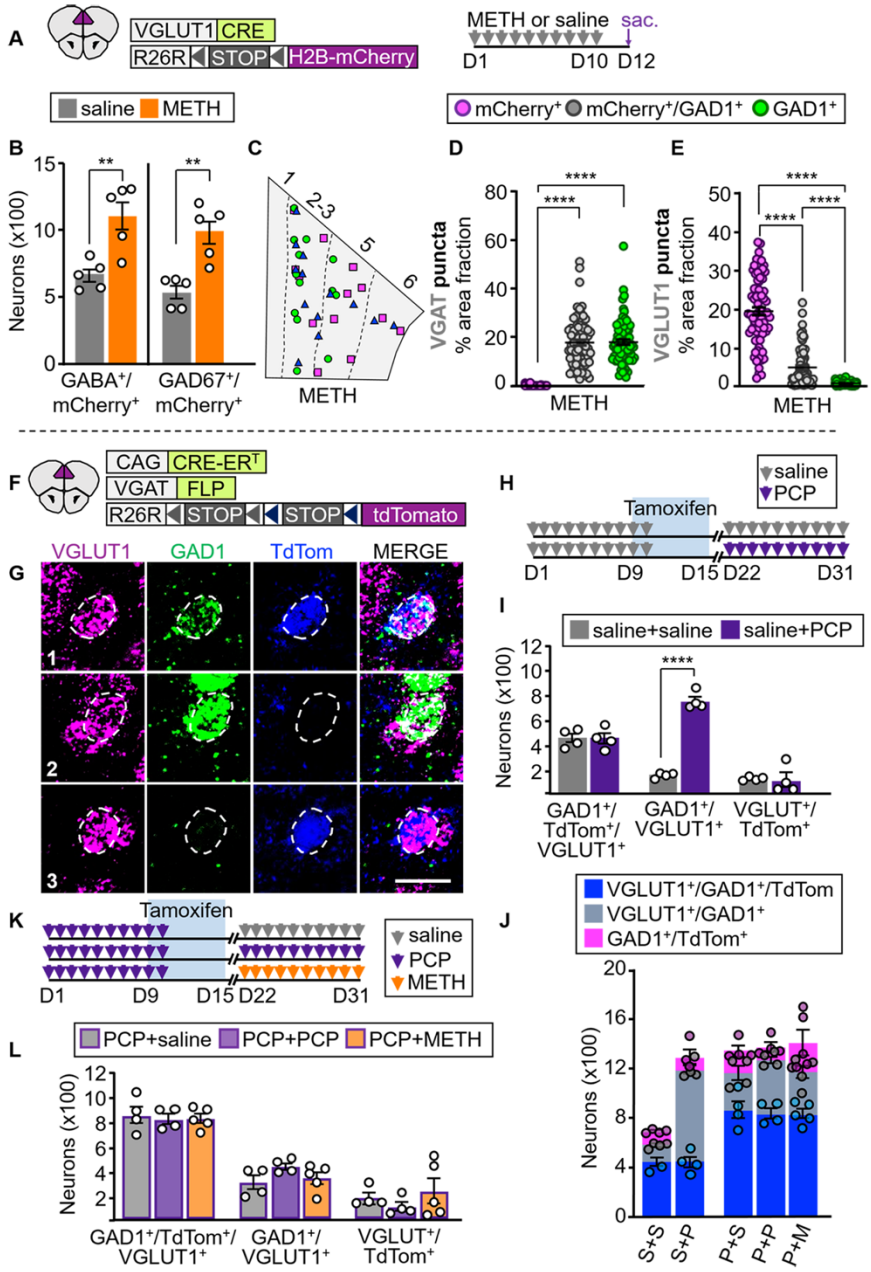

Fig. 2. METH causes the same change in PL glutamatergic neuron transmitter phenotype. (**A**) Experimental protocol. (**B**) Quantification of PL neurons co-expressing mCherry and GABA or GAD67 (n=5 mice per group). (**C**) PL locations of glutamatergic neurons that co-expressed GABA or changed transmitter identity upon treatment with METH generated as in Fig. 1D. (**D** and **E**) VGAT and VGLUT1 expression measured as percent of cell area occupied by mRNA puncta (n=25 cells/type/mouse for 3 mice). (**F**) Mouse line used for tamoxifen-inducible genetic labeling. (**G**) Examples of neurons that are VGLUT1$^+$/GAD1$^+$/TdTom$^+$ (1), VGLUT1$^+$/GAD1$^+$ (2), and VGLUT1$^+$/TdTom$^+$ (3). (**H**) Experimental protocol to test the efficiency and specificity of TdTomato labeling and distinguish VGLUT1 neurons co-expressing GAD1 in drug-naïve conditions from those gaining GAD1 upon PCP treatment. (**I**) Quantification of the neurons shown in (G) in mice treated as described in (H) (n=4 mice per group). (**J**) Cumulative number of neurons that are VGLUT1$^+$/GAD1$^+$/TdTom$^+$, VGLUT1$^+$/GAD1$^+$, and VGLUT1$^+$/TdTom$^+$ from (I) and (L) (S+S: saline+saline; S+P: saline+PCP, P+S: PCP+saline; P+P: PCP+PCP; P+M: PCP+METH). (**K**) Experimental protocol to learn whether serial administration of PCP and METH changes the transmitter phenotype of the same number of neurons as PCP alone, causes neurons that have gained GAD1 to lose it, or enables other neurons to gain GAD1. (**L**) Quantification of these neurons in mice treated as described in (K) (n=4 to 5 mice per group). Scale bar, (G) 20 µm. Statistical significance (**P<0.01, ****P<0.0001) was assessed using unpaired t-test or Mann Whitney test (B), Kruskal-Wallis followed by Dunn's test (D and E), and two-way ANOVA followed by Tukey's test (I and L). Data are mean ± SEM. Further statistical details are presented in Table S2.

in controls (~0.3% of the total number of FG$^+$ neurons) (fig. S8D), indicating that neurons changing transmitter identity with drug-treatment project to the NAc.

Since both PCP and METH affect the transmitter phenotype of PL glutamatergic neurons that have the NAc as a shared downstream target, we asked whether both drugs change the transmitter identity of

the same cells. If PCP and METH changed the transmitter identity of different cells, administering the two drugs one after the other should induce gain of GABA in neurons that have not gained it after treatment with the first drug. To determine whether this was the case, we genetically labeled neurons expressing GABAergic markers during the interval between the delivery of PCP and METH, using VGAT^{FLP}::CreER^{T}::TdTomato$^{cON/fON}$ mice in which neurons expressing VGAT at the time of tamoxifen administration are permanently labeled with TdTomato (Fig. 2F). We first injected tamoxifen in saline-treated controls and determined that TdTomato labels neurons co-expressing VGLUT1 and GAD1 in drug-naïve conditions with 77% efficiency and 79% specificity (Fig. 2G, and fig. S9).

We then used this labelling approach to distinguish neurons expressing GAD1 in drug-naïve mice from those gaining it upon drug-exposure, by administering mice with PCP after saline- and tamoxifen-treatment (Fig. 2H). PCP administration increased the total number of PL VGLUT1^{+}/GAD1^{+} neurons 2-fold compared to controls (1188±23, saline+PCP; 582±27, saline+saline) (Fig. 2I), in line with previous findings (Fig. 1K). We detected no differences in the number of VGLUT1^{+}/GAD1^{+}/TdTomato^{+} neurons (441±43, saline+PCP; 447±34, saline+saline) and VGLUT1^{+}/TdTomato^{+} neurons (99±61, saline+PCP; 120±8, saline+saline) between saline+PCP mice and saline+saline controls (Fig. 2, G to J). Changes in these numbers would have indicated that drug-treatment caused some glutamatergic neurons co-expressing GAD1 in drug-naïve conditions to lose expression of GAD1. The results indicate that PCP induces expression of GAD1 in a population of PL neurons that were not previously expressing it, without affecting the transmitter phenotype of cells co-expressing GAD1 and VGLUT1 in drug-naïve conditions.

We next used VGAT^{FLP}::CreER^{T}::TdTomato$^{cON/fON}$ mice to determine if PCP and METH cause the same neurons to switch transmitter phenotype. Mice were treated first with PCP followed by tamoxifen administration, and then treated with either saline, METH or PCP (Fig. 2K). Across treatment groups the total number VGLUT1^{+}/GAD1^{+} neurons was unchanged (1169±46, PCP+saline; 1273±69, PCP+PCP; 1177±45, PCP+METH) (Fig. 2, J and L), indicating that consecutive administration of drugs does not cause additional glutamatergic neurons to gain GAD1. Furthermore, we did not detect differences in the number of VGLUT1^{+}/GAD1^{+}/TdTomato^{+} neurons (861±73, PCP+saline; 831±43, PCP+PCP; 832±38, PCP+METH) nor in the number of VGLUT1^{+}/TdTomato^{+} neurons (180±44, PCP+saline; 108±41, PCP+PCP; 237±103, PCP+METH) (Fig. 2, J and L). A decrease in the first population and an increase in the second population would have indicated loss of GAD1 from some VGLUT1^{+} neurons and gain of GAD1 in another population of VGLUT1^{+} neurons. These results indicated that consecutive administration of PCP and METH does not cause gain of GAD1 by additional neurons, nor induces neurons that gained GAD1 upon PCP-treatment to revert to their original transmitter phenotype. Thus, PCP and METH change the transmitter identity of a largely overlapping population of PL neurons.

Drug-induced prelimbic hyperactivity mediates the change in transmitter phenotype and linked cognitive deficits

Demonstration that both PCP and METH have the same effect on the transmitter phenotype of the same PL glutamatergic neurons prompted investigation of the underlying mechanism of drug action. Increased neuronal activity can cause neurons to change the transmitter they express (*8, 27, 28*). Could PCP and METH induce alterations in PL activity that mediate the switch in PL neuron transmitter phenotype? Both PCP and METH increased c-fos expression in PL glutamatergic neurons by 3.8- and 3.5-fold after a single injection and by 2.6- and 3.7-fold throughout a 10-day treatment (fig. S10, A to D, and F and G). This PL hyperactivity was still present 2 days after the end of drug-treatment (fig. S10, I to K). To determine whether this increase in activity promoted the switch in transmitter phenotype, we tested whether suppression of PL hyperactivity would prevent glutamatergic neurons from gaining GABA.

Glutamatergic cells in the PL receive perisomatic inhibition from local PV^+ interneurons, which do not show changes in c-fos expression after administration of PCP or METH (fig. S10, E and H). We hypothesized that chemogenetic activation of PV^+ neurons would suppress drug-induced hyperactivity of glutamatergic cells (*29, 30*). To test this idea, we expressed the chemogenetic receptor PSAML-5HT3HC in mPFC PV^+ neurons and administered $PSEM^{308}$ immediately before acute injection of either PCP or METH (fig. S11, A to E). While c-fos was increased in GFP^+ neurons infected with the virus (fig. S11F), consistent with their expected activation, both PCP- and METH-induced hyperactivity were suppressed in PL glutamatergic neurons (fig. S12).

We then combined chemogenetic activation of PL PV^+ interneurons with either PCP- or METH-administration for the duration of drug-treatment (Fig. 3, A and B). The number of $VGLUT1^+/GAD1^+$ neurons in the PL of PCP- and METH-treated mice that received $PSEM^{308}$ was half of that of mice that did not (586±18 and 611±23 vs 1262±66 and 1222±12) and was indistinguishable from that of saline-treated controls (Fig. 3, C and D). Thus, suppression of drug-induced PL hyperactivity is sufficient to prevent glutamatergic neurons from switching their transmitter identity, indicating that hyperactivity mediates the change in transmitter phenotype.

We now tested whether blocking the change in transmitter phenotype through chemogenetic activation of PV^+ neurons was sufficient to prevent drug-induced cognitive deficits. In mice treated with $PSEM^{308}$, drug-induced hyperlocomotion was absent on both the first and last days of treatment (fig. S13, A to C, and fig. S14, A to C), consistent with suppression of acute drug-induced hyperactivity of PL glutamatergic neurons (*31*). Suppressing PL activity prevented both PCP- and METH-induced appearance of memory deficits in both the NORT and the SAT (Fig. 3, E to H, fig. S13, F and G and fig. S14, F and G), without influencing exploratory behaviors (fig. S13, D and E, and fig. S14, D and E). The results indicate that chemogenetic activation of PV^+ neurons in the PL has no effect on the behavior of saline controls and specifically affects the performance of drug-treated mice.

Normalizing prelimbic neuron activity after drug-exposure reverses the change in transmitter identity and the associated behavioral alterations

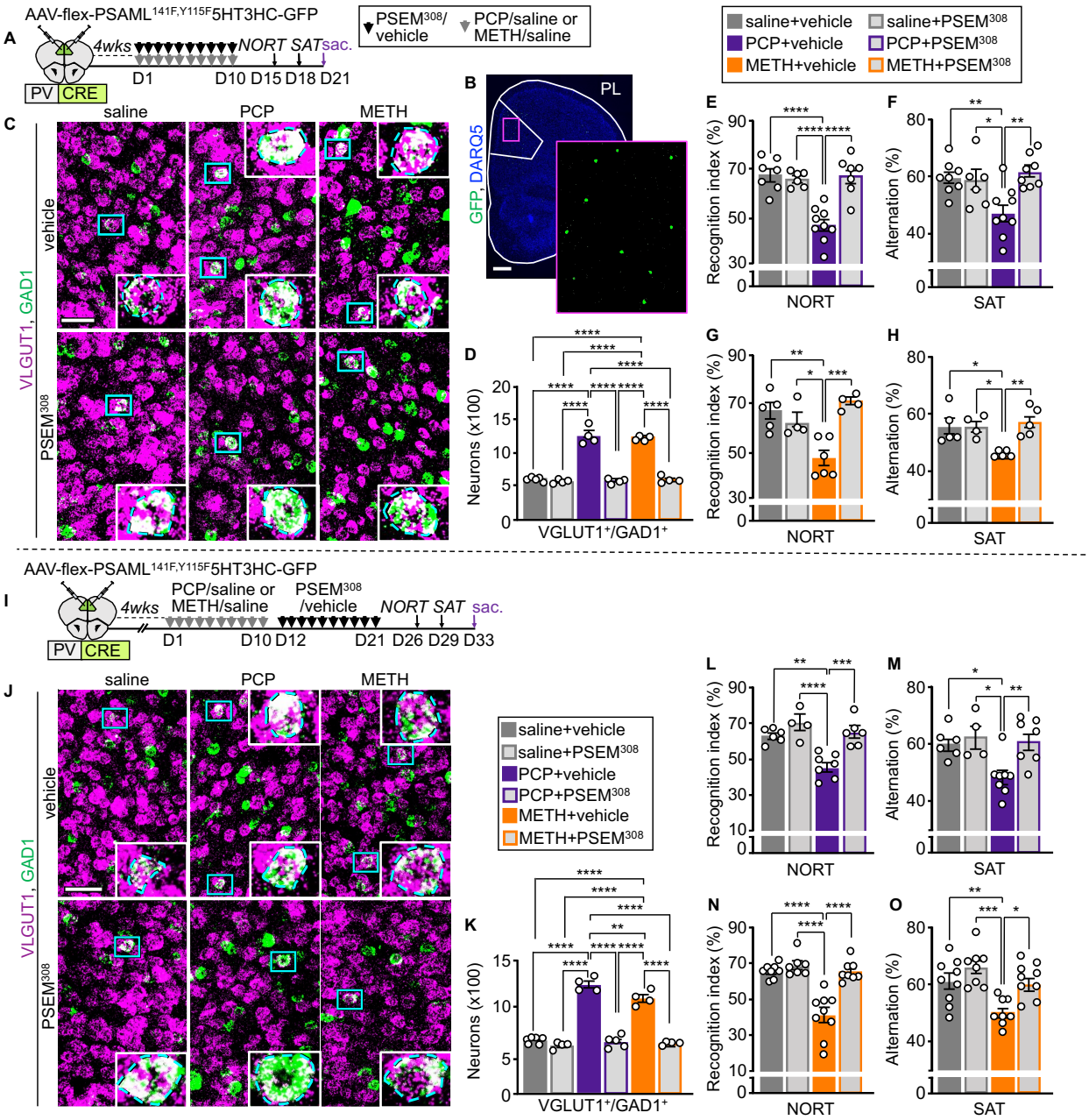

Fig. 3. Suppression of drug-induced PL hyperactivity prevents or reverses the change in transmitter identity and drug-induced behavioral alterations. (**A**) Experimental protocol. (**B**) Expression of PSAML-5HT3HC-GFP in the PL. (**C**) VGLUT1 and GAD1 expression in the PL following saline/drug treatment combined with chemogenetic activation of PV^{+} neurons. (Blue rectangles) neurons co-expressing VGLUT1 and GAD1 illustrated at high magnification in the insets. (**D**) Quantification of (C) (n=4 to 5 mice per group). (**E** to **H**) Chemogenetic activation of PV^{+} neurons during drug treatment prevents the deficits in the NORT and SAT induced by both PCP and METH (n=4 to 9 mice per group). (**I**) Experimental protocol. (**J**) PL expression of VGLUT1 and GAD1 after exposure to saline/drug treatment followed by chemogenetic activation of PV^{+} neurons. (Insets) Higher magnification of VGLUT1^{+}/GAD1^{+} neurons. (**K**) Quantification of (J) (n=4 to 6 mice per group). (**L** to **O**) PCP- and METH-induced deficits in the NORT (n=4 to 7 mice per group) and SAT (n=4 to 9 mice per group) are reversed by sustained activation of PV+ neurons. Scale bars, (B) 500 µm, (C and J) 50 µm. Statistical details are presented in Table S3.

PL glutamatergic neurons that change their transmitter upon exposure to PCP or METH retain the GABAergic phenotype for at least 11 days of drug washout (Fig. 3D, and PCP+saline group in Fig. 2L). As PCP- and METH-induced memory deficits are also long-lasting (*13, 32*), we asked whether the persistence of behavioral deficits is linked to retention of the GABAergic phenotype and whether both can be reversed.

Clozapine, a powerful antipsychotic drug, reverses PCP-induced deficits in the NORT (*13*), leading us to investigate whether it also reverses the change in transmitter phenotype. VGLUT1^{CRE}::mCherry mice that received PCP displayed 1.94 fold more mCherry+/GABA+ PL neurons than controls, 17 days after the end of PCP-treatment, indicating that neurons had maintained the newly acquired GABAergic phenotype (fig. S15, A to C). In mice that received clozapine treatment after PCP, the number of mCherry$^+$/GABA$^+$ neurons was reduced compared to that of mice treated with PCP alone (559±55 vs 1124±94) and not different from that of saline-treated controls (fig. S15, A to C). Clozapine did not affect the number of mCherry$^+$/GABA$^+$ neurons in saline-treated mice, suggesting that this drug selectively reverses the PCP-induced change in glutamatergic neuron transmitter identity. We confirmed that clozapine rescued PCP-induced memory deficits in the NORT and SAT, without affecting the behavioral performance of controls (fig. S15, D to J).

We next investigated the mechanisms underlying clozapine-induced reversal of the switch in transmitter identity. Because clozapine suppresses the acute PCP-induced increase in PL c-fos expression (*33, 34*), we asked whether reversal of the gain of GABA depends on neuronal activity. After drug-treatment, the number of c-fos$^+$ glutamatergic neurons was 2.1- and 2.8-fold higher in PCP- and METH-treated mice compared to controls (fig. S10, I to K). Administration of clozapine after PCP-treatment returned c-fos expression to baseline (fig. S16), suggesting that PL hyperactivity during drug-washout is necessary to maintain the newly acquired transmitter phenotype. If this were the case, suppressing PL hyperactivity after the transmitter switch has occurred should reverse the change. To test this hypothesis, we chemogenetically activated PV$^+$ neurons for 10 days to normalize c-fos expression in the PL of PCP- and METH-treated mice after the change in transmitter phenotype had taken place (fig. S17). More than 3 weeks after the end of drug-treatment, glutamatergic neurons in the PL of both PCP and METH-treated mice still displayed the drug-induced GABAergic phenotype (Fig. 3, I to K). Normalizing PL activity decreased the number of VGLUT1$^+$/GAD1$^+$ neurons in the PL of PCP and METH-treated mice to the level of controls (588±27 and 575±9 vs 1225±38 and 1071±42) (Fig. 3, I to K). Thus, PL neuronal activity maintains the transmitter switch once it has been induced. Chemogenetically activating PL PV$^+$ interneurons after the change in transmitter phenotype had occurred also rescued memory deficits in the NORT and SAT and suppressed locomotor sensitization to both PCP and METH (Fig. 3, L to O, fig. S18 and fig. S19). Overall, these data show that suppressing PL hyperactivity following drug-exposure reverses the change in transmitter phenotype and the associated behavioral alterations.

Dopamine signaling is necessary and sufficient to change the transmitter identity of prelimbic neurons

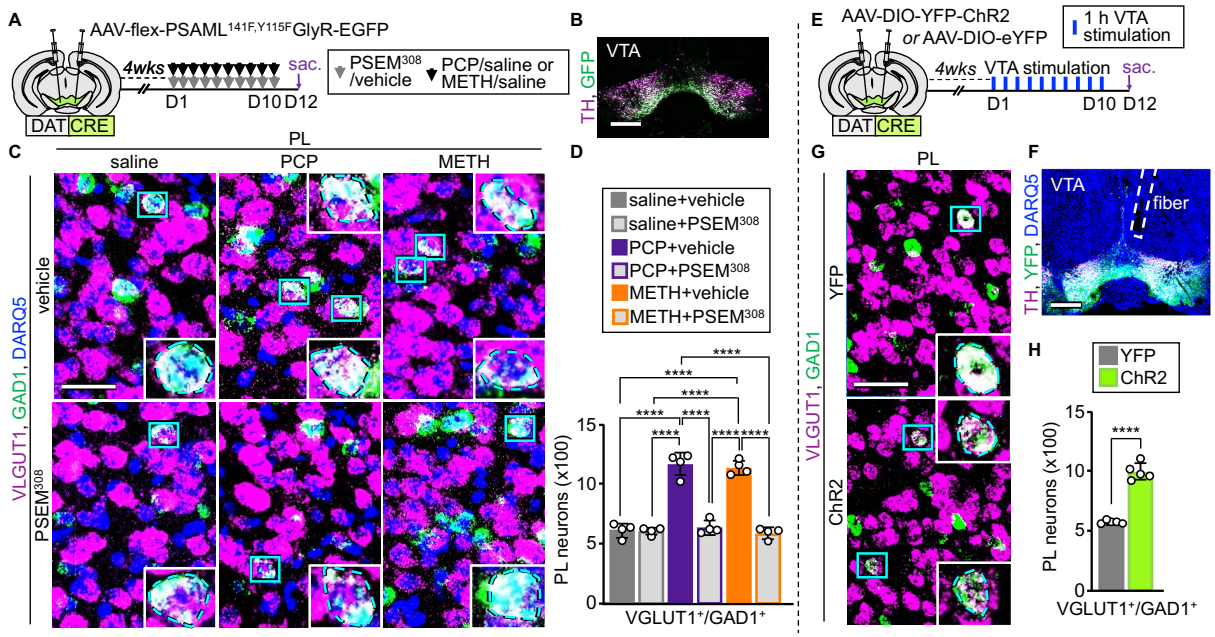

Fig. 4. Activity of VTA DA neurons is necessary and sufficient to induce PL glutamatergic neurons to switch their transmitter identity. (A) Experimental protocol. (**B**) Expression of PSAML-GlyR-GFP in the VTA. (**C**) VGLUT1 and GAD1 expression in the PL following saline/drug treatment combined with chemogenetic inhibition of VTA dopaminergic neurons. (Blue rectangles) neurons co-expressing VGLUT1 and GAD1 illustrated at high magnification in the insets. (**D**) Quantification of VGLUT1$^+$/GAD1$^+$ neurons in the PL (n=4 to 5 mice per group). (**E**) Experimental protocol. (**F**) Expression of ChR2-YFP in the VTA and fiber location. (**G**) VGLUT1 and GAD1 expression in the PL following 10 days of VTA optogenetic stimulation. (Insets) Higher magnification of neurons co-expressing VGLUT1 and GAD1. (**H**) Quantification of VGLUT1$^+$/GAD1$^+$ neurons in the PL (n=5 mice per group). Scale bars, (B and F) 500 µm, (C and G) 50 µm. Statistical significance (****P<0.0001) was assessed using two-way ANOVA with Tukey's multiple-comparisons test (D) and unpaired t-test (H). All data are presented as mean ± SEM. Further statistical details are presented in Table S4.

PCP, METH, and other addictive substances increase phasic firing of dopaminergic neurons in the ventral tegmental area (VTA) (*35, 36*) and increase the levels of extracellular dopamine (DA) in the striatum and prefrontal cortex (*37, 38*). Could DA signaling be a common mediator for the PCP- and METH-induced transmitter switch? To address this question, we tested whether suppressing the activity of VTA dopaminergic neurons during treatment with PCP or METH affects the number of PL neurons that switch transmitter phenotype. We expressed the PSAML-GlyR chemogenetic receptor in the VTA of DAT^{CRE} mice (fig. S20, A to D). Administration of PSEM^{308} before drug-injection suppressed the acute PCP- and METH-induced increase in c-fos$^+$ dopaminergic neurons (fig. S20, E and F). Combining VTA suppression with drug administration for the entire duration of treatment, by co-administration of PSEM^{308} and PCP or METH, prevented the increase in the number of PL VGLUT1$^+$/GAD1$^+$ neurons (Fig. 4, A to D). These results show that drug-induced increase in activity of VTA dopaminergic neurons is required for PL neurons to change their transmitter phenotype upon treatment with PCP or METH.

It remained unclear, however, whether DA signaling alone is sufficient to induce PL neurons to switch transmitter phenotype, or whether non-dopaminergic effects of PCP or METH are involved. Phasic firing of VTA neurons can be mimicked by optogenetic stimulation of VTA dopaminergic neurons (*39*). We

asked whether repeated optogenetic stimulation of VTA dopaminergic neurons is sufficient to induce PL glutamatergic neurons to switch transmitter identity. We expressed ChR2-YFP (or YFP as control) in VTA DAT^{CRE} neurons and implanted an optic fiber above the VTA (Fig. 4, E and F). Administration of 80 sets of laser stimulation (each consisting of 30 bursts of 5 pulses of 4 ms at 20 Hz) over the course of 1h increased the number of VTA dopaminergic neurons expressing c-fos by 7.3-fold in mice expressing ChR2 (fig. S21). Expression of c-fos in PL glutamatergic neurons was also increased by 2.7-fold, resembling the effect of a single dose of PCP or METH (fig. S22, A to D). We then exposed mice to 1h of VTA stimulation per day for 10 days and analyzed the transmitter phenotype of PL glutamatergic neurons (Fig. 4E). Remarkably, the number of $VGLUT1^{+}/GAD1^{+}$ was 1.7-fold higher in ChR2-expressing mice compared to controls (Fig. 4, G and H, and fig. S22, E to G), demonstrating that phasic firing of dopaminergic neurons in the VTA changes the transmitter phenotype of PL glutamatergic neurons. These findings establish DA signaling as a common mediator for PCP- and METH-induced gain of GABA in PL glutamatergic neurons and suggest that exposure to other addictive substances that activate the VTA could produce similar effects.

DISCUSSION

We show that a change in the transmitter identity of PL glutamatergic neurons is a shared mechanism underlying both PCP- and METH-induced cognitive deficits. Both drugs cause the same PL neurons to acquire a new transmitter phenotype characterized by expression of GABA, GAD67, and VGAT, combined with lower levels of VGLUT1. Other PL neurons show the same transmitter phenotype in drug naïve conditions, as suggested by earlier studies (*40, 41*). This change in transmitter phenotype causes locomotor sensitization and memory deficits in the NORT and SAT, consistent with the involvement of the PL and the NAc, which receives input from PL neurons that change their transmitter identity (*19, 20, 24-26, 42*). Given the role of GABAergic long-range projections in modulating brain oscillation and synchronization (*43*), gain of GABA by PL neurons projecting to the NAc may contribute to the reduction of NAc firing rates and disruption of cortex-accumbens synchronization after PCP-treatment (*44*).

Neuronal activity mediates this drug-induced change in transmitter identity, as expected for activity-dependent neurotransmitter switching (*8, 27, 28*), and is necessary to maintain the newly acquired transmitter phenotype after the end of drug-treatment. Midbrain cholinergic neurons that change transmitter identity in response to sustained exercise spontaneously revert to expression of their original transmitter within a week of cessation of the stimulus (*8*). In contrast, PL glutamatergic neurons maintain their GABAergic phenotype for more than 3 weeks after the end of drug-treatment and the linked cognitive deficits are long-lasting (*13, 32*). c-fos expression in the PL increases after acute treatment with PCP or METH (*33, 45*), as well as after one hour of phasic stimulation of VTA dopaminergic neurons, and remains elevated for at least two weeks (fig. S16, fig. S17, and *46, 47*). Suppressing drug-induced hyperactivity during or after drug-treatment, respectively, prevents or rescues the switch in transmitter phenotype and the coupled behavioral alterations, indicating that PL hyperactivity is necessary to produce and maintain

these changes. Because exposure to either PCP or METH decreases expression of PV and GAD67 (*17, 48*), impaired function of PFC PV^+ interneurons may contribute to PL glutamatergic neuron hyperactivity and maintenance of the newly acquired transmitter phenotype. Chronic treatment with clozapine also reduces c-fos expression in the PL of PCP-treated mice and reverses PCP-induced changes in transmitter phenotype and behavior. This effect may be mediated by increased inhibitory input to PL glutamatergic neurons (*49*).

DA signaling is necessary for PL neurons to change their transmitter identity, because suppression of dopaminergic hyperactivity in the VTA during PCP- or METH-treatment prevents it. Optogenetic stimulation of phasic firing of dopaminergic neurons in the VTA is sufficient to cause PL hyperactivity and induce the switch in transmitter phenotype. Many addictive substances promote phasic firing of these dopaminergic neurons (*50*). Reproducing this firing pattern optogenetically enhances DA release in the NAc (*51*) and replicates some of the neuroplastic and behavioral effects of cocaine, including conditioned place preference and acquisition of self-administration that can lead to compulsive-like behavior (*39, 51*). This stimulation only models some aspects of drug intake (*52*) and does not account for the non-dopaminergic mechanisms through which multiple drugs of abuse differentially impact brain function and behavior. Evidence that multiple drugs of abuse acutely promote mPFC hyperactivity (*53, 54*), and that stimulation of VTA dopaminergic neurons is sufficient to change the transmitter identity of PL neurons, raises the possibility that drugs other than METH and PCP may induce cognitive deficits by switching the transmitter phenotype of PL glutamatergic neurons.

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Supplementary Materials

Materials and Methods

Figs. S1 to S22

Tables S1 to S24