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Inhibition of GABA interneurons in the mPFC is sufficient and necessary for rapid antidepressant responses

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Abstract

Major depressive disorder (MDD) is associated with alterations of GABAergic interneurons, notably somatostatin (Sst) as well as parvalbumin (Pvalb), in cortical brain areas. In addition, the antidepressant effects of rapid acting drugs are thought to occur via inhibition of GABA interneurons. However, the impact of these interneuron subtypes in affective behaviors as well as in the effects of rapid acting antidepressants remains to be determined. Here, we used a *Cre*-dependent DREADD-chemogenetic approach to determine if inhibition of GABA interneurons in the mPFC of male mice is sufficient to produce antidepressant actions, and conversely if activation of these interneurons blocks the rapid and sustained antidepressant effects of scopolamine, a non-selective acetylcholine muscarinic receptor antagonist. Chemogenetic inhibition of all GABA interneurons (Gad1+), as well as Sst+ and Pvalb+ subtypes in the mPFC produced dose and time-dependent antidepressant effects in the forced swim and novelty suppressed feeding tests, and increased synaptic plasticity. In contrast, stimulation of Gad1, Sst or Pvalb interneurons in mPFC abolished the effects of scopolamine and prevented scopolamine-induction of synaptic plasticity. The results demonstrate that transient inhibition of GABA interneurons promotes synaptic plasticity that underlies rapid antidepressant responses.

Keywords

depression; somatostatin; parvalbumin; interneuron; DREADD; VGLUT1; medial prefrontal cortex; scopolamine

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Author contributions

M.V.F. designed the study, performed the experiments, analyzed the data and wrote the manuscript. M.W. performed the electrophysiological experiments and analyzed the data. X-Y. L. performed the genotyping of the animals. C. L. helped to perform the CUS experiments. M.R.P. provided scientific input for experiments added in revision and contributed to editing and revising the manuscript. R.S.D. designed the study, revised and contributed to writing the manuscript.

Introduction

Major Depressive Disorder (MDD) is a recurring illness that is among the leading contributors to social and economic burden, estimated to be the second leading cause of disability world-wide (1, 2). Current pharmacological treatments, notably the monoaminergic medications, take weeks to months to produce a therapeutic response, and a third of patients fail to respond to two or more trials and are regarded as treatment resistant (3). Considering the secondary health implications, as well as the time lag and low efficacy of currently medications, there has been tremendous interest in rapid-acting antidepressants, notably ketamine, an N-methyl-D-aspartate (NMDA) receptor blocker, and scopolamine, a muscarinic receptor (ACh-M) antagonist (4–7). Low doses of ketamine or scopolamine induce rapid (within 2 to 24 h), sustained (up to 7 days) antidepressant effects in MDD patients (4–6, 8, 9), stimulating interest in the molecular mechanisms underlying these rapid antidepressant effects and how they relate to the pathophysiology of MDD.

Accumulating evidence indicates that dysfunction of GABA, as well as glutamate systems contributes to depression-related behavior (10–18). GABA interneurons have a critical role in orchestrating excitation:inhibition balance within the mPFC, as well as excitatory outputs to projection areas, by targeting specific subcellular domains of glutamatergic pyramidal neurons, and controlling cortical firing rate, bursting, timing, synchrony and rhythms. Three major non-overlapping interneuron groups in the neocortex can be identified accordingly to their expression profile of neurochemical markers, including those that express the calcium-binding protein parvalbumin (Pvalb, ~40%), the neuropeptide somatostatin (Sst, ~30%) and the ionotropic serotonin receptor 3 (~30%) (19, 20). MDD subjects and rodent stress models show alterations in markers of cortical GABA interneurons, notably Sst and, less consistently Pvalb interneurons, (14, 15, 19–26). Sst interneurons have low-threshold regular spiking properties, high basal firing activity, and synapse onto the dendritic tufts of pyramidal neurons, thereby acting as “burst and rate detectors” to regulate synaptic input integration in pyramidal cells (21, 27). Pvalb cells are fast spiking and, through axo-somatic synapses with principal neurons, gate the spiking output of the cell, its timing and thus firing synchrony (21, 28). Considering the pivotal role of GABA in orchestrating excitatory signaling, a leading hypothesis for the rapid actions of ketamine and scopolamine is that these agents act via blockade of NMDA and ACh-M receptors, respectively, on tonic firing GABA interneurons, resulting in disinhibition of excitatory neurons and a subsequent glutamate burst that stimulates rapid and sustained synaptic changes in the medial prefrontal cortex (mPFC) (29, 30). However, there is also evidence that these drugs may act directly on excitatory neurons to produce rapid antidepressant actions (7, 30, 31).

Although both Sst and Pvalb interneurons have been implicated in the pathophysiology of MDD, and direct manipulation of these interneuron subtypes has been shown to influence affective behaviors, there are conflicting reports, and the role of GABA interneuron subtypes remain unknown (15, 32–36). In the present study, we investigate if blockade of GABA interneuron subtypes is sufficient and necessary for rapid antidepressant responses in male mice.

Materials and Methods

Animals

Male transgenic mice and WT C57BL/6 littermates (8–12-week-old) were obtained from in-house breeders. *Glutamic acid decarboxylase* (*Gad1)-Cre* mice were obtained from Marina Piccioto laboratory (37). *Pvalb-Cre* (#008069) and *Sst-Cre* (#013044) mice were obtained from Jackson Laboratories. All animals were group-housed with a 12/12h light-dark cycle and food and water *ad libitum*. All procedures were conducted in compliance with the National Institute of Health (NIH) guidelines for the care and use of laboratory animals and were approved by the Yale Animal Care and Ethics Committee.

Viral constructs and surgery

Adeno-associated viruses AAV2-hSyn-DIO-hM3D(Gq)-mCherry (hM3DGq), AAV2-hSyn-DIO-hM4D(Gi)-mCherry (hM4DGi) and AAV2-hSyn-DIO-mCherry (mCherry), $\geq 4 \times 10^{12}$ vg/ml) were obtained from Addgene (USA). Anesthetized mice (ketamine, 100 mg/kg; and xylazine, 10 mg/kg) received bilateral infusions of stimulatory hM3DGq, inhibitory hM4DGi or control mCherry virus (0.5 μl/side; 0.1μl/min) into the mPFC; mice were used after a 2–3 week recovery period.

Drug administration

Clozapine-N-oxide (CNO) was administered alone (1.0 or 2.5 mg/kg i.p.) at different time points as indicated; these doses were chosen based on reports that higher doses (5–10 mg/kg) produce off-target effects, either directly or via metabolism to clozapine (38, 39). Scopolamine (Sigma-Aldrich, 25 μg/kg, i.p.) was administered every 48 h for 5 days and behavior conducted 24 h after the last injection, based on clinical and previous pre-clinical studies (6, 37, 40).

Chronic Unpredictable Stress

Animals were exposed to a sequence of two random unpredictable stressors per day for 21 days, accordingly to a previous established protocol (41, 42). CUS continued in the days between the behavioral tests. However, to avoid acute effects of stress, animals were not exposed to any stressors on the day of treatments or prior to behavioral tests. Control animals (non-stressed) were handled daily but were not exposed to any stressors.

Behavioral studies

Animals were habituated to test rooms 30 min before each experiment. All behavioral tests were conducted between 10 a.m. and 3 p.m. The Forced Swimming Test (FST) was conducted as previously described (43). Video recorded sessions were scored for total immobility time during minutes 2 to 6. The Novelty Suppressed Feeding Test (NSFT) was also conducted as previously described (44), and latency to feed was measured with a time limit of 12 min. Additional tests were performed following CUS exposure: the Sucrose Splash Test (SUST) and the Female Urine Sniffing Test (FUST), in which the grooming time and the urine or water sniffing time, respectively, was measured during 5 min, as previously described (41, 45). Animals were randomly assigned to the treatment groups and the FST,

NSFT, SUST and FUST were scored by an experimenter blind to treatments. Home cage food intake was measured as a control. Locomotor Activity (LMA) was determined using an infrared automated tracking system (Med Associates) (41).

Western blot

Western blots were conducted as previously described (46) using primary (rabbit anti-PSD95 #2507, rabbit anti-Synapsin1 #6710, rabbit anti-GluA1 #13185, rabbit anti-VGLUT1 #12331, 1:1000, Cell Signaling) and secondary antibodies (anti-rabbit, 1:3000 for VGLUT1 and 1:5000 for all the others, #PI-1000–1, Vector Laboratories); labeled bands were visualized with chemiluminescent reagent (ECL, GE Healthcare). Preserved bands were quantified using the ImageJ Software. GAPDH (#5174, Cell Signaling, 1:5000) was used for loading control and normalization.

Viral infection efficiency and immunofluorescence

Viral expression and placement were visualized by fluorescence microscope (Zeiss, Germany). c-Fos and VGLUT1 immunofluorescence was conducted using primary (rabbit anti-c-Fos #ab190289, Abcam, 1:1000; guinea-pig anti-VGLUT1 #135304, Synaptic Systems, 1:2000) and secondary antibodies (AlexaFluor® 488 goat anti-rabbit #A21206, AlexaFluor® 546 goat anti-rabbit #A11010 or AlexaFluor® 647 goat anti-guinea-pig #A21450, 1:1000). For quantification, 3–4 sections containing the mPFC were analyzed by an experimenter blind to treatments using a confocal microscope to obtain z-stack image sequences (Leica TSE-SPE). See supplemental methods for further details.

Electrophysiological recordings

Coronal slices containing the mPFC were prepared from male mice that received viral infusion of mCherry (control), hM4DGi or hM3DGq (12 to 16 weeks old) for whole-cell recording as previously described (47). Neurons expressing mCherry were visualized using the appropriate filter. Inward or outward currents induced by CNO (10 μM), delivered by y-tube for 15 seconds, were recorded with voltage-clamp technique at a holding potential of −70mV. Pyramidal neurons in mPFC were visualized by videomicroscopy using a microscope (40x IR lens) with infrared differential interference contrast (IR/DIC). Postsynaptic currents were measured in continuous single-electrode voltage-clamp mode (3000 Hz low-pass filter) at −65 mV to separate IPSCs from EPSCs.

Statistical analysis

The results were analyzed by Student’s t-test (two-tailed) and one- or two-way ANOVA, followed by Duncan’s posthoc test, $p \leq 0.05$. All distributions were tested for homogeneity of variance using the Levene’s test and the sampling distribution of the data was assumed to be normal. Sample sizes were chosen based on previous experience with the behavioral tests employed and power analyses (Cohen’s d power analysis, > 0.8 effect size, n = 10/group) conducted following a pilot study (not shown). Because of the possibility of off target viral infusion and loss of animals, we have estimated the number of mice per group to 12. Animals that received infusion outside the mPFC were excluded from analysis (a criteria decided on prior to initiating the experiments). The details of statistical tests and

their outcomes are presented in the figure legends. For all analyses, data are expressed as the mean ± standard error and analyzed using SPSS Software (Version 20.0). Each experiment was replicated at least twice under our experimental conditions.

Results

Chemogenetic inhibition of Gad1 interneurons produces rapid antidepressant effects

Based on evidence that cortical GABAergic interneurons play a role in depression-related behaviors and rapid antidepressant responses (36), we tested the behavioral effects of chemogenetic inhibition of Gad1 cells. The Cre-dependent hM4DGi virus was infused into the mPFC of *Gad1-Cre* and WT littermate controls (Figure 1A). Patch clamp recordings demonstrate that CNO incubation of slices from *Gad1-Cre* mice increased outward currents and hyperpolarized labeled Gad1 interneurons as expected (Figure 1B). There were no significant effects in pre-swim or locomotor tests before CNO, indicating that there are no effects of genotype or viral vector infusions (supplemental Figure 1A). Given the short half-life of CNO in mice (~1 h) (48) we examined several different protocols to identify an optimal dosing regimen (Figure 1C); behavioral analysis was conducted 24 h after dosing when CNO is largely metabolized to avoid acute drug effects, and thereby allow for observation of sustained behavioral effects, similar to scopolamine and ketamine. For this and all experiments both *Gad1-Cre* and WT mice received CNO to control for off-target effects. Behavioral tests included the FST, a measure of behavioral despair responsive to acute antidepressant administration, and NSFT, a measure of anxiety responsive to chronic, but not acute administration of monoaminergic antidepressants. The results demonstrate that a single CNO injection in *Gad1-Cre* mice infused with hMD4Gi virus significantly decreased immobility time in the FST but was ineffective in the NSFT (Figure 1D). There was no effect of CNO administration on LMA (Figure 1D) or home cage food consumption (supplemental Figure 1B).

Next, we tested the effects of 3 CNO injections for more sustained inhibition of Gad1 neurons (Figure 1C). Behavioral testing was conducted 4 h after the last dose when ~94% of the CNO is metabolized, based on a half-life of 1 h. The results demonstrate that 3x CNO dosing in mice infused with hMD4Gi virus produced significant antidepressant actions, reducing immobility time in the FST as well as latency to feed in the NSFT (Figure 1E). To determine if the effects observed with the 3x CNO dosing are due to the shorter time after the last injection (4 h), mice received a single injection of CNO and tested 4 h later (Figure 1C). This regimen had no effect on immobility time in the FST or latency to feed in the NSFT in *Gad1-Cre* mice expressing hM4DGi (Figure 1F). There were no effects on LMA or home cage food consumption for either of these regimens (Figure 1E, F and supplemental Figure 1B).

To investigate whether the antidepressant-like effects observed might be correlated with changes in synaptic activity in the mPFC, *Gad1-Cre* mice were infused with hMD4Gi virus or a control virus expressing mCherry. All mice were subjected to the same 3x CNO dosing regimen previously described (Figure 1E), and slice recordings of pyramidal and Gad1 interneurons were performed (Figure 1G and 1H). The results demonstrate that chemogenetic inhibition of Gad1 interneurons produced an increase in excitatory post-

synaptic currents (EPSCs) in pyramidal neurons and a decrease in inhibitory post-synaptic currents (IPSCs) in Gad1 interneurons in the mPFC (Figure 1G and 1H). Although not statistically significant, there was a trend for increased IPSCs in pyramidal cells. There was no difference in EPSCs in Gad1 interneurons.

Next, we tested if the antidepressant-like effects of 3x CNO could be reproduced using the CUS protocol, a well-validated animal model of depression-like behavior. CUS exposure produced depressive-like effects in the SUST, NSFT and FST in WT-hMD4Gi animals as compared to non-stressed WT-hMD4Gi mice (Figure 1I). These effects were completely reversed by chemogenetic inhibition of Gad1 interneurons using the 3x CNO dosing regimen in *Gad1-Cre* mice infused with hMD4Gi virus. In addition, CNO produced antidepressant-like effects in the FUST test in these animals. No changes in locomotor activity (Figure 1I) or food consumption (supplemental Figure 1B) were observed.

A previous study has reported that acute inhibition of Sst interneurons in mouse precingulate/prelimbic cortex increases emotionality 30 min after a single CNO dose (35), when CNO is still present in the brain. Here, we found that a single injection of CNO had no effect in the FST or NSFT in *Gad1-Cre* mice 30 min after dosing (Supplemental Figure1C).

Chemogenetic inhibition of Sst or Pvalb interneurons produces rapid antidepressant effects

Using the same chemogenetic approach, we determined the effects of CNO-inhibition of Sst and Pvalb interneurons. hM4DGi virus was infused into the mPFC of *Sst-Cre or Pvalb-Cre* mice (Figure 2A). WT, *Cre negative* littermate controls were used for *Sst-Cre* and were injected with hM4DGi virus; for *Pvalb-Cre* experiments, controls were *Pvalb-Cre* positive mice that were infused with a control virus, mCherry. Infusions of hM4DGi virus resulted in Cre-dependent expression in the mPFC of *Sst-* or *Pvalb-Cre* mice (Figure 2B, E), and patch clamp recordings demonstrate functional effects of viral hM4DGi expression (Figure 2C, F).

For these experiments we used the CNO dosing schedule (3x) that produced the most robust effects in the *Gad1-Cre* mice. There were no significant effects in pre-swim or locomotor tests before CNO dosing (supplemental Figure 2A, C). CNO 3x inhibition of *Sst-Cre* or *Pvalb-Cre* mice infused with hMD4Gi virus significantly reduced immobility time in the FST and latency to feed in the NSFT (Figure 2D, G). In contrast, CNO 1x + 4 h or 30 min after dosing had no effects on immobility time in the FST or latency to feed in the NSFT in either *Sst-Cre* or *Pvalb-Cre* mice (Figure 2D, G, supplemental Figure 2E, F). No changes in locomotor activity or food consumption (supplemental Figure 2B, D) were observed

Chemogenetic inhibition of Sst or Pvalb interneurons increases c-Fos+ cell number and VGLUT1

Previous studies demonstrate that ketamine and scopolamine increase the number of c-Fos+ cells, a marker of neuronal activity, in the mPFC (37, 49, 50). Here we examined the effects of CNO inhibition of GABA interneurons on c-Fos+ cell number at 60 min after dosing, a time typically used for studies of cFos (Figure 2A). CNO administration significantly increased the number of c-Fos+ cells in the mPFC of both *Sst-Cre* and *Pvalb-Cre* mice

(Figure 2H). Most Sst and Pvalb positive cells infected with hM4DGi virus did not express c-Fos, suggesting that these cells were effectively inhibited by CNO injection (Figure 2H).

Enhancement of synaptic function via up-regulation of excitatory synaptic proteins is associated with the rapid and sustained antidepressant actions of ketamine and scopolamine (43, 51, 52). This increase in synaptic function is activity dependent and is thought to result from inhibition of GABA function and a burst of glutamate (7). Here we examined the effects of Sst or Pvalb inhibition on VGLUT1 as previously reported (42), allowing for assessment of localized synaptic changes. *Sst-Cre* or *Pvalb-Cre* mice were infused with hM4DGi or control mCherry virus into the mPFC, and received the CNO 3x injection regimen as described for the behavioral tests (Figure 3A). The results demonstrate that chemogenetic inhibition of either Sst or Pvalb interneurons in the mPFC (layer II/III) increased the expression of VGLUT1 (Figure 3B, C). In contrast, CNO 1x + 4 h had no effect on VGLUT1 levels in either *Sst-Cre* or *Pvalb-Cre* mice (Figure 3E, F).

Chemogenetic activation of Gad1, Sst or Pvalb interneurons abolishes the rapid antidepressant-like effects of scopolamine

Previous studies report that scopolamine induces rapid antidepressant effects via blockade of ACh-M1 receptors (37, 49, 52), and viral-mediated cell specific knockdown of ACh-M1 in Gad1 or Sst, but not in Pvalb interneurons in the mPFC, blocks the rapid antidepressant effects of scopolamine (37). Here, we used a chemogenetic approach to determine if the antidepressant effects of scopolamine are mediated by inhibition of GABAergic interneurons in the mPFC. The Cre-dependent stimulatory hM3DGq virus was infused into the mPFC of *Gad1-, Sst-, or Pvalb-Cre* mice and two to three weeks later the effects of CNO-activation (1 mg/kg) of GABA interneurons ± scopolamine (25 μg/kg) were tested (Figure 4A). We used the same scopolamine regimen (3 injections, once every 48 h) as that used for clinical studies and for our previous preclinical research (6, 37, 40). WT littermate controls were used for *Gad1-* and *Sst-Cre* mice and were injected with hM3DGq virus; for *Pvalb-Cre* experiments, controls were *Pvalb-Cre* positive mice that were infused with a control mCherry virus (Figure 4B, E and H). Electrophysiological recordings show that incubation of mPFC slices with CNO induced depolarization (inward currents) in Gad1, Sst and Pvalb interneurons in the corresponding Cre mice infused with hM3DGq (Figure 4C, F and I).

There were no effects on pre-swim or LMA before drug dosing (Supplemental Figure 3A–C). Administration of scopolamine produced antidepressant effects in control mice, decreasing immobility time in the FST and latency to feed in the NSFT (Figure 4D, G and J), and these antidepressant responses were completely abolished by administration of CNO prior to scopolamine in *Gad1-Cre, Sst-Cre* and *Pvalb-Cre* mice infused with hM3DGq virus (Figure 4D, G and J). Interestingly, in the hM3DGq-*Pvalb-Cre* group, mice receiving CNO plus scopolamine showed significantly increased immobility time in the FST in comparison to the WT control group (Figure 4J). There were no differences in locomotor activity (Figure 1D, G and J) or home cage food consumption (supplemental Figure 3D–F) in any of the groups tested.

Chemogenetic activation of Sst and Pvalb interneurons abolishes scopolamine-induction of c-Fos+ and VGLUT1

Scopolamine increases c-Fos expression in rodent mPFC and this effect is abolished by viral-mediated knockdown of the ACh-M1 receptor in Sst interneurons (37, 49). Here, we tested if chemogenetic activation of Sst or Pvalb interneurons in the mPFC abolishes scopolamine-induction of c-Fos. Mice received a single injection of CNO (1 mg/kg), followed 20 min later by vehicle or scopolamine, and 60 min later the animals were perfused for c-Fos immunofluorescence (supplemental Figure 4A). Scopolamine increased the number of c-Fos+ cells in the mPFC of control mice (supplemental Figure 4B–D, supplemental Figure 5), and these effects were completely blocked by CNO in *Sst-Cre* and *Pvalb-Cre* mice infused with the hM3DGq virus. Although most c-Fos labeling observed in the mPFC sections was not located in Sst and Pvalb positive cells, we did observe co-localization of hM3DGq virus and c-Fos, indicating that Sst and Pvalb interneurons were activated by CNO injection.

Previous reports show that scopolamine rapidly increases synaptic number and function in the mPFC (51, 52), and here we determined if scopolamine-induction of synaptic changes are blocked by activation of Sst or Pvalb interneurons. First, we tested the effects of scopolamine in C57BL/6J mice and measured levels of synaptic proteins 24 h after the last of 3 injections (Figure 5A). Western blot analysis shows that scopolamine administration significantly increased levels of PSD95, Syn1 and VGLUT1 and induced a trend for increased GluA1 in the mPFC (Figure 5B and C). The increase in VGLUT1 expression was also confirmed by immunofluorescence (layer II/III) (Figure 5D).

Next, we tested if the increase in VLGUT1 expression induced by scopolamine could be blocked by chemogenetic activation of Sst or Pvalb interneurons. Excitatory hM3DGq or control mCherry viruses were infused into the mPFC of *Sst-Cre* or *Pvalb-Cre* mice, using the same protocol as described for behavioral tests (Figure 5E). CNO was administered 20 min before scopolamine, and 24 h after the last of 3 scopolamine injections mice were perfused for immunofluorescence. The results demonstrate that chemogenetic activation of either Sst or Pvalb interneurons in the mPFC (layer II/III) blocked scopolamine-induction of VGLUT1 expression (Figure 5F, G).

Discussion

Here we find that chemogenetic inhibition of Gad1, Sst or Pvalb interneurons in the mPFC produced dosing and time dependent antidepressant behavioral responses, and increased EPSCs in pyramidal neurons as well as c-Fos and VGLUT1 expression (Table 1). These results demonstrate that both length of GABA inhibition and time afterward are required for the synaptic plasticity alterations underlying antidepressant responses. Importantly, inhibition of Gad1 interneurons also reversed the depression-like effects induced by CUS, suggesting involvement of GABA interneurons in the pathophysiology induced by stress, and therefore relevant to depression. We also found that chemogenetic activation of Gad1, Sst or Pvalb interneurons in the mPFC completely abolished the antidepressant effects of scopolamine and blocked the induction of c-Fos+ and VGLUT1. These results indicate that the initial trigger for the actions of scopolamine is inhibition of GABAergic interneurons,

which results in a burst of glutamate that stimulates neuronal activity and promotes synaptic plasticity in the mPFC (supplemental Figure 6).

Postmortem studies of MDD and rodent chronic stress report reductions in the expression of Sst and less prominently Pvalb in PFC subregions, although there are also conflicting reports (14, 15, 23, 24, 33, 36, 53). Only a few studies have explored the behavioral and molecular consequences of chemogenetic or optogenetic intervention of GABAergic interneurons, and the majority of these have not investigated different dosing or temporal protocols after dosing (Table 1). Also, although the use of constitutive global manipulations has been informative, interpretation of these studies is limited by the lack of regional and temporal control. For example, whereas global deletion of Sst or chemogenetic inhibition of Sst interneurons 30 min prior the test increased emotionality (a z-score measurement of anxiety- and depressive-like behaviors) (33, 35), chronic chemogenetic inhibition of Sst interneurons reduced emotionality [35] (Table 1). Another study reported that global deletion of the $GABA_A$-receptor-$\gamma 2$ subunit, which increases GABA inhibition, produced anxiolytic- and antidepressant-like behaviors (54) (Table 1). There is also a report that chemogenetic inhibition of mPFC Pvalb interneurons produced an increase in learned helplessness behavior, a model of behavioral despair (55) (Table 1). These studies demonstrate different outcomes, increased or decreased anxiety and depression related behaviors depending on the type and/or length of manipulation, as well as interneuron subtype targeted.

Here we examined the effects of direct inhibition of Gad1, Sst, and Pvalb interneurons in the mPFC using different dosing and time after dosing regimens to address this issue (Table 1). We found that a single dose of CNO in *Gad1-Cre* mice produced a partial response (FST, but not NSFT), but that repeated dosing (3x) produced robust antidepressant actions in both the FST and NSFT. These effects were dependent on a time lag of 21–24 h after the first CNO dose, to allow time for synaptic changes, as a single dose of CNO in *Gad1-Cre* mice tested 4 h or 30 min later had no effect on FST or NSFT behaviors. In addition, inhibition of Gad1 interneurons by repeated CNO dosing (3x) completely reversed the depression-like behavioral consequences induced by CUS in tests that evaluate anhedonia, motivation, anxiety and despair. Repeated CNO dosing produced similar antidepressant effects in Sst- and Pvalb-Cre mice, whereas a single dose had no effects 4 h or 30 min later. These findings indicate that sustained inhibition of either Sst interneurons, which control spiking input to principle neurons via synapses onto dendrite tufts (21, 27), or Pvalb cells, which control synchronization and spike timing of excitatory neurons via somatic synapses (21, 28), can produce antidepressant-like effects. Given the heterogeneity of GABA interneurons in the neocortex, additional studies should be performed to differentiate specific subpopulations of Sst and Pvalb interneurons in these responses based on further neurochemical markers that can overlap with these two larger groups (such as calretinin, calbindin and/or cholecystokinin, among others), as well as electrophysiological and morphological features.

The negative findings of a single CNO dose at 30 min differ from a previous study (35), which reported that a single, higher dose of CNO (5 mg/kg) targeting inhibition of Sst interneurons in more dorsal mPFC (prelimbic (PL)/cingulate) increased emotionality measured 30 min after dosing. Notably, emotionality was measured as a z-score that

combines the results of 4 tests (NSFT, elevated plus maze, open field, and cookie test), but there were no significant effects in the NSFT or open field test, and the FST was not performed. Our negative results of Pvalb interneurons 30 min after single CNO dose differ from another study (55) reporting that inhibition of Pvalb interneurons in prelimbic PFC increased depression-like behavior in a learned helplessness model. The current study targeted a more ventral aspect of mPFC that included infralimbic (IL) as well as PL subregions, raising the possibility that regulation of GABA interneurons in the ventral mPFC, which plays an important role in affect regulation and social cognition (56), could contribute to the behavioral effects observed. However, since the PL and IL are anatomically and functionally distinct subregions with different projection patterns to target structures, including the amygdala, bed nucleus of the stria terminalis and hypothalamus (57), it is possible that GABA interneurons in these subregions have different roles in modulating rapid antidepressant responses.

Rapid-acting antidepressants, including ketamine and its metabolite (*2R,6R*)-hydroxynorketamine, as well as scopolamine produce rapid, activity dependent increase in EPSCs, synaptic proteins as well as synapse number and function in the mPFC (40, 43, 52, 58). Here we found that the repeated CNO dosing regimen also increased EPSCs in pyramidal neurons and decreased IPSCs in Gad1 interneurons, suggesting that the cortical excitation produced by a more sustained inhibition of GABA interneurons is long-lasting. Interestingly, similar to our recent findings with ketamine administration (42), there was also a trend for increased IPSCs in pyramidal neurons, probably induced by adaptive self-tuning mechanisms to promote excitation:inhibition balance in the mPFC. Since Gad1+ cells are heterogeneous, additional studies will be necessary to investigate the involvement of different subtypes of interneurons in EPSCs and IPSCs, including Sst and Pvalb cells. We also found that the repeated CNO dosing regimen increased VGLUT1 expression, a marker of presynaptic glutamate terminals in the mPFC of both *Sst-Cre* and *Pvalb-Cre* mice infused with hM4DGi virus. In contrast, a single dose of CNO examined 4 h after dosing had no effect onVGLUT1 levels, consistent with its lack of effect on antidepressant behaviors. These findings indicate that like rapid acting antidepressants, chemogenetic inhibition of Sst and Pvalb interneuron subtypes causes synaptic changes that are associated with antidepressant behavioral responses. The ability of a single CNO dose to rapidly increase c-Fos+ neurons, but not induce changes in synaptic proteins, indicates that neuronal activity alone is insufficient to produce synaptic and antidepressant behavioral responses at the 4 h time tested.

In a recent study we reported that the antidepressant actions of ketamine are blocked by optogenetic stimulation of Gad1 interneurons in the mPFC (42). Here we demonstrate that chemogenetic activation of Gad1, Sst or Pvalb interneurons prior to administration of scopolamine completely abolished the antidepressant effects of scopolamine. These findings provide further support for the disinhibition hypothesis (7, 31), indicating that scopolamine blocks muscarinic receptors on GABA interneurons leading to a glutamate burst that promotes changes in synaptic plasticity. These findings are also consistent with a previous report that ACh-M1 receptor knockdown on GABAergic interneurons, but not principle neurons in the mPFC blocks the antidepressant effects of scopolamine (37). One difference is that the latter study found that knockdown of ACh-M1 on Sst, but not Pvalb interneurons

blocked the antidepressant effects of scopolamine (37), while the current study found that inhibition of either Sst or Pvalb produced an antidepressant response. A possible explanation for this difference is that Sst interneurons show a more robust response to cholinergic stimulation and express higher levels of ACh-M1 in the mPFC (37) compared to Pvalb interneurons (59, 60). In contrast, CNO-stimulation of either Sst or Pvalb cells could inhibit principle neurons via inhibition of terminals or somatic output, respectively and block the effects of scopolamine. This is also consistent with the results that inhibition of either Sst or Pvalb neuronal activity reproduces the antidepressant effects of scopolamine. Further studies should be performed to determine whether, and how, cholinergic signaling onto GABA interneurons influences circuits that lead to the antidepressant actions of scopolamine.

Scopolamine administration increases the expression of c-Fos and synaptic proteins, as well as synaptic function (40, 49, 52), and prevents CUS-mediated reductions of synaptic proteins, including VGLUT1 in the mPFC (51). In addition, VGLUT1 knockdown in the mPFC induces depressive behaviors that are not reversed by scopolamine, indicating that up-regulation of VGLUT1 is required (51). The results of the current study demonstrate that activation of Sst or Pvalb interneurons prevents the increase in c-Fos+ cells number and the up-regulation of VGLUT1 that is associated with the synaptic changes in the mPFC, including the PL and IL areas. Although these are distinct structures, previous studies have shown that administration of scopolamine increases markers of c-Fos in both PL and IL, and that neuronal silencing of either of these subregions blocks the rapid antidepressant-like effects of systemic scopolamine administration (49).

Taken together, our results demonstrate that direct chemogenetic inhibition of Gad1 interneurons, including both the Sst or Pvalb subtypes is sufficient to produce synaptic and behavioral actions, and provide evidence for a key role for mPFC GABAergic neurocircuitry in rapid antidepressant responses (supplemental Figure 6). These findings are consistent with the hypothesis that the initial cellular trigger for the antidepressant actions of scopolamine is inhibition of GABAergic interneurons in the mPFC, which leads to activity dependent increases in synaptic plasticity. Importantly, the results demonstrate that the observed antidepressant responses are dependent on both the length of GABA inhibition and time after dosing to allow for synaptic plasticity. While it is difficult to extrapolate, these findings indicate that it would also be beneficial to optimize dosing schedules for different types of interventions (e.g., pharmacological, deep brain stimulation, and transcranial magnetic stimulation) to achieve maximal therapeutic responses. Finally, these findings will help guide future studies to identify novel cellular targets, for example other neurotransmitter receptors and channels that modulate GABAergic neuronal activity, for development of the next generation of fast-acting antidepressants with fewer side effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Dr. Duman passed away on February 1st, 2020. This article is dedicated to Dr. Duman in memory of his great mentorship and scientific leadership.

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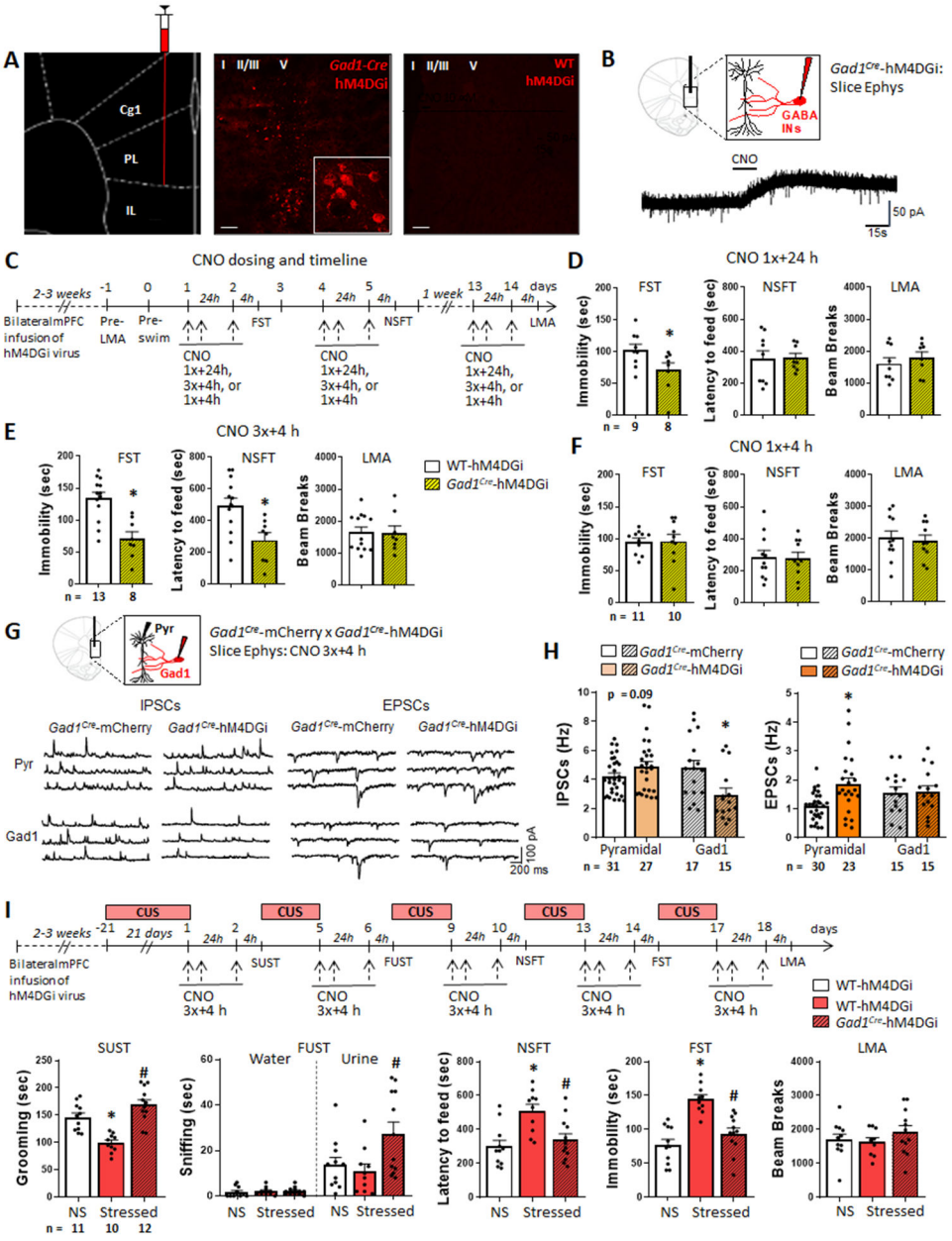

Figure 1. Chemogenetic activation of Gad1 interneurons in the mPFC produces time- and dosing-dependent rapid antidepressant-like effects.

(A) Representative images of the mPFC from *Gad1-Cre* and WT mice that received bilateral infusion of hM4DGi virus (magnification: 10X; inset: 60X). (B) Voltage-clamp recording (−70 mV) of Gad1 interneurons expressing hM4DGi virus in the mPFC. The representative trace shows that CNO (10 μM) application causes hyperpolarization of Gad1 cells (n = 5 Gad1+ neurons were recorded, all of which displayed inhibition by CNO incubation). (C) Experimental time course for surgery, CNO injections (2.5 mg/kg) and the behavioral tests. Animals were submitted to different CNO dosing regimens: mice received a single CNO injection and 24 h later were exposed to the behavioral test (CNO 1x+24 h), three CNO injections (3, 9 p.m and 8 a.m) and 4 h after the last injection were tested (CNO 3x+4 h),

or two vehicle injections (3, 9 p.m.) followed, on the next day, by a single CNO injection (8 a.m.) and 4 h later were tested (CNO 1x+4 h). (D) CNO injection 24 h before the test produced an antidepressant-like effect in the FST (n = 8–9/group; Student's t-test, t_{15} = 2.19, $p < 0.05$) but not in the NSFT ($t_{15} = 0.12$, $p > 0.05$). (E) Chemogenetic inhibition of Gad1 interneurons in the mPFC using the three-dosing regimen produced antidepressant-like effects in the FST (n = 8–13/group; Student's t-test, $t_{19} = 4.30$, $p < 0.05$) and in the NSFT ($t_{19} = 2.99$, $p < 0.05$). (F) No effect was found after a single CNO injection 4 h before the test in the FST (10–11/group; Student's t-test, $t_{19} = 0.03$, $p > 0.05$) and NSFT (t_{19} = 0.14, $p > 0.05$). (D, E and F) CNO injections did not change locomotor activity at any of the time points analyzed (CNO 1x+24 h: $t_{15} = 0.70$, $p > 0.05$); (CNO 3x+4 h: $t_{19} = 0.08$, $p > 0.05$); (CNO 1x+4 h: $t_{19} = 0.34$, $p > 0.05$). (G) Representative traces of postsynaptic currents (PSCs) in pyramidal and Gad1 cells in the mPFC from *Gad1-Cre* mice infused with mCherry (control) or hM4DGi virus, and treated with CNO (3x). (H) Chemogenetic inhibition of Gad1 interneurons in the mPFC using the three-dosing regimen (CNO 3x+4 h) increased frequency of excitatory postsynaptic currents (EPSCs) in pyramidal neurons (n = 23–30 cells/group from 6–7 animals/group; $t_{51} = 3.57$, $p < 0.05$) and decreased frequency of inhibitory postsynaptic currents (IPSCs) in Gad1 interneurons (n = 15–17 cells/group from 5 animals/group; $t_{30} = 2.64$, $p < 0.05$). There was a trend toward an increase in IPSCs in pyramidal neurons (n = 27–31 cells/group from 6–7 animals/group; $t_{56} = 1.68$, $p = 0.09$) and no alteration in EPSCs in Gad1 interneurons (n = 15 cells/group from 5 animals/group; t_{28} = 0.11, $p > 0.05$). (I) WT- hM4DGi (control) and *Gad1-Cre*-hM4DGi mice were exposed to CUS for 21 days and received 3 CNO injections before each behavioral test. Exposure to CUS continued between test days, with the exception of the days of treatment and on days of behavioral testing. Chemogenetic inhibition of Gad1 interneurons in the mPFC completely reversed the depression-like effects of CUS in the SUST (n = 10–12/group; One-Way ANOVA followed by Duncan test, $F_{2,30} = 20.49$, $p < 0.05$), NSFT ($F_{2,30} = 8.34$, $p < 0.05$) and FST ($F_{2,30} = 19.02$, $p < 0.05$). In the FUST, although there was no statistical difference between WT-hM4DGi stressed and non-stressed mice, *Gad1-Cre*-hM4DGi mice showed increased urine sniffing time in comparison to the stressed control group ($F_{2,30}$ = 4.62, $p < 0.05$). There were no differences in water sniffing time ($F_{2,30} = 0.17$, $p > 0.05$) or in locomotor activity ($F_{2,30} = 0.85$, $p > 0.05$). Each bar represents the mean ± standard error of the mean (S.E.M.). * $p < 0.05$ compared to the non-stressed control group; # $p < 0.05$ compared to the stressed control group. Scale bar: 100 μm.

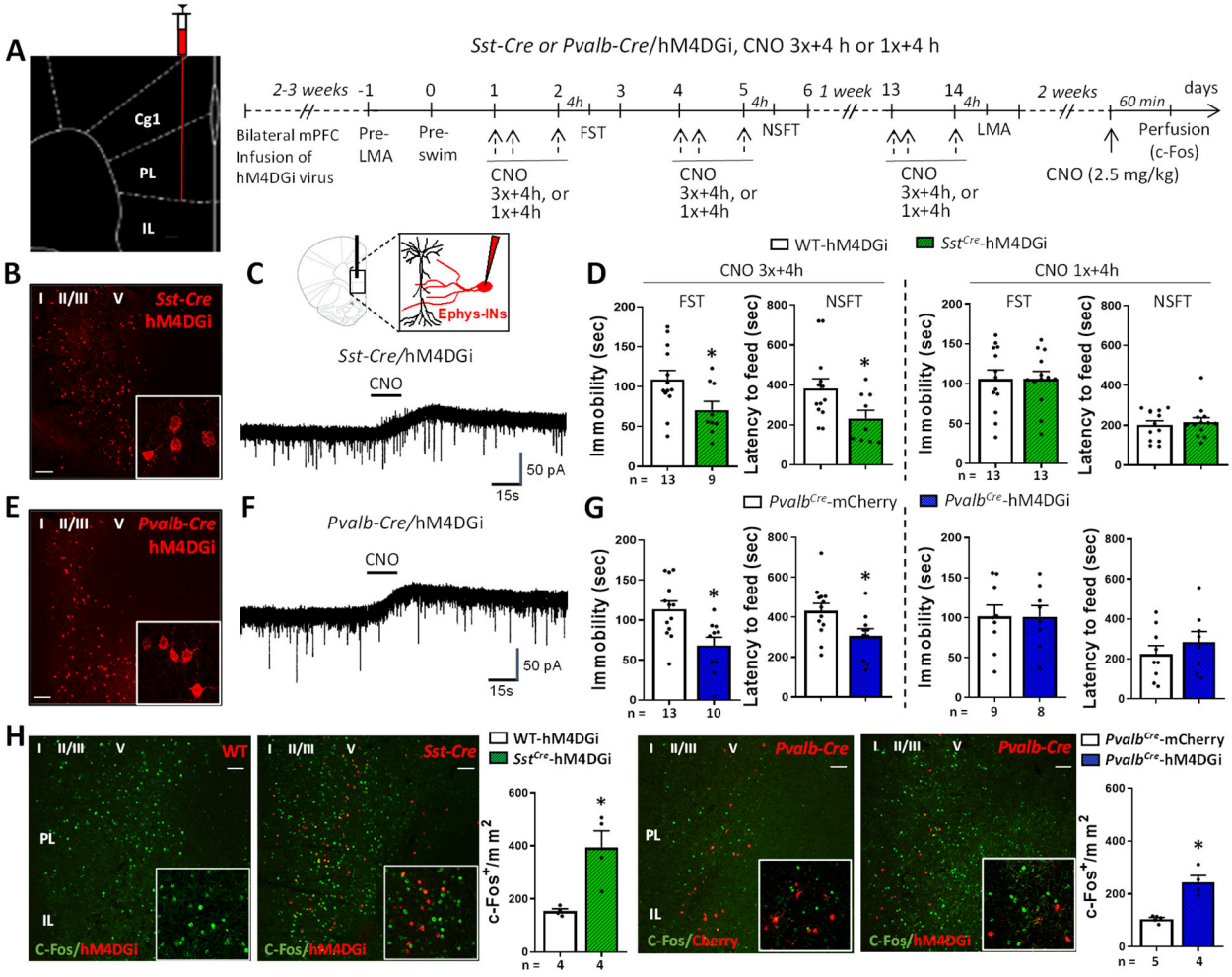

Figure 2. Chemogenetic inhibition of Sst or Pvalb interneurons in the mPFC produces fast antidepressant-like effects and increases c-Fos expression.

(A) Experimental time course for surgery, CNO injections and the behavioral tests. Animals received three CNO injections (2.5 mg/kg, 3x CNO+4h), and 4 h after the last injection were exposed to behavioral testing. In a control experiment, animals received two injections of vehicle followed, on the next day, by a single CNO injection (2.5 mg/kg) 4 h before the behavioral test (1x CNO+4h). (B and E) Representative images of the mPFC from *Sst-Cre and Pvalb-Cre* mice that received bilateral infusion of hM4DGi virus (magnification: 10X; inset: 60X). WT, *Cre* negative littermate controls were used for *Sst-Cre* mice and were injected with hM4DGi virus; for *Pvalb-Cre*, controls were also *Pvalb-Cre* positive mice and were infused with a control virus (mCherry). (C and F) Voltage-clamp recording (−70 mV) of Sst and Pvalb interneurons expressing hM4DGi virus in the mPFC. The representative traces show that CNO (10 μM) application causes hyperpolarization of labeled Sst and Pvalb cells (n = 6 Sst+ and n = 7 Pvalb neurons were recorded, all of which displayed inhibition by CNO incubation). (D and G) Chemogenetic inhibition of Sst and Pvalb interneurons in the mPFC using the 3x CNO + 4h dosing regimen produced antidepressant-like effects in the FST (*Sst-Cre*: n = 9–13/group. Student's t-test, $t_{20} = 2.32$, $p < 0.05$); (*Pvalb-Cre*: n = 10–13/group; $t_{21} = 3.14$, $p < 0.05$) and in the NSFT (*Sst-Cre*: $t_{20} = 2.13$, $p < 0.05$);

(*Pvalb-Cre*: $t_{21} = 2.40$, $p < 0.05$). CNO injection 4 h before testing did not produce any behavioral effects in the FST (*Sst-Cre*: n = 13/group, Student's t test, $t_{24} = 0.01$, $p > 0.05$); (*Pvalb-Cre*: n = 8–9/group, $t_{15} = 0.01$; $p > 0.05$) or in the NSFT (*Sst-Cre*: $t_{24} = 0.48$, $p > 0.05$); (*Pvalb-Cre*: $t_{15} = 0.90$, $p > 0.05$). (H) After the last behavioral test, the animals were submitted to a 2-week washout period and then treated with a single injection of CNO (2.5 mg/kg) followed, 60 min later, by perfusion for c-Fos immunofluorescence. Chemogenetic inhibition of Sst (n = 4/group. Student's t-test, $t_6 = 3.70$, $p < 0.05$) or Pvalb interneurons (n = 4–5/group. $t_7 = 5.90$, $p < 0.05$) increased the number of c-Fos+ cells in the mPFC (magnification: 10X; inset: 20X). Each bar represents the mean ± standard error of the mean (S.E.M.). * $p < 0.05$ compared to the control group. Scale bar: 100 μm.

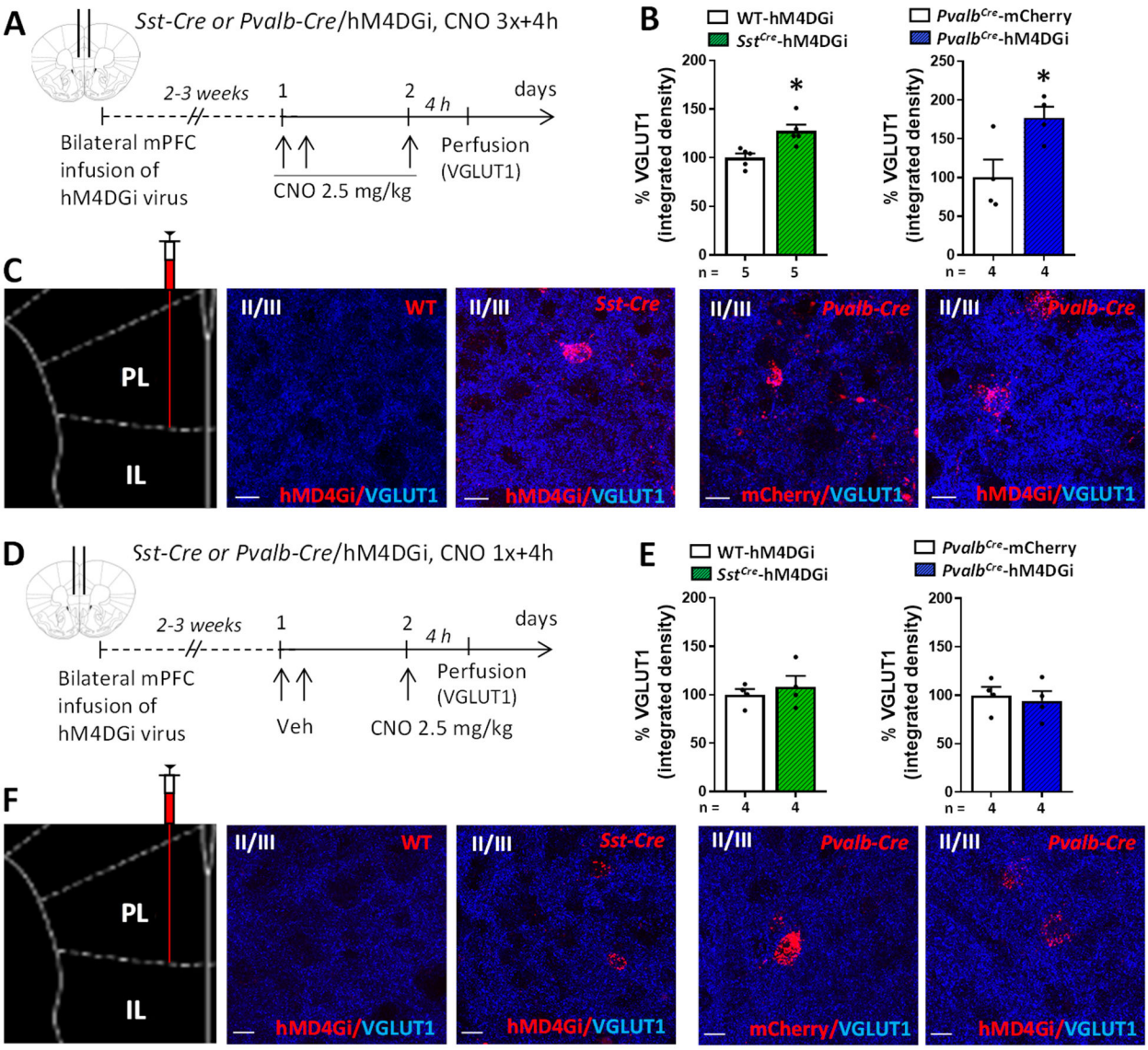

Figure 3. Chemogenetic inhibition of Sst or Pvalb interneurons in the mPFC increases VGLUT1 expression.

(A) Experimental time course for surgery, CNO injections and immunofluorescence. *Sst-Cre* and *Pvalb-Cre* mice received bilateral infusions of hM4DGi virus into the mPFC. WT, *Cre negative* littermate controls were used for *Sst-Cre* mice and were injected with hM4DGi virus; for *Pvalb-Cre*, controls were *Pvalb-Cre* positive mice infused with a control virus (mCherry). Animals received three CNO injections (2.5 mg/kg), and 4 h after the last injection were perfused (CNO 3x+4h). (D) In a control experiment, animals received two injections of vehicle followed, on the next day, by a single CNO injection (2.5 mg/kg) 4 h before the perfusion (CNO 1x+4h). (B and C) The 3x+4h CNO dosing regimen increased the expression of VGLUT1 in the layer II/III mPFC of *Sst-Cre* (n = 5/group. Student-s t-test, $t_6 = 2.83$, $p < 0.05$) and *Pvalb-Cre* mice (n = 4/group. $t_8 = 3.46$, $p < 0.05$). (E and F) A single 4 h-CNO injection (1x+4h) did not change VGLUT1 expression in the mPFC of *Sst-Cre* (n = 4/group. Student-s t-test, $t_6 = 0.66$, $p > 0.05$) or *Pvalb-Cre* mice (n = 4/group. t_6 =

0.44, $p > 0.05$). Results are expressed as a percent of the respective control groups. Each bar represents the mean ± standard error of the mean (S.E.M.). * $p < 0.05$ compared to the control group. Magnification: 60X+2X zoom. Scale bar: 10 μm.

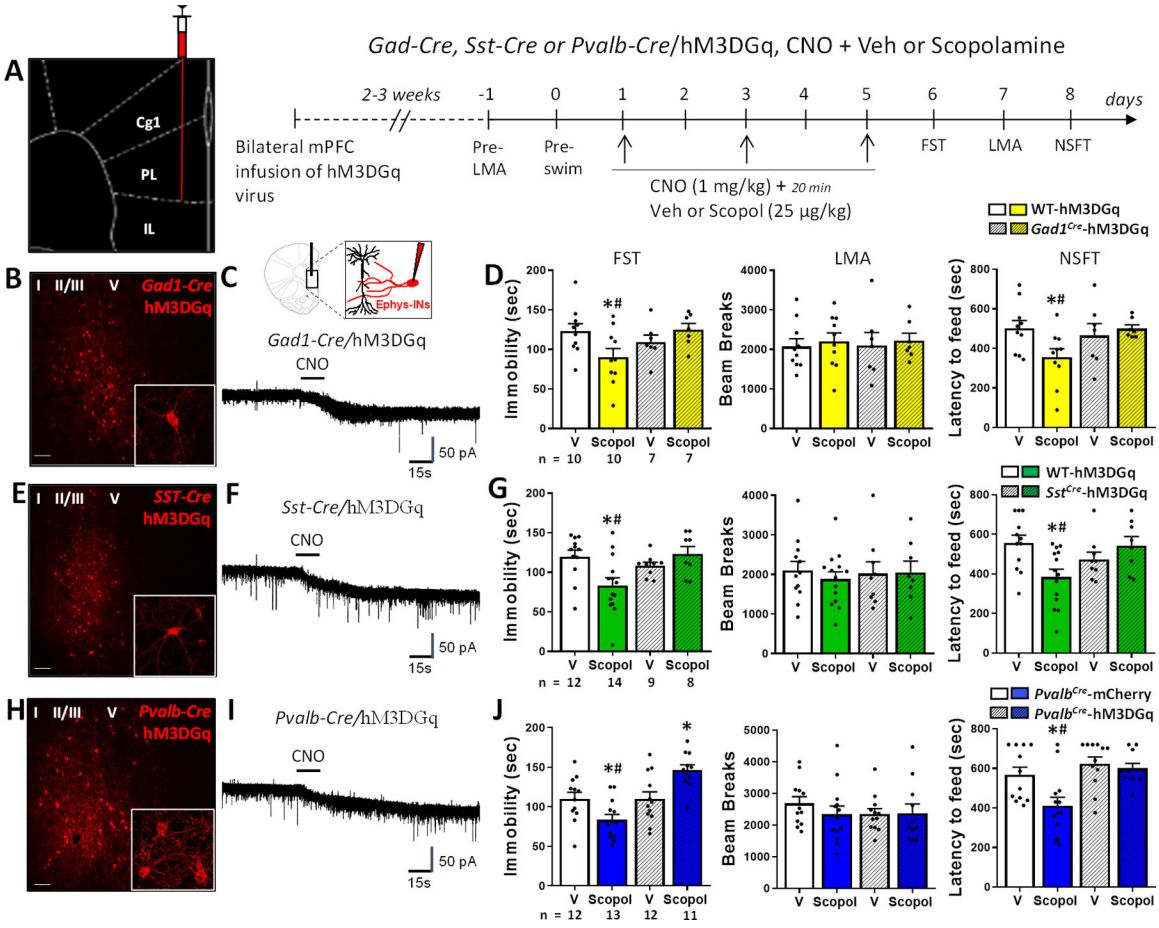

Figure 4. Chemogenetic activation of Gad1, Pvalb or Sst interneurons in the mPFC abolishes the rapid antidepressant-like effects of scopolamine.

(A) Experimental time course for surgery, CNO/scopolamine injections and behavioral testing. (B, E and H) Representative images of the mPFC from *Gad1-Cre, Sst-Cre and Pvalb-Cre* mice that received bilateral infusion of hM3DGq virus (magnification: 10X; inset: 60X). WT littermate controls were used for *Gad1-* and *Sst-Cre* mice and were injected with hM3DGq virus; for *Pvalb-Cre* mice, controls were also *Pvalb-Cre* positive mice and were infused with a control virus (mCherry). (C, F and I) Voltage-clamp recording (−70 mV) of Gad1, Sst and Pvalb interneurons expressing hM3DGq virus in the mPFC. The representative traces show that CNO (10µM) incubation causes depolarization of interneurons (Gad1+, n=4, Sst+, n=8, and Pvalb, n=8 neurons were recorded and all were excited by CNO incubation). (D, G and J) CNO injection (1 mg/kg, 20 min prior to scopolamine dosing) abolished the antidepressant-like effects of scopolamine (25 µg/kg, total of 3 injections, every 48 h) in the FST in all three lines (*Gad1-Cre*, n = 7–10/group; Two-way ANOVA followed by Duncan test; Genotype: $F_{1,31} = 1.10$, $p > 0.05$; Treatment: $F_{1,31} = 0.72$, $p > 0.05$; Treatment*Genotype: $F_{1,31} = 5.86$, $p < 0.05$); (*Sst-Cre*, n = 8–14/group; Genotype: $F_{1,39} = 2.43$, $p > 0.05$; Treatment: $F_{1,39} = 1.45$, $p > 0.05$; Treatment*Genotype: $F_{1,39} = 8.18$, $p < 0.05$); (*Pvalb-Cre* mice, n = 11–13/group; Virus: $F_{1,44} = 15.43$, $p < 0.05$; Treatment: $F_{1,44} = 0.41$, $p > 0.05$; Treatment*Virus: $F_{1,44} = 16.19$, $p < 0.05$). CNO injection also abolished the anxiolytic/antidepressant effect of scopolamine

in the NSFT in in all three lines (*Gad1-Cre*; Two-way ANOVA followed by Duncan test; Genotype: $F_{1,31} = 1.39$, $p > 0.05$; Treatment: $F_{1,31} = 1.94$, $p > 0.05$; Treatment*Genotype: $F_{1,31} = 4.07$, $p < 0.05$); (*Sst-Cre*; Genotype: $F_{1,39} = 0.83$, $p > 0.05$; Treatment: $F_{1,39} = 1.53$, $p > 0.05$; Treatment*Genotype: $F_{1,39} = 8.53$, $p < 0.05$); (*Pvalb-Cre* mice; Virus: $F_{1,44} = 9.11$, $p < 0.05$; Treatment: $F_{1,44} = 7.74$, $p < 0.05$; Treatment*Virus: $F_{1,44} = 4.45$, $p < 0.05$). There was no effect of treatments on locomotor activity (Two Way ANOVA, *Gad1-Cre*, Treatment*Genotype: $F_{1,31} = 0.001$, $p > 0.05$. *Sst-Cre*: $F_{1,39} = 0.24$, $p > 0.05$. *Pvalb-Cre*: $F_{1.44} = 0.62$, $p > 0.05$). Each bar represents the mean ± standard error of the mean (S.E.M.). * $p < 0.05$ compared to the control group; # $p < 0.05$ compared to the *Cre*-positive group treated with scopolamine. Scale bar: 100 μm.

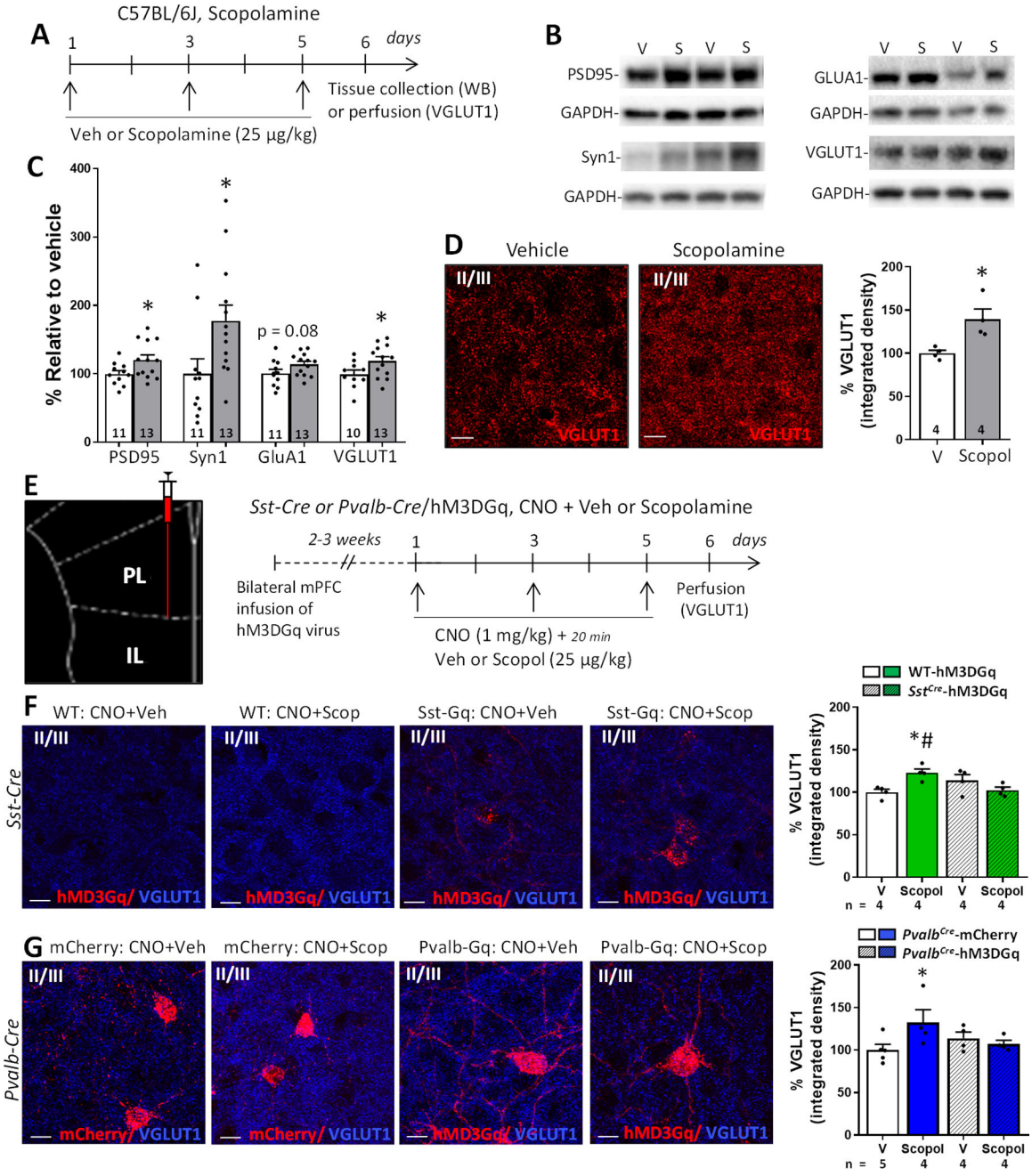

Figure 5. Chemogenetic activation of Sst or Pvalb interneurons abolishes scopolamine-induction of VGLUT1 in the mPFC.

(A) Experimental time course for scopolamine injections. C57BL/6J mice received three injections of either vehicle or scopolamine (25 µg/kg, every 48 h) and 24 h later mPFC was isolated for Western blot analysis of synaptic proteins or mice were perfused for VGLUT1 immunofluorescence. (B) Representative bands of Western blots (n = 10–13/group). (C) Scopolamine treatment significantly increased the levels of PSD95 (Student's t-test, t_{22} = 2.19, $p < 0.05$), Synapsin 1 (t_{22} = 2.39, $p < 0.05$) and VGLUT1 (t_{21} = 2.10, $p < 0.05$), and induced a trend for increased GluA1 levels (t_{22} = 1.80, p = 0.08) in the mPFC.

(D) Representative immunofluorescence images showing VGLUT1 staining in the mPFC (layer II/III) of animals treated with vehicle or scopolamine (n = 4/group). Scopolamine treatment increased VGLUT1 levels in mPFC (Student's t-test, $t_6 = 3.24$, $p < 0.05$). (E) Experimental time course for surgery and CNO (1 mg/kg) ± scopolamine (25 μg/kg, every 48 h) treatments. *Sst-Cre* and *Pvalb-Cre* mice received hM3DGq viral infusions into the mPFC. WT littermate controls were used for *Sst-Cre* mice, and were injected with hM3DGq virus; for *Pvalb-Cre*, controls were also *Pvalb-Cre* positive mice and were infused with a control virus (mCherry). (F) CNO injection blocked the scopolamine-induction of VGLUT1 expression in the mPFC of *Sst-Cre* (n = 4/group; Two-way ANOVA followed by Duncan test. Genotype: $F_{1,12} = 0.47$, $p > 0.05$; Treatment: $F_{1,12} = 1.41$, $p > 0.05$; Treatment*Genotype: $F_{1,12} = 12.41$, $p < 0.05$) and (G) in *Pvalb-Cre* mice (Genotype: $F_{1,13} = 0.36$, $p > 0.05$; Treatment: $F_{1,13} = 2.16$, $p > 0.05$; Treatment*Genotype: $F_{1,13} = 4.66$, $p < 0.05$). Results are expressed as the percent of vehicle group. Each bar represents the mean ± standard error of the mean (S.E.M.). * $p < 0.05$ compared to the control group. # $p < 0.05$ compared to the *Cre*-positive group treated with scopolamine. Magnification: 60X+2X zoom. Scale bar: 10 μm.

Table 1.

Chemogenetic, optogenetic, or gene deletion studies of cortical GABA interneurons and antidepressant/anxiety related behaviors.

Current study		Previous studies	
Approach	**Effect**	**Approach**	**Effect**
Inhibition of GABA INs		**Inhibition of GABA INs**	
Gad1, hM4DGi (mPFC)		***Gad1***, Opto inhib (mPFC)	
1x CNO[a] + 24h 3x CNO[a] + 4 h	Adt in FST, not NSFT Adt in FST and NSFT	1 h inhibition + 24 h	No effect FST, NSFT [42]
3x CNO[a] + 4 h ± CUS	Adt in SUST, FUST, NSFT and FST		
1 × CNO[a] + 4 h 1 × CNO[a] + 30 min	No effect FST, NSFT No effect FST, NSFT		
Sst, hM4DGi (mPFC)		***Sst***, hM4DGi (PL/Cg)	
3x CNO[a] + 4 h 1 × CNO[a] + 4 h 1 × CNO[a] + 30 min	Adt in FST and NSFT No effect FST, NSFT No effect FST, NSFT	1 × CNO[c] + 30 min 42x/21 d CNO[d] + 30 min	Axg EPM, not NSFT; FST, nt [35]; Axl EPM, not NSFT; FST, nt [35]
		Sst knockout	Axg in NSFT [33]
Pvalb, hM4DGi (mPFC)			
3x CNO[a] + 4 h 1 × CNO[a] + 4 h 1 × CNO[a] + 30 min	Adt in FST and NSFT No effect FST, NSFT No effect FST, NSFT	***Pvalb***, hM4DGi (PL) 3x/3 d CNO[e] + 30 min	Increased LH behavior [55]
Stimulation-GABA Ins		**Stimulation-GABA INs**	
Gad1, hM3DGq (mPFC) CNO[b] ± Scopol	Blocks scopol FST, NSFT; No effect bsl FST, NSFT	***Gad1***, Opto stim (mPFC) 1 h stimulation + 24 h	Blocks ketamine FST, NSFT; no effect bsl FST, NSFT [42]
Sst, hM3DGq (mPFC) CNO[b] ± Scopol	Blocks scopol FST, NSFT; No effect bsl FST, NSFT	***Sst***, Deletion of γ2-$GABA_A$ (disinhibition of GABA)	Axl EPM, NSFT; Adt FST, LH [54]
Pvalb, hM3DGq (mPFC) CNO[b] ± Scopol	Blocks scopol FST, NSFT; No effect bsl FST, NSFT	***Pvalb***, hM3DGq (mPFC) 21x/21 d CNO[d] (CUS) 1x CNO[d] + 30 min	Axg OF, NSFT (only in females) No effect OF [61]

Adt, antidepressant; Axl, anxiolytic; Axg, anxiogenic; bsl, baseline behavior; mPFC, medial PFC (includes infralimbic and prelimbic subregions); PL, prelimbic PFC; Cg, cingulate; FST, forced swim test; NSFT, novelty suppressed feeding test; LH, learned helplessness; EPM, elevated plus maze; OF, open field; SUST, sucrose splash test; FUST, female urine sniffing test; CUS, chronic unpredictable stress; INs, interneurons; nt, not tested; CNO dosing:

[a] 2.5 mg/kg;

[b] 1.0 mg/kg;

[c] 5.0 mg/kg;

[d] 0.5 mg/kg;

[e] 10.0 mg/kg.