Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction

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Amphetamine-induced augmentation of striatal dopamine and its blunted release in prefrontal cortex (PFC) is a hallmark of schizophrenia pathophysiology. Although *N*-methyl-D-aspartate receptor (NMDAR) hypofunction is also implicated in schizophrenia, it remains unclear whether NMDAR hypofunction leads to dopamine release abnormalities. We previously demonstrated schizophrenia-like phenotypes in GABAergic neuron-specific NMDA hypofunctional mutant mice, in which *Ppp1r2*-Cre dependent deletion of indispensable NMDAR channel subunit *Grin1* is induced in corticolimbic GABAergic neurons including parvalbumin (PV)-positive neurons, in postnatal development, but not in adulthood. Here, we report enhanced dopaminomimetic-induced locomotor activity in these mutants, along with bidirectional, site-specific changes in in vivo amphetamine-induced dopamine release: nucleus accumbens (NAc) dopamine release was enhanced by amphetamine in postnatal *Ppp1r2*-Cre/*Grin1* knockout (KO) mice, whereas dopamine release was dramatically reduced in the medial PFC (mPFC) compared to controls. Basal tissue dopamine levels in both the NAc and mPFC were unaffected. Interestingly, the magnitude and distribution of amphetamine-induced c-Fos expression in dopamine neurons was comparable between genotypes across dopaminergic input subregions in the ventral tegmental area (VTA). These effects appear to be both developmentally and cell-type specifically modulated, since PV-specific *Grin1* KO mice could induce the same effects as seen in postnatal-onset *Ppp1r2*-Cre/*Grin1* KO mice, but no such abnormalities were observed in somatostatin-Cre/*Grin1* KO mice or adult-onset *Ppp1r2*-Cre/*Grin1* KO mice. These results suggest that PV GABAergic neuron-NMDAR hypofunction in postnatal development confers bidirectional NAc hyper- and mPFC hypo-sensitivity to amphetamine-induced dopamine release, similar to that classically observed in schizophrenia pathophysiology.

Key words: amphetamine/microdialysis/striatum/prefrontal cortex/GABAergic neurons/parvalbumin

Introduction

A current version of the dopamine hypothesis of schizophrenia posits that striatal hyperdopaminergia contributes to positive symptoms, and frontal cortical hypodopaminergia contributes to negative symptoms and cognitive dysfunctions.[1] Indeed, receptor imaging studies have repeatedly demonstrated increased amphetamine-induced striatal dopamine release in subjects with schizophrenia relative to healthy controls.[2-4] Conversely, a recent imaging study demonstrated that amphetamine-induced presynaptic dopamine release is robustly decreased in most brain regions, including prefrontal cortex (PFC), in patients with schizophrenia.[5]

Several precedent mechanisms to elicit striatal hyperdopaminergia have been proposed.[6-10] One plausible hypothesis is that dopamine malfunction could be secondary to altered glutamatergic function, particularly *N*-methyl-D-aspartate receptor (NMDAR) hypofunction.[11,12] Supporting this hypothesis, preclinical studies showed NMDA antagonists, such as ketamine and phencyclidine (PCP), enhance amphetamine-induced striatal

dopamine release.[13–16] Human brain imaging studies agree with these findings.[17] Since systemic administration of an NMDAR antagonist alone (with no psychostimulant challenge) resulted in mixed results in both clinical[18,19] and preclinical studies,[11,20] the effect of NMDAR hypofunction on striatal dopamine release appears to be more pronounced in heightened dopaminergic states.

On the other hand, acute systemic administration of PCP,[21,22] ketamine,[23–25] or MK-801[26] alone increases medial PFC (mPFC) dopamine release, while chronic administration results in negligible elevation.[11,15,24] Conversely, few studies have investigated the impact of NMDAR blockade on amphetamine-induced dopamine release in mPFC. Balla et al demonstrated that systemic and subchronic administration of PCP in rats results in a potentiation of amphetamine-induced mPFC dopamine release.[15,16] However, these findings are inconsistent with recent PET imaging findings.[5] Therefore, it is of critical importance to determine whether NMDAR hypofunction leads to similar dopamine abnormalities as observed in schizophrenia, as well as which cell-types are involved in this phenomenon.

We previously reported no exacerbation of amphetamine-induced hyperlocomotion, a behavioral correlate of dopamine reinforcing effect,[27] when genetic ablation of NMDAR indispensable subunit type 1 (GRIN1), encoded by *Grin1* gene, is targeted selectively to cortical pyramidal neurons.[28] NMDAR hypomorph mice, in which GRIN1 expression levels are globally 5–10% of wild type mice, also show no excessive amphetamine-induced hyperlocomotion.[29] Conversely, when *Grin1* ablation is targeted to a subset of corticolimbic GABAergic neurons during the early postnatal period (but not, interestingly, during adulthood), we found several schizophrenia-like phenotypes.[30] This led us to test a hypothesis that NMDAR hypofunction in GABAergic neurons in postnatal development, but not in adulthood, is necessary for abnormal dopamine release. To this end, we evaluated the amphetamine-induced dopamine release in both nucleus accumbens (NAc) and mPFC by in vivo brain microdialysis, using 4 different conditional *Grin1* KO mouse strains, in which *Grin1* is selectively eliminated in a subset of GABAergic neurons during either postnatal development or in adulthood.

Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee at University of Alabama at Birmingham and Universidad de Buenos Aires in Argentina. For extensive details, see supplementary material.

Animals

We employed 2 types of *Ppp1r2*-Cre/floxed-*Grin1* knockout mice, in which genetic deletion of obligatory *Grin1* subunit occurs in ~50% of cortical and hippocampal GABAergic interneurons from either the postnatal second week (namely postnatal *Grin1* KO mice) or postnatal 8th week (namely adult *Grin1* KO mice), respectively, as previously described.[30] Additionally, we used cell-type specific *Grin1* knockout mice of 2 major groups of GABAergic interneurons, namely PV-Cre/*Grin1* KO mice and SST-Cre/*Grin1* KO mice.

Behavior

Acute dopamine-dependent locomotor effect was assessed in an open field after intraperitoneal injection of *d*-amphetamine (2.5 mg/kg) or methamphetamine (1.5 mg/kg).

Microdialysis

A microdialysis CMA7 probe was inserted into left mPFC or NAc lateral shell through the guide cannula, and the mouse was placed into a test chamber. Following a 2-h equilibration period, samples were collected every 20 min at 1 μl/min perfusion rate into a microcentrifuge tube containing antioxidants. Three baseline samples were collected before treatment with *d*-amphetamine (2.5 mg/kg, i.p.) and 5 additional samples were collected post-treatment. Samples were analyzed by HPLC. Basal tissue dopamine and its metabolite levels were also assessed using untreated mice.

Immunocytochemistry

Brain immunostaining was conducted to determine the cell-types of *Pppp1r2*-Cre positive neurons in mPFC, striatum, and midbrain including VTA. c-Fos immunomapping was employed to assess the activity of VTA neurons following amphetamine challenge. Coronal brain sections of postnatal *Grin1* KO and their floxed-control male mice, which received amphetamine injection (2.5 mg/kg, i.p.) 90 min before, were co-labeled with anti-tyrosine hydroxylase (TH) and anti-Fos to assess neuronal activity. The average number of the cells expressing c-Fos was assessed in the anterior, medial, and posterior part of bilateral VTA, respectively.

Statistical Analyses

Student's *t*-tests and ANOVA were employed where appropriate. Post hoc Tukey–Kramer analyses were also completed as indicated by main effects and interactions. Data were presented as mean ± SEM. Significance was considered at $P < .05$.

Results

Amphetamine-Induced Excess Dopamine Release in Striatum and Blunted Dopamine Release in mPFC of Postnatal Grin1 KO Mice

We first examined psychostimulant-induced locomotor activity in response to administration of *d*-amphetamine

(2.5 mg/kg, i.p.) or methamphetamine (1.5 mg/kg, i.p.). Both amphetamine (figure 1A) and methamphetamine (figure 1B) augmented open field horizontal locomotor activity. However, postnatal *Grin1* KO mutant mice showed much higher activities in response to both psychostimulants compared to the littermate floxed-controls. As psychostimulant-induced locomotor hyperactivity can be attributed to abnormality of dopaminergic function,[31] we measured the brain tissue levels of dopamine and its metabolites of postnatal *Grin1* KO mice. These revealed a slight increase in mutant 3,4-dihydroxyphenylacetic acid (DOPAC) levels in mPFC (supplementary figure S1B), while no genotypic difference was detected in dopamine (supplementary
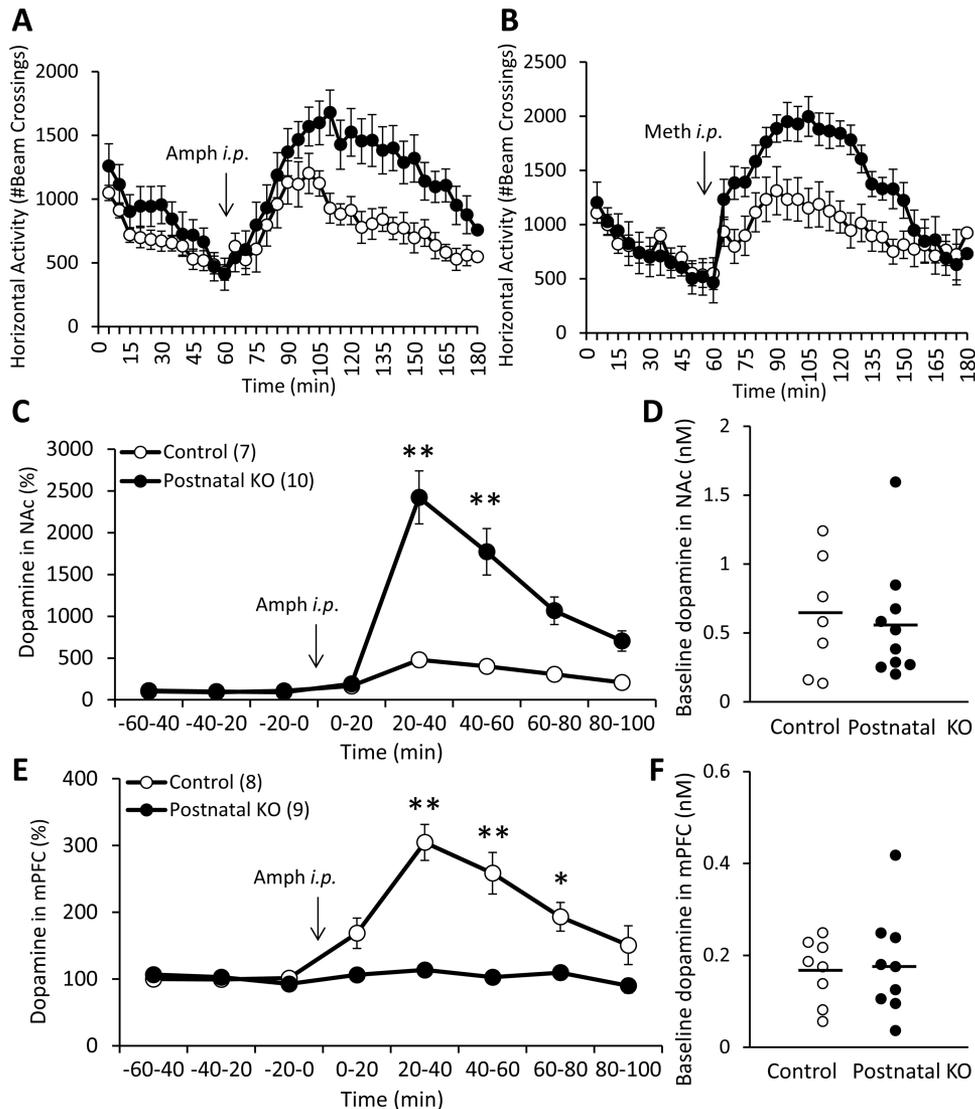

Fig. 1. Psychostimulant-induced hyper-dopaminergic response in nucleus accumbens and hypo-dopaminergic response in mPFC of postnatal *Grin1* KO mice. (A) Horizontal locomotor hyperactivity in open field elicited by *d*-amphetamine administration (2.5 mg/kg, i.p.) was higher in postnatal *Grin1* KO mice (black circle) compared to the floxed-*Grin1* line-A controls (white circle, repeated-measures ANOVA, $F_{1,368} = 1.793$, $P < .02$). No genotypic difference noted before treatment. (B) Postnatal *Grin1* KO mice (black circle) also showed a higher locomotor activity in response to methamphetamine (1.5 mg/kg, i.p.) than the controls (white circle, $F_{1,299} = 4.93$, $P < .001$). No genotypic difference before treatment. (C) Amphetamine-induced extracellular dopamine concentration increased 24.2-fold from the baseline level in the NAc lateral shell of postnatal *Grin1* KO mice (Student's paired *t*-test between −20 to 0 min and 20 to 40 min, $t_9 = 3.96$, $P < .004$), whereas only 4.8-fold increase (paired *t*-test between −20 to 0 min and 20 to 40 min, $t_6 = 3.32$, $P < .02$) in floxed-*Grin1* control mice. Repeated-measures ANOVA for genotype effect, $F_{1,60} = 16.88$, $P < .0001$, followed by Tukey–Kramer post hoc test, **$P < .01$. *$P < .05$. (D) No genotypic difference in baseline dopamine levels in NAc lateral shell before treatment. (E) No obvious amphetamine-induced dopamine increase was detected from baseline levels in mPFC of postnatal *Grin1* KO mice (paired *t*-test between −20 to 0 min and 20 to 40 min, $t_8 = −2.62$, $P = .985$), whereas 3.0-fold increase was observed in the control mPFC (paired *t*-test between −20 to 0 min and 20 to 40 min, $t_7 = −6.56$, $P = .00032$). Repeated-measures ANOVA for genotype effect, $F_{1,60} = 7.11$, $P < .0001$, followed by Tukey–Kramer post hoc test, **$P < .01$. *$P < .05$. (F) No genotypic difference in baseline dopamine levels in mPFC before treatment. Each dot represents individual animals in D and F. Solid lines in D and F give the mean. Data are mean ± SEM.

figure S1A) or homovanillic acid (HVA) levels (supplementary figure S1C).

We then measured the extracellular brain dopamine levels by in vivo microdialysis from awake, behaving mice. When the probe was targeted to the NAc lateral shell (supplementary figure S2), we found that postnatal *Grin1* KO mice had enhanced amphetamine-stimulated dopamine concentrations, up to 24-fold by 20–40 min following injection, which then declined by 100 min after the treatment. In contrast, amphetamine increased the dopamine level by only 4.8-fold in the control mice (figure 1C). No baseline dopamine difference was observed between genotypes (figure 1D). In contrast, when sampling from mPFC (supplementary figure S2) in a different cohort of animals, control mice showed augmented dopamine levels within the first 20 min, reaching up to 3-fold higher 20–40 min after amphetamine treatment (figure 1E), as previously reported.[32] Remarkably, the same amphetamine treatment failed to alter dopamine levels in postnatal *Grin1* KO mice, regardless of gender, throughout the post-treatment period. Before the treatment, no genotypic difference was observed in baseline dopamine levels (figure 1F). There was also no genotypic difference in amphetamine-induced 5-HT release in either mPFC or NAc (supplementary figure S3). These results suggest that postnatal NMDAR hypofunction in GABAergic neurons results in excessive dopamine release in NAc and blunted dopamine release in mPFC in response to amphetamine.

No Genotypic Difference in Amphetamine-Induced c-Fos-Positive Dopamine Neurons Across VTA

To explore whether preferential activity of dopamine neurons in particular VTA subregions,[33] which may differentially project to NAc or mPFC,[34,35] could explain the region-based differences in dopamine release of postnatal *Grin1* KO mice, we conducted various immunocytochemical analyses. No genotypic differences were observed in the protein levels of dopamine D1 receptor (D1R), D2R, or dopamine transporter (DAT) in either striatum (figure 2A) or mPFC (figure 2B) before amphetamine treatment. The TH fiber density in mPFC, which may reflect the degree of axonal innervation of mostly dopaminergic fibers but not noradrenergic fibers,[36] was comparable between the genotypes (figures 2C and 2D). There was also no genotypic difference of TH fiber density in NAc (figure 2E). Moreover, we conducted double immunostaining for TH and c-Fos 90 min after systemic amphetamine administration (figure 2F) to assess excitability of VTA neurons. It is noted that anti-TH-immunostaining using the *Ppp1r2*-Cre/floxed-R26R-EYFP reporter line, revealed virtually no Cre-dependent YFP expression in VTA/substantia nigra, including TH+ neurons (supplementary figures S4A and S4B), and little YFP expression was observed in amygdala and thalamus (data not shown). Our immunocytochemical analysis revealed that amphetamine induces higher c-Fos expression in TH+ dopamine neurons in the caudal VTA, which may preferentially project to NAc shell[34] (figure 2H), while the number of TH+ neurons was similar among the VTA subregions (figure 2G). However, we also found that TH+ neurons are equally activated by amphetamine between genotypes, including in caudal VTA neurons (figure 2H). Therefore, excessive amphetamine-induced NAc dopamine release may not be attributed to over-activation of caudal VTA neurons in mutants. Interestingly, the number of c-Fos+/TH− cells in VTA increased in the mutants throughout the VTA subregions (figure 2I), which is concordant with a previous report showing that activity of VTA non-dopaminergic neurons correlates with amphetamine-induced locomotor hyperactivity.[37]

Cre Expression Is in Mostly Parvalbumin-Positive GABAergic Interneurons

The finding of abnormal dopamine release in NAc and mPFC of the postnatal *Grin1* KO mutants led us to examine the cell-type specificity of Cre recombination in each brain area. Immunocytochemistry using *Ppp1r2*-Cre/floxed-tdTomato mice revealed that Cre+ neurons were virtually all Gad67+ and partially positive for PV in anterior cingulate cortex (ACC) (supplementary figure S5). Among the Cre+ neurons in the *Ppp1r2*-Cre/floxed-R26R-EYFP mice, approximately 40% of them were positive for PV in the premotor cortical area, ACC and medial orbital cortex; however, PV-positivity was decreased in prelimbic and infralimbic area (supplementary figure S6), which may reflect a weaker expression of PV, as previously reported.[38,39] Conversely, among PV+ neurons, over 74% of PV+ cells were YFP+ expressed by Cre recombination ($74.2 \pm 1.6\%$, 51 sections, 3 mice) regardless of the mPFC subareas, suggesting that Cre recombination occurs in a majority of PV+ interneurons in mPFC. We also found that 10–16% of Cre-expressing YFP+ cells were positive for Reelin and 8–12% of YFP+ cells were positive for SST across the mPFC subareas, although the Cre-positivity among entire Reelin-positive cells and SST+ cells were $33.5 \pm 1.8\%$ (21 sections from 3 animals) and $22.8 \pm 1.2\%$ (33 sections from 3 animals), respectively.

In the striatum including NAc area, sporadically observed *Ppp1r2*-Cre positive cells were mostly non-medium spiny neurons, because few tdTomato+ cells in *Ppp1r2*-Cre/floxed-tdTomato line were positive for Ctip2 (supplementary figure S7), a marker for striatal medium spiny neurons.[40] While $42.5 \pm 4.3\%$ of those cells was PV+ interneurons, the remaining Cre+ cell-types are unknown, since none of the following striatal interneuron markers colocalized with the Cre-recombination marker tdTomato: SST, calretinin, or choline-acetyltransferase (ChAT) (supplementary figure S7).
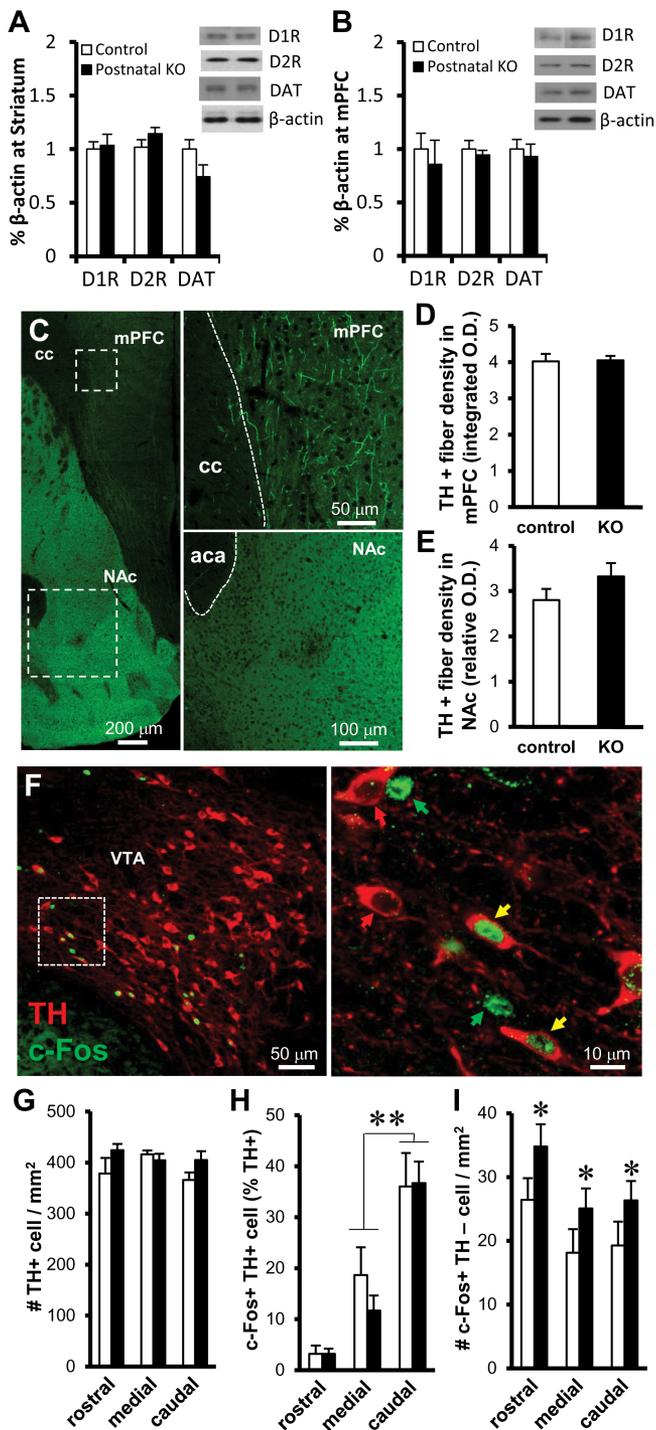
NMDAR Hypofunction in PV Neurons, But Not SST Neurons, Mimics Dopamine Abnormalities

To examine whether *Grin1* deletion in PV neurons are responsible for the abnormal dopamine release observed in the mutants, we generated a novel PV-Cre/*Grin1* KO mice by crossing a PV-Cre line[41] to a floxed-*Grin1* line-A.[42] First, we confirmed that NMDA currents are missing in PV-Cre-positive EYFP neurons in ACC area of mutant mPFC (supplementary figure S8). PV-Cre/*Grin1* KO mice grew normally with no gross abnormality. However, amphetamine-induced locomotor activity was higher in PV-Cre/*Grin1* KO mice compared to controls, while baseline locomotion was comparable (figure 3A). Furthermore, NAc dopamine concentration increased by 8-fold in PV-Cre/ *Grin1* KO mice, which was about 1.5-fold higher than control mice after amphetamine treatment (figure 3B). Similarly to postnatal *Grin1* KO mice, the dopamine level did not increase in the PV-Cre/*Grin1* KO mPFC after treatment, whereas the dopamine level was augmented in control mPFC (figure 3D). There was no genotypic difference in basal dopamine levels in NAc (figure 3C) or mPFC (figure 3E). These results indicated that "dual" dopamine release phenotypes observed in postnatal *Grin1* KO mice were at least partially replicated following PV neuron-selective NMDAR deletion.

To further examine the cell-type specificity of these effects, we generated SST-Cre/*Grin1* KO mice by crossing a SST-Cre line[43] to a floxed-*Grin1* line-A[42] to generate mice with SST neuron-selective NMDAR deletion, and confirmed that NMDA currents were missing in Cre-positive EYFP neurons in the ACC area of mPFC

tyrosine hydroxylase (TH, green) depicting the regions of interest used for TH-fiber quantification. Right upper panel: TH-fibers innervating deep layers of the mPFC (cc: corpus callosum). Right lower panel: NAc region (aca: anterior commissure). No genotypic differences ($n = 4$–7/group) in TH fiber density in either mPFC (D, $t_9 = 0.13$, $P = .89$) or NAc (E, $t_9 = 1.12$, $P = .26$). (F) VTA neuron excitability 90 min after amphetamine (2.5 mg/kg, i.p.) was assessed by anti-Fos immunoreactivity (green). Coronal sections were co-labeled with anti-TH (red) to visualize dopaminergic neurons. White boxed area indicates the enlarged region presented in the right panel. Red arrow: c-Fos−/TH+ neurons, Green arrow: c-Fos+/TH− neurons, Yellow arrow: c-Fos+/TH+ neurons. (G–I) Quantitative analysis shows higher amphetamine-induced c-Fos expression in TH-positive dopamine neurons in caudal VTA, compared to middle or rostral VTA (in H, 2-way ANOVA, $F_{\text{rostral-caudal factor}(2,28)} = 37.1$, $P < .0001$, Newman Keuls post hoc analysis, $**P < .001$). No genotypic differences ($n = 5$–7/group each) in the number of TH+ neurons (in G, $F_{\text{genotype}(1,28)} = 3.2$, $P = .09$) or the number of TH+ neurons immunoreactive for c-Fos (in H, $F_{\text{genotype}(1,28)} = 0.46$, $P = 0.5$). The number of TH−/c-Fos+ neurons was increased in mutants (in I, $F_{\text{genotype}(1,28)} = 6.82$, $*P < .02$) irrespective of the subregions of the VTA analyzed ($F_{\text{interaction}(2,28)} = 0.025$, $P = .97$). Note that the number of c-Fos+ VTA neurons was close to zero regardless of the rostro-caudal axis (c-Fos+ cells/mm², 3.0 ± 0.97 for 6 control mice vs 2.6 ± 1.2 for 5 KO mice, t-test $t_9 = 0.22$, $P = .83$).
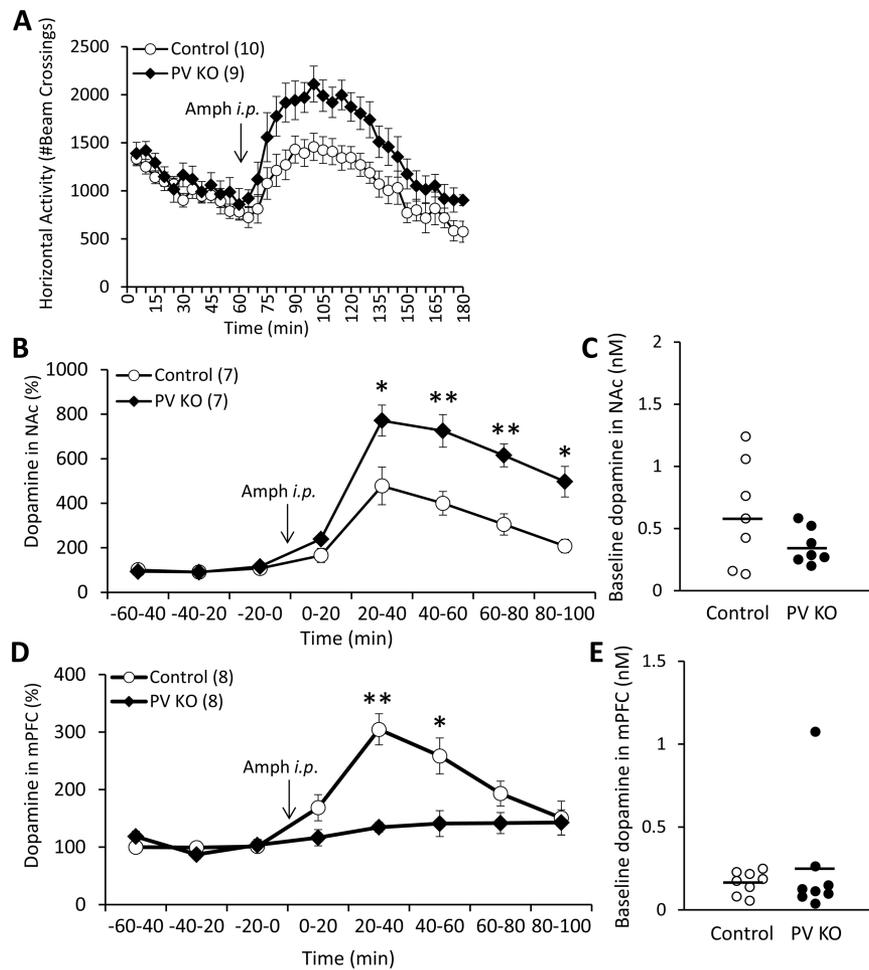
Fig. 2. No alterations in the levels of dopamine receptors, transporter or tyrosine hydroxylase, or amphetamine-evoked VTA dopamine neuron activity in postnatal *Grin1* KO mice. (A) Representative western blot bands and their quantification showing no genotypic differences ($n = 7$–9/group; 10–12 weeks old) in protein levels of striatal dopamine receptor D1 (D1R, $t_{14} = 0.27$, $P = .79$), D2 receptor (D2R, $t_{14} = 1.43$, $P = .17$), or dopamine transporter (DAT, $t_{14} = 1.73$, $P = .11$). (B) Representative western blot bands and their quantification showing no genotypic differences ($n = 6$/group; 10–12 weeks old) in mPFC protein levels of D1R ($t_{10} = 0.53$, $P = .61$), D2R ($t_{10} = 0.6$, $P = .56$), or DAT ($t_{10} = 0.48$, $P = .64$). (C–E) Left: Representative photograph of a coronal section stained against

Fig. 3. Parvalbumin (PV)-Cre/*Grin1* KO mice show similar amphetamine-induced dopamine release abnormalities. (A) PV-Cre/*Grin1* KO mice exhibited higher numbers of horizontal beam crossings in response to amphetamine compared to their floxed controls (repeated-measures ANOVA, $F_{1,391} = 1.22$, $P = 0.22$, post hoc planned comparisons at 80 to 130 min, $P < 0.05$). No genotypic difference in locomotor activity before injection. (B) Extracellular NAc dopamine concentrations of PV-Cre/*Grin1* KO mice increased after amphetamine, and were much higher than those of control mice. $F_{1,48} = 3.46$, $P < .02$, Tukey–Kramer post hoc test, $**P < .01$. $*P < .05$. (C) No genotypic difference in NAc baseline dopamine levels before treatment ($P = 0.25$). (D) Amphetamine-induced mPFC dopamine release of PV-Cre/*Grin1* KO mice was disturbed (*Student's* paired *t*-test between −20 to 0 min vs 20 to 40 min, $t_7 = -2.09$, $P = .075$), and significant difference was detected between genotypes. Repeated-measures ANOVA, $F_{1,56} = 6.55$, $P < .0002$, Tukey–Kramer post hoc test, $**P < .01$. $*P < .05$. (E) No genotypic difference in mPFC baseline dopamine levels before treatment ($P = .55$). Each dot represents individual animals in C and E. Solid lines in C and E give the mean. Data are mean ± SEM.

(supplementary figure S8). SST-Cre/*Grin1* KO mice grew normally with no gross abnormality, while the body weight of the mutants was somewhat lower than floxed-control littermates (data not shown). However, we did not observe genotypic differences in amphetamine-induced locomotor activity, extracellular dopamine levels in NAc or mPFC before and after amphetamine-treatment (supplementary figure S9). These results supported the role of NMDAR deletion in PV+ GABAergic neurons on dopamine release abnormalities in response to amphetamine.

Adult-Onset NMDAR Deletion Has No Impact on Amphetamine-Induced Dopamine Release

Subchronic PCP treatment in adulthood results in a potentiation of amphetamine-induced dopamine release in the mPFC as well as in the NAc.[15,16] To explore whether adult onset of NMDAR hypofunction in corticolimbic GABAergic neurons leads to the emergence of a dopamine release abnormality, we used a floxed-*Grin1* line-B,[44] which is different from the floxed-*Grin1* line-A[42] in figure 1, to elicit NMDAR deletion in adulthood by crossing to the *Ppp1r2*-Cre line. The onset of NMDAR deletion in *Ppp1r2*-positive neurons of this adult *Grin1* KO mice is 8 weeks of age.[30] Adult *Grin1* KO mice (20–24 weeks old) showed comparable increases in amphetamine-stimulated locomotor activity (figure 4A) and extracellular dopamine levels in both NAc (figure 4C) and mPFC (figure 4E), in comparison to floxed-control littermates. These results showed that adult-onset NMDAR deletion in cortical GABA neurons does not alter amphetamine-stimulated dopamine release, suggesting that early-postnatal onset
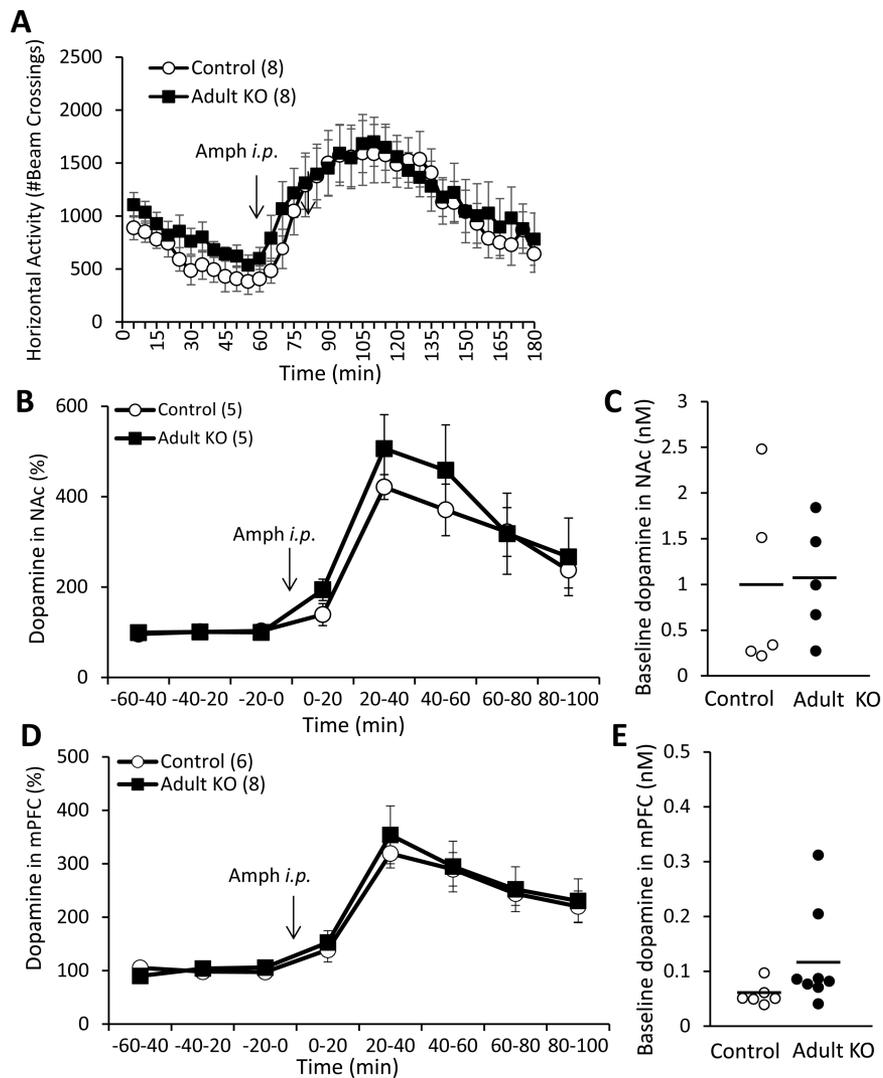
Fig. 4. Normal amphetamine-induced dopamine response in NAc and mPFC of adult *Grin1* KO mice. (A) No differences between adult-onset *Grin1* KO mice and their floxed-control littermates in the open field horizontal locomotor activities before (repeated-measures ANOVA, $F_{1,154} = 0.54$, $P = .87$) or after amphetamine ($F_{1,322} = 0.99$, $P = .47$). (B, C) No genotypic difference in NAc extracellular dopamine concentrations before ($t_{(8)} = -0.16$, $P = .88$) in C, or after amphetamine ($F_{1,32} = 0.66$, $P = .624$) in B. (D, E) No genotypic difference in mPFC extracellular dopamine concentrations before ($t_{12} = -1.62$, $P = .131$) in E, or after amphetamine ($F_{1,48} = 0.31$, $P = .872$) in D. Each dot represents individual animals in C and E. Solid lines in C and E give the mean. Data are mean ± SEM.

of NMDAR hypofunction is crucial for later emergence of the dual dopamine dysregulation observed in both mPFC and NAc.

Discussion

Here, we demonstrate that genetic *Grin1* deletion in corticolimbic GABAergic neurons during early postnatal development causes striatal hyper- and mPFC hypo-sensitivity to amphetamine-induced dopamine release, which recapitulates the characteristic "dual" dopamine phenotypes reported in human schizophrenia. Specifically, we found that *Ppp1r2*-Cre-mediated *Grin1* deletion in ~50% of corticolimbic GABAergic neurons in postnatal development leads to locomotor hyperactivity and excessive dopamine release in the NAc shell in response to

amphetamine administration. Concomitantly, amphetamine failed to induce enhancement of dopamine release in the mPFC of these mutant mice, as observed in control mice. Interestingly, *Grin1* knockout selectively in PV+, but not SST+, GABAergic neurons, also simultaneously induced augmented dopamine release in NAc and blunted dopamine release in mPFC, following amphetamine challenge. Moreover, such "dual" dopamine abnormalities were undetectable when *Grin1* deletion occurred in adulthood. These results suggested that NMDAR hypofunction under tight spatial (ie, cell-type specific) and temporal (ie, early postnatal onset) constraints is crucial for the emergence of the "dual" dopamine phenotype characteristic of schizophrenia.

Previous studies have shown that subchronic PCP treatment leads to a robust increase in amphetamine-triggered

dopamine release in both mPFC and striatum.[15,16] Therefore, it was unexpected that postnatal *Grin1* deletion in corticolimbic GABAergic neurons hampered an increase in the amphetamine-induced mPFC dopamine release. A major difference from systemic PCP administration is that our genetic manipulation confers GABAergic neuron-selective blockade, so that NMDAR-mediated glutamatergic transmission to cortical excitatory neurons is spared. Interestingly, theoretical analysis in a spiking network model for working memory predicts that GABAergic neuron-selective NMDAR blockade is more effective in eliciting abnormally elevated excitatory/inhibitory balance in mPFC seen in schizophrenia, compared to NMDAR blockade of both pyramidal neurons and interneurons.[45] Our study further suggested the candidate cell population for NMDAR hypofunction was PV neurons. Moreover, among the PV neurons, striatal PV+ fast-spiking interneurons may be excluded from the candidate cell-types, because they appear to carry no synaptic NMDA currents.[46] While contribution of PV neurons in other subcortical areas cannot be dismissed, it is tempting to assume that NMDAR hypofunction in cortical and hippocampal PV neurons could be responsible for the emergence of dopamine phenotypes.[12,47–49]

Another notable difference from the subchronic PCP experiments[15,16] is that activity of VTA dopamine neurons is normal in postnatal *Grin1* KO mice. This is at least partly because Cre expression is spared in the VTA of *Ppp1r2*-Cre strain. For example, *Grin1*-deletion in VTA dopamine neurons results in impaired phasic firing.[50] On the other hand, NMDAR blockade in VTA GABAergic neurons causes behavioral sensitization.[51] Subchronic PCP treatment elicits long-lasting activation of VTA dopamine neurons,[52] thereby releasing more dopamine in both striatum and mPFC in response to amphetamine. In the present study, despite little Cre expression in the VTA, we still expected abnormal VTA activation upon cortical disinhibition, based on the finding by Carr and Sesack[53] that mPFC glutamatergic efferents to the VTA make synaptic connections with dopamine neurons that project back to mPFC. Unexpectedly, we failed to detect any genotypic difference in the number of amphetamine-stimulated c-Fos+ dopamine neurons regardless of VTA subregion. It appears that disinhibition of corticotegmental efferents as a consequence of *Grin1* deletion in cortical GABAergic neurons has little effect on dopamine neuron activity, while it may exert some impact on the non-TH (presumably GABAergic or glutamatergic) neurons that also receive glutamatergic projection from mPFC.[53] We predict that disinhibition-induced activation of the mPFC glutamatergic pathway directly projecting to the NAc triggers exacerbation of striatal dopamine release, independently of dopaminergic cell-body firing in postnatal *Grin1* KO mice.

The onset of GABAergic NMDAR hypofunction also appears to be crucial for the dopamine phenotypes, because we failed to detect dopamine abnormality when *Grin1* deletion was introduced in adulthood. While the mechanism of age-dependent sensitivity to GABAergic NMDAR hypofunction remains elusive, it may be attributed to the maturation and increased resiliency of the GABAergic function during adolescence.[54] It is noted that the striatal phenotype of amphetamine-induced excessive dopamine release is somewhat milder in the PV-cre/*Grin1* mutants compared to the postnatal *Grin1* KO mice. This weaker phenotype could be due to the fact that onset of PV-Cre mediated recombination[55] appears to be slower than that of *Ppp1r2*-Cre mediated *Grin1* deletion, which occurs from postnatal day 7.[30] Alternatively, although not mutually exclusive, the stronger phenotype in postnatal *Grin1* KO mice could be due to the additional *Ppp1r2*-Cre-mediated NMDAR deletion in non-PV type GABAergic neurons.

Altogether, our results suggest the critical importance of spatial and temporal constraints of NMDAR hypofunction[56] for the "dual" dopamine phenotypes seen in patients with schizophrenia. It is of paramount importance to uncover the mechanisms underlying the emergence of aberrant dopamine release in the mPFC and striatum for the NMDAR hypofunction model of schizophrenia.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

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