Cholinergic Neurons of the Medial Septum Are Crucial for Sensorimotor Gating

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Hypofunction of NMDA receptors has been considered a possible cause for the pathophysiology of schizophrenia. More recently, indirect ways to regulate NMDA that would be less disruptive have been proposed and metabotropic glutamate receptor subtype 5 (mGluR5) represents one such candidate. To characterize the cell populations involved, we demonstrated here that knock-out (KO) of mGluR5 in cholinergic, but not glutamatergic or parvalbumin (PV)-positive GABAergic, neurons reduced prepulse inhibition of the startle response (PPI) and enhanced sensitivity to MK801-induced locomotor activity. Inhibition of cholinergic neurons in the medial septum by DREADD (designer receptors exclusively activated by designer drugs) resulted in reduced PPI further demonstrating the importance of these neurons in sensorimotor gating. Volume imaging and quantification were used to compare PV and cholinergic cell distribution, density, and total cell counts in the different cell-type-specific KO lines. Electrophysiological studies showed reduced NMDA receptor-mediated currents in cholinergic neurons of the medial septum in mGluR5 KO mice. These results obtained from male and female mice indicate that cholinergic neurons in the medial septum represent a key cell type involved in sensorimotor gating and are relevant to pathologies associated with disrupted sensorimotor gating such as schizophrenia.

Key words: cholinergic; gating; mGluR5; NMDA; sensorimotor

Significance Statement

The mechanistic complexity underlying psychiatric disorders remains a major challenge that is hindering the drug discovery process. Here, we generated genetically modified mouse lines to better characterize the involvement of the receptor mGluR5 in the fine-tuning of NMDA receptors, specifically in the context of sensorimotor gating. We evaluated the importance of knocking-out mGluR5 in three different cell types in two brain regions and performed different sets of experiments including behavioral testing and electrophysiological recordings. We demonstrated that cholinergic neurons in the medial septum represent a key cell-type involved in sensorimotor gating. We are proposing that pathologies associated with disrupted sensorimotor gating, such as with schizophrenia, could benefit from further evaluating strategies to modulate specifically cholinergic neurons in the medial septum.

Introduction

Hypofunction of ionotropic NMDA receptors has been hypothesized to underlie for the pathophysiology of schizophrenia (Snyder and Gao, 2013; Jada et al., 2016). Previous reports dem-

onstrated that administration of NMDA receptor antagonists, such as ketamine and phencyclidine, induced schizophrenia symptoms in human subjects (Bubeniková-Valesová et al., 2008; Driesen et al., 2013). In animals, administration of a noncompetitive NMDA receptor antagonist, MK801, leads to schizophrenia-like behaviors, including disruption of prepulse inhibition of the startle response (PPI), social interaction deficit, and enhanced locomotor activity (Gururajan et al., 2010). Therefore, small molecular agents capable of increasing NMDA receptor function have been considered as a therapeutic option for the treatment of schizophrenia. However, direct activation of NMDA receptors triggers rapid Ca^{2+} influx that can lead to excitotoxic neuronal death. Indirect potentiation of NMDA receptor signaling could represent another approach for the development of antipsychotic drugs.

Metabotropic glutamate receptor subtype 5 (mGluR5) is closely associated, both physically and physiologically, with NMDA receptors (Conn et al., 2009; Lindsley and Stauffer, 2013).

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Global genetic deletion of mGluR5 or modification of its cell-surface expression triggers behaviors categorized as schizophrenia-like phenotypes, such as decreased PPI and increased sensitivity to locomotor hyperactivity induced by NMDA receptor antagonists (Kinney et al., 2003; Brody et al., 2004; Wang et al., 2009). Consistent with the study of global mGluR5 knock-out (KO) mice, mGluR5 antagonists potentiate the psychotomimetic effects of NMDA receptor antagonists in animals (Henry et al., 2002). In the CNS, mGluR5 is predominantly expressed in glutamatergic, GABAergic, and cholinergic neurons (Pisani et al., 2001; López-Bendito et al., 2002; Wu et al., 2004; Sun et al., 2009). For all these reasons, mGluR5 has emerged as a potential target for the treatment of schizophrenia (Lindsley et al., 2004; Kinney et al., 2005; Stefani and Moghaddam, 2010; Rook et al., 2015; Maksymetz et al., 2017).

Disruption of sensorimotor gating, a measure of which is PPI of startle, contributes to cognitive disorganization and represents an important symptom in schizophrenia. PPI deficits remain mostly unaffected by most antipsychotics (Geyer, 2006). The relationship between mGluR5 function in different cell types and the pathophysiology of disrupted sensorimotor gating remains largely unknown. In general, the cell-type specificity associated with sensorimotor gating deficiencies is not well characterized. In this study, we started by generating three cell-type-specific mGluR5 KO mouse lines to address this question and further confirm, using DREADD (designer receptors exclusively activated by designer drugs) technology, the nature and location of the neurons involved. Conditional KO of mGluR5 specifically in cholinergic neurons reduced PPI and enhanced sensitivity to MK801-induced activity. Along these lines, interference with cholinergic neurons in the medial septum using DREADD also lead reduced PPI validating the role of these neurons in sensorimotor gating. Furthermore, using mGluR5 KO mice, electrophysiology recordings of cholinergic neurons of the medial septum showed reduced NMDA receptor-mediated currents. All together, these results indicate that cholinergic neurons in the medial septum are important for sensorimotor gating and that mGluR5 mediates some of these effects.

Materials and Methods

Animals. All animal procedures were approved by the Rockefeller University Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health guidelines. The floxed mGluR5 mouse line (generous gift from Dr. A. Contractor, Northwestern University; Xu et al., 2009, 2014; Lee et al., 2015) were crossed with EMX1-cre (stock 005628, The Jackson Laboratory; Gorski et al., 2002), parvalbumin (PV)-cre (stock 008069, The Jackson Laboratory; Hippenmeyer et al., 2005), and choline acetyl transferase (ChAT)-cre (stock 006410, The Jackson Laboratory; Rossi et al., 2011) mouse lines to delete mGluR5 in glutamatergic, PV-positive GABAergic, and cholinergic neurons, respectively. The ChAT-cre mouse line (stock 006410, The Jackson Laboratory) was used for DREADD-mediated manipulation of cholinergic neurons.

For each line, enough animals of the same age were generated, taking advantage of *in vitro* fertilization and embryo transfer techniques (Transgenic Facility, Rockefeller University), and housed four per cage with a 12 h light/dark cycle and *ad libitum* access to food and water was provided. The 10- to 16-week-old male mice were used for behavioral testing and assigned to different experimental groups based on their genotype. Other experiments were performed on males and females.

PPI. Startle was measured using a San Diego Instruments SR-Lab Startle Response System as described previously (Wang et al., 2009). Mice were placed into nonrestrictive Plexiglas cylinders resting on a Plexiglas platform. High-frequency speakers (33 cm above the cylinders) produced all acoustic stimuli. Piezoelectric accelerometers mounted under the cylinders transduced movements of the mice, which were digitized

and stored by an interface and computer assembly. Beginning at startle stimulus onset, 65 consecutive 1 ms readings were recorded to obtain the amplitude of the mouse's startle response. The house light remained on throughout all testing sessions. For the acoustic startle sessions, the intertrial interval between stimulus presentations averaged 15 s (range: 7–23 s). A 65 dB background was presented continuously throughout the session. Startle pulses were 40 ms in duration, prepulses were 20 ms in duration, and prepulses preceded the pulse by 100 ms (onset–onset). The Plexiglas holders were wiped clean and allowed to dry between runs.

The acoustic startle sessions consisted of three blocks. Sessions began with a 5 min acclimation period followed by delivery of five startle pulses (120 dB). This block allowed startle to reach a stable level before specific testing blocks. The next block tested response threshold and included four each of five different acoustic stimulus intensities: 80, 90, 100, 110, and 120 dB (data not shown) presented in a pseudorandom order. The third block consisted of 42 trials including 12 startle pulses (120 dB) and 10 each of 3 different prepulse trials (i.e., 68, 71 and 77 dB preceding a 120 dB pulse). We focused only on the 77 dB prepulse trial because we did not see a difference between the three cell-type-specific mGluR5 KO and WT groups at 68 dB and 71 dB. PPI was calculated as follows using the trials in the third block: $100 - ((\text{average startle of the prepulse} + \text{pulse trials}) / \text{average startle in the pulse alone trial}) \times 100$. In all experiments, the average startle magnitude over the record window (i.e., 65 ms) was used for all data analysis. For AAV-injected animals, clozapine-*N*-oxide (CNO; 1 mg/kg, i.p.; Sigma-Aldrich) was given 1 h before the test.

MK801-induced locomotor activity. MK801-induced locomotor activity was measured in open-field arenas (42 × 42 × 22.5 cm) as described before (Wang et al., 2009). Automated Accuscan software was used to calculate total distance traveled. Mice were habituated to the chamber for 30 min before MK801 injection (0.3 mg/kg, i.p.; Sigma-Aldrich). Their activity was monitored for 1 h immediately after injection. For AAV-injected animals, CNO (1 mg/kg, i.p.; Sigma-Aldrich) was given 1 h before the test.

Stereotaxic viral injection. Cre-dependent AAV8-hSyn-DIO-mCherry and AAV8-hSyn-DIO-hM4D(Gi)-mCherry were obtained from the Vector Core Facilities of University of North Carolina (Chapel Hill, NC). Mice were anesthetized with 2.5% avertin (250 mg/kg, i.p.) and placed onto a stereotaxic device (Leica). AAV vectors (1 μ l for medial septum, 0.8 μ l per side for nucleus accumbens) were microinjected using a Micro4 injector (World Precision Instruments) into medial septum (coordinates AP/DV/ML = 0.98/−4.34/0.00 mm) or nucleus accumbens (coordinates AP/DV/ML = 1.18/−4.67/±1.10 mm). After surgery, animals received ibuprofen in drinking water (50 mg/kg/d) for 3 d and were allowed to recover for at least 2 weeks before behavioral assessments.

Immunohistochemistry. Animals were deeply anesthetized and transcardially perfused with PBS, followed by 4% paraformaldehyde (PFA) in PBS. Brains were postfixed in 4% PFA overnight at 4°C, and then cryoprotected using 30% sucrose for at least 24 h, followed by freezing in OCT medium (Sakura Finetek). A cryostat was used to collect coronal sections of 40 μ m thickness. Free-floating sections were washed in PBS and incubated in blocking buffer (0.5% Triton X-100, 5% normal goat serum or 5% normal donkey serum, in PBS) at room temperature for 1 h. Sections were then incubated with the primary antibodies diluted in the blocking buffer at 4°C overnight. Primary antibodies: anti-ChAT (goat polyclonal, EMD Millipore; 1:250), anti-VGLUT2 (guinea pig polyclonal, Synaptic Systems; 1:250), anti-parvalbumin (mouse monoclonal, Swant; 1:1000), anti-mGluR5 (rabbit monoclonal, Abcam; 1:500). After incubation, sections were washed in PBS three times and incubated with AlexaFluor-conjugated secondary antibodies (Invitrogen). After secondary incubation, sections were washed in PBS three times and mounted on glass slides with DAPI (Life Technologies). Confocal images were obtained on a Zeiss LSM 710 confocal imaging system (Carl Zeiss).

Microscopy. AAV vector-injected mice were deeply anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg, i.p.) and transcardially perfused with 4% PFA. Brains were rapidly removed and further fixed in 4% PFA overnight. Brains were cryoprotected with 30% (w/v) sucrose solution, sectioned (50 μ m), and mounted on glass slides. Confocal images were acquired on a Zeiss LSM 710 confocal imaging system (Carl Zeiss)

using a 10×/0.3 objective lens. Images were analyzed using the Zen 2010 software (Carl Zeiss).

Volume imaging and quantification. Volume staining was performed using the bulk tissue labeling and clearing method, iDISCO, as described previously (Renier et al., 2014; Liebmann et al., 2016). Mice were first perfused with 4% PFA and postfixed overnight at 4°C, followed by washing with PBS and coronal sectioning into 3 mm blocks centered on the respective regions-of-interest. Tissue blocks were then gradually dehydrated in methanol, bleached and rehydrated in PBS. After a 0.3 M glycine wash and serum blocking, each tissue block was incubated both with rabbit anti-PV (Swant PV27; 1:100) and goat anti-choline acetyltransferase (Millipore ab144p; 1:50) for 4 d. Unbound antibodies were washed for 2 d in PBS containing heparin with repeated solution change before incubation with donkey anti-rabbit AlexaFluor 647 and donkey anti-goat AlexaFluor 568 for 4 d, each at 20 µg/ml. Excess secondary antibody was washed as previously described and tissue was cleared using methanol, dichloromethane and dibenzylether.

Volume imaging was performed on a LaVision light sheet microscope with a 4× objective in the Bio-Imaging Resource Center at The Rockefeller University. For optimal axial resolution, images were taken every 3 µm with horizontal focus applied at 10 positions across the sample. Automated cell counting was performed with Imaris object detection, with filter settings adjusted for background intensity and minimum cell size according to the respective region. For the dentate gyrus and prefrontal cortex, analysis was restricted to 1 mm tissue thickness. The entire medial septum and all of the nucleus accumbens contained within the slice were counted. ChAT staining in the dentate gyrus and prefrontal cortex was almost exclusively from axons, therefore cells were not counted.

Slice preparation. Mice were deeply anesthetized using carbon dioxide and decapitated. The brain was removed quickly and placed into the ice-cold NMDG solution containing the following (in mM): 93 NMDG (N-methyl-D-glucamine, supports neurons in brain slice preparations), 93 HCl, 10 MgSO₄, 2.5 KCl, 1.2 NaH₂PO₄, 30 NaHCO₃, 0.5 CaCl₂, 20 HEPES, 25 glucose, 5 Na-ascorbate, 3 Na-pyruvate, and 2 Thiourea, pH 7.4, 300 mOsm. Coronal slices (300 µm) were cut with a vibratome (VP1000S; Leica Microsystems) and incubated at 37°C for 15 min in NMDG solution. Then brain slices were transferred to ACSF containing the following (in mM): 130 NaCl, 26 NaHCO₃, 1 CaCl₂, 2 MgCl₂, 2.5 KCl, 1.25 NaH₂PO₄, 25 glucose, pH 7.4, 300 mOsm, bubbled with 95% O₂ and 5% CO₂, and kept at room temperature (22–24°C) for 2 h before recordings.

Electrophysiological recordings. Whole-cell voltage-clamp recording was performed in cholinergic projection neurons in the medial septum slices as reported previously (Cheng et al., 2014). Mouse slices were positioned in a perfusion chamber attached to the fixed stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated ACSF. The firing rate of cholinergic neurons in the medial septum/diagonal band of Broca (MSDB) was recorded by using cell-attached patch-clamp. The patch electrodes were filled with internal solution (in mM: 126 K-gluconate, 10 KCl, 2 MgSO₄, 0.1 BAPTA, 10 HEPES, 4 ATP, 0.3 GTP, and 10 phosphocreatine, pH 7.3, 290 mOsm). To examine the regulation by DREADD on neuronal activity, cholinergic neurons were recorded 10 min as baseline and then CNO (1 µM) was bath applied to the brain slices. Bicuculline (20 µM) and CNQX (20 µM) were added in NMDAR mediated EPSC recordings. Bicuculline (20 µM) and TTX (5 µM) were added in mEPSC recordings. Patch electrodes contained the following internal solution (in mM): 130 Cs-methanesulfonate, 10 CsCl, 4 NaCl, 10 HEPES, 1 MgCl₂, 5 EGTA, 2 QX-314 (Na⁺ channel blocker), 12 phosphocreatine, 5 MgATP, 0.2 Na₃GTP, pH 7.2–7.3, 265–270 mOsm. Medial septal cholinergic neurons were visualized with a 40× water-immersion lens and recorded with the MultiClamp 700B amplifier (Molecular Devices). For NMDAR-EPSC, the cell (clamped at –70 mV) was depolarized to +60 mV for 3 s before stimulation to fully relieve the voltage-dependent Mg²⁺ block. Evoked EPSCs were generated with a series of pulses with different stimulation intensities (120–150 µA) from a stimulation isolation unit controlled by a S48 pulse generator (Grass Technologies). A bipolar stimulating electrode (FHC) was placed ~80

µm from the neuron under recording. For mEPSC recordings, the membrane potential was held at –70 mV.

Data analyses were performed with Clampfit (Molecular Devices), Mini Analysis Program (Synaptosoft), and GraphPad Prism 6 (GraphPad Software). Amplitude of the NMDAR-EPSC was subjected to two-way ANOVA followed by *post hoc* Bonferroni tests. Statistical comparisons of the mEPSC amplitude and frequency were made by using unpaired Student's *t* test.

Statistical analysis. Statistical tests were performed by two-tailed unpaired *t* test or ANOVA using Prism 6 software (GraphPad). Statistical significance was set at *p* ≤ 0.05. All behavioral data are representative of at least two experiments using different cohorts of animals.

Results

To identify key neuronal populations involved in sensorimotor gating, relevant for the pathophysiology of psychiatric disorders such as schizophrenia, in the context of mGluR5 signaling, we used three cell-type-specific mGluR5 KO mouse lines and analyzed their sensorimotor gating properties. We generated the three conditional mouse lines by crossing existing and well characterized mouse lines. Briefly, floxed mGluR5 mice (Xu et al., 2009, 2014; Lee et al., 2015) were crossed with three different Cre lines (1) empty spiracles homeobox 1' (EMX)-Cre (Gorski et al., 2002; Lee et al., 2015), (2) PV-positive-Cre (Hippenmeyer et al., 2005; Lee et al., 2015), and (3) ChAT-Cre (Rossi et al., 2011) to delete mGluR5 in glutamatergic, PV-positive GABAergic, and cholinergic neurons, respectively. For each mouse line, immunohistochemical labeling studies were performed to ensure that the KO of mGluR5 was effective. Several anti-mGluR5 antibodies were tested and the one producing the least background was chosen (see Materials and Methods for details). Representative images are shown in Figure 1A–C. First, we ensured that baselines corresponding to basic behaviors such as locomotor activity in the open field were not altered. Next, we evaluated PPI in three cell-type-specific mGluR5 KO mouse lines according to standard protocols (Wang et al., 2009). A significant PPI deficit was observed in ChAT-mGluR5 KO mice compared with wild-type (WT) littermates (unpaired two-tailed *t* test, *t* = 2.274, *p* = 0.0341; *n* = 10; **p* < 0.05; Fig. 1D). In contrast, EMX-mGluR5 and PV-mGluR5 KO mice showed normal PPI at a prepulse intensity of 77 dB compared with WT littermates (Fig. 1E,F; *n* = 9 and *n* = 12, respectively). There was no significant difference in the startle response to 120 dB pulses within the PPI block between the three mGluR5 KO and WT groups. MK801-induced locomotor activity in cell-type-specific mGluR5 KO mice was then assessed. After MK801 treatment, ChAT-mGluR5 KO mice showed a significant enhanced sensitivity to MK801-induced locomotor activity (two-way ANOVA for the interaction between genotype and block; *F*_(17,252) = 2.442; *n* = 8; **p* < 0.05 and ****p* = 0.0015, two-way ANOVA followed by the Bonferroni *post hoc* test; Fig. 1G). In contrast, there was no difference in locomotor activity in either EMX- or PV-mGluR5 KO mice compared with their WT littermates (Fig. 1H,I; *n* = 11 and *n* = 8, respectively).

These results suggest that the KO of mGluR5 specifically in cholinergic neurons recapitulates phenotypic characteristics observed in global mGluR5 KO mice, and highlight cholinergic neurons as a component of the circuitry involved in sensorimotor gating.

The cholinergic system consists of two main cell types: (1) cholinergic interneurons in the striatum and (2) projection neurons from the pedunclopontine/lateral tegmental areas and the basal forebrain complex including medial septum (MSDB) (Scarr et al., 2013). Several lines of evidence suggest that mGluR5 is involved in the excitability of cholinergic interneurons in the

Figure 1. Analysis of prepulse inhibition of the startle response and locomotor response to MK801 in ChAT, EMX, and PV cell-type-specific mGluR5 KO mouse lines. **A–C**, Representative images after immunohistochemical stainings of ChAT, VGLUT2 (EMX marker), PV, and mGluR5 in WT and conditional KO neurons. Scale bars, 50 μ m. Arrowheads indicate some mGluR5-positive cells (yellow) and some fibrous processes (pink). Prepulse inhibition of the startle response and average startle magnitudes at 120 dB were measured in ChAT-mGluR5 (**D**), EMX-mGluR5 (**E**), and PV-mGluR5 (**F**) KO mice. The results obtained for each KO line are compared with their cre(-) mGluR5^{f/f} littermates. MK801-induced locomotor activity was analyzed for ChAT-mGluR5 (**G**), EMX-mGluR5 (**H**), and PV-mGluR5 (**I**) KO mice. Mice were injected with MK801 (0.3 mg/kg, i.p.) at the indicated time (arrow) and total distance traveled was measured using the open-field paradigm. Bar graphs are presented as mean values $\pm$ SEM. * p < 0.05. *** p = 0.0015.

striatum and septohippocampal cholinergic projection neurons originating from the MSDB (Wu et al., 2004; Bonsi et al., 2005).

We hypothesized that inhibition of cholinergic neurons in the nucleus accumbens or the MSDB might be required for the behavioral differences observed in ChAT-mGluR5 KO mice. Therefore, an inhibitory DREADD, where a Gi coupled G-protein coupled receptor (GPCR) can be activated by a ligand that does not target other endogenous GPCRs (Urban and Roth, 2015), was used to identify the type(s) of cholinergic neurons responsible for mGluR5-mediated behaviors. We found that inhibition of cholinergic neurons in the nucleus accumbens of ChAT-cre mice after stereotaxic injection of cre-dependent AAV-Gi-mCherry (Fig. 2A) resulted in normal PPI and startle reactivity compared with the control AAV-mCherry-injected group (Fig. 2C; n = 8). We also targeted the septohippocampal cholinergic projection in

the MSDB, a major cholinergic input to the hippocampus. Interestingly, mice subjected to stereotaxic injection of AAV-Gi-mCherry into the MSDB (Fig. 2D) displayed disrupted PPI compared with the control AAV-mCherry-injected group (Fig. 2F, left graph). There was no significant difference in the startle response to 120 dB pulses between control AAV-mCherry and AAV-Gi-mCherry groups (unpaired two-tailed t test; F ; t = 2.564, ** p = 0.0058; n = 9; Fig. 2F, right graph). Images obtained after IHC and confocal microscopy analysis of cre-dependent control AAV-mCherry (CTRL) or AAV-Gi-mCherry (Gi)-infected neurons did not show significant differences (Fig. 2B, E). Next, electrophysiological recordings were performed to assess the neuronal modulation by DREADD on the cholinergic neurons in the MSDB. Control AAV-mCherry (CTRL) had no effect on the firing rate of cholinergic neurons in the absence or pres-

Figure 2. Chemogenetic reduction of medial septum ChAT cell activity. Selective reduction of cholinergic neuron activity was obtained by injecting cre-dependent DREADD AAV viruses to the nucleus accumbens (NAc; **A–C**) or MSDB (**D–F**) of ChAT-cre mice. **A**, Schematic view of a coronal mouse brain section indicating the NAc injection zone (red rectangles). **B**, Representative images obtained after IHC and confocal microscopy analysis of cre-dependent control AAV-mCherry (CTRL) or AAV-Gi-mCherry (Gi)-infected neurons in the NAc of ChAT-cre mice. AC, anterior commissure. Scale bar, 200 μ m. **C**, Prepulse inhibition of the startle response and average startle magnitudes at 120 dB. **D**, Schematic view of a coronal mouse brain section indicating the MSDB injection zone (red rectangle). **E**, Representative images obtained after IHC and confocal microscopy analysis of cre-dependent control AAV-mCherry (CTRL) or AAV-Gi-mCherry (Gi)-infected neurons in the MSDB of ChAT-cre mice. Scale bar, 200 μ m. **F**, PPI of the startle response and average startle magnitudes at 120 dB. **G**, **H**, Representative traces (**G**) and summary graph (**H**) showing the firing rate of cholinergic neurons in the MSDB expressing Control AAV-mCherry (CTRL) or AAV-Gi-mCherry (Gi). CNO was applied to activate DREADD. Bar graphs are presented as mean values $\pm$ SEM. * p < 0.05. ** p = 0.0058.

ence of the DREADD agonist, CNO. Expression of AAV-Gi-mCherry itself did not alter the basal activity of cholinergic neurons. Importantly, CNO activation of the Gi DREADD significantly reduced the firing rate of cholinergic neurons in the MSDB (paired two-tailed t test; H ; t = 3.75, * p = 0.033; n = 3 mice per group; Fig. 2*G,H*). These results suggest that septohippocampal cholinergic projection neurons in the MSDB could be one of the cell types involved in the circuitry relevant for behaviors involving sensorimotor gating.

To ensure that general characteristics of the brain regions studied were not altered in the mouse lines used, quantification of PV and ChAT cells in multiple brain regions were performed using volume imaging. Representative iDISCO visualization of optical sections from the indicated brain regions stained for parvalbumin (cyan) and acetylcholine esterase (magenta) did not show significant differences in the medial septum (Fig. 3*A*). The quantification for the entire medial septum is shown in Figure 3*C*. Similar quantifications were performed for the other regions studied (Fig. 3*B*).

Figure 3. Quantification of PV and ChAT cells in multiple brain regions using volume imaging. **A**, Representative iDISCO visualization of optical sections from medial septum stained for PV (cyan) and ChAT (magenta) for both WT and KO animals. **B**, Representative iDISCO visualization of optical sections from three different brain regions costained for PV (cyan) and ChAT (magenta). Prefrontal cortex and dentate gyrus were restricted to 1 mm optical sections. **C**, Total cell counts of PV-positive and ChAT cells in the entire medial septum from volume images. Each group included four animals; both hemispheres were used. Scale bars, 500 μ m.

Next, we examined the NMDAR function in cholinergic projection neurons in the medial septum of WT and mGluR5 KO mice. NMDAR-mediated EPSCs (NMDAR-EPSCs) induced by a series of stimulus intensities were recorded. As shown in Figure 4A and B, NMDAR-EPSC was markedly reduced in cholinergic neurons in mGluR5 KO mice (55–60% decrease, WT: $n = 5$ cells/3 mice, mGluR5 KO: $n = 6$ cells/3 mice). Two-way ANOVA revealed a significant main effect of genotype and stimulation intensity ($F_{(1,44)} = 30.3$, $*p < 0.01$) and stimulation intensity ($F_{(3,44)} = 9.8$, $*p < 0.01$). Furthermore, we examined the AMPAR function and synaptic glutamate release by recording mEPSC in cholinergic neurons. The amplitude and frequency of mEPSC were relatively small in the cholinergic cells and were not remarkably altered in mGluR5-KO mice [Fig. 4C–G; amplitude (pA), WT: 12.6 ± 1.1 , $n = 5$ cells/3 mice, mGluR5 KO: 15.2 ± 0.7 , $n = 6$ cells/3 mice; frequency (Hz), WT: 0.63 ± 0.11 , mGluR5 KO: 0.57 ± 0.04 , $p > 0.05$]. Together, these results suggest that the

function of NMDARs is reduced in medial septal cholinergic neurons in mGluR5 KO mice.

Discussion

Several *In vivo* studies focusing on mGluR5 (Kinney et al., 2003; Brody et al., 2004; Wang et al., 2009, 2018) raised the possibility that mGluR5 plays a role in the pathophysiology of schizophrenia. Yet most studies report little or no change of mGluR5 mRNA expression or protein levels in postmortem samples from schizophrenic patients in the prefrontal cortex, striatum, and hippocampus (Volk et al., 2010; Corti et al., 2011; Matosin and Newell, 2013). Assuming that the quality of these postmortem samples was satisfactory, this observation can be explained by various factors. First, GPCRs undergo various modes of regulation that do not involve necessarily transcriptional and translational modifications (e.g., cell-surface expression, allosteric modulation,

phosphorylation, recycling). Second, GPCRs, including mGluR5, often have more than one variant/isoform. Third, closely related receptors (e.g., mGluR1) might contribute partially to some of the physiological differences described (Brody et al., 2003; Gupta et al., 2005; Pietraszek et al., 2007). Finally, the studies performed with patients on postmortem samples lack the anatomical precision that would be required to analyze specific cell types (e.g., ChAT interneurons). Without this level of specificity, a discreet specific differential expression might be diluted out and overlooked.

Here, we have shown that EMX-mGluR5 KO mice displayed normal PPI and normal response to MK801-induced locomotor activity, suggesting that mGluR5 in excitatory glutamatergic neurons, in the cortex and hippocampus, is not crucial for the behavioral differences mentioned earlier. This might be one explanation why most studies performed on postmortem tissues from schizophrenic patients did not observe significant mGluR5 expression level changes in these regions. However, based on the data presented here, we believe that studying mGluR5 in the context of schizophrenia by focusing on the MSDB region might be helpful in identifying procognitive cotreatments.

Intriguingly, an increased sensitivity to MK-801 after mGluR5 KO in cholinergic neurons was observed. According to the simplest model, the loss of mGluR5 should impair NMDA receptors and, considering the mode of action of MK-801, this should translate into a decreased sensitivity to MK-801. Several hypotheses could explain these results but the two main ones are as follows: (1) mGluR5 loss leads to a different NMDAR outcome, either because the signaling pathways (e.g., adaptor proteins, receptor dimers), feedback loops and the receptors' dynamic are different in this cell type, and/or because the channel properties are modified (e.g., decrease in channel opening probability or channel number); (2) MK-801 through its non-NMDA targets might allow for compensatory mechanisms and complicate the mGluR5/NMDAR model. Because of the physical absence of mGluR5 in our system we believe that it is more likely that the absence of synaptic surface co-clustering between mGluR5 and NMDAR would affect functional channel number (Aloisi et al., 2017), rather than the opening probability. Regarding MK-801 non-NMDAR known and unknown molecular targets (e.g., dopamine and se-

Figure 4. Glutamatergic neurotransmission in cholinergic neurons in the medial septum. **A**, Representative NMDAR-EPSC traces at 150 μ A stimulation. Scale bars: 20 pA, 100 ms. **B**, Input/output curves (mean $\pm$ SEM) of NMDAR-EPSC in response to a series of stimulation intensities in medial septal cholinergic neurons from WT and mGluR5 KO mice. **C**, Representative traces of mEPSC. Scale bars: 5 pA, 5 s. **D, F**, Cumulative plots of the distribution of mEPSC amplitude (**D**) and frequency (**F**) in WT and mGluR5 KO mice. **E, G**, Bar graphs (mean $\pm$ SEM) showing the amplitude (**E**) and frequency (**G**) of mEPSC. * $p < 0.05$.

rotonin receptors and transporters, nicotine acetylcholine $\alpha 7$ receptors; Ramoa et al., 1990; Clarke and Reuben, 1995; Iravani et al., 1999; Seeman et al., 2005) more work will be needed to elucidate these alternative pathways and their possible contribution. Finally, one can imagine a higher level of specificity or a greater diversity of interneurons as it was recently demonstrated for the dorsal striatum (Muñoz-Manchado et al., 2018).

In contrast to PPI, we found that selective inhibition of cholinergic neurons by AAV-Gi-mCherry in the MSDB did not affect MK801-induced locomotor activity (data not shown). The absence of an effect on locomotor activity after injection in the MSDB suggests that other circuits such as the brainstem pedunclopontine/laterodorsal tegmental nuclei may be involved in MK801-induced locomotor activity (Mori et al., 2016). Interestingly, MSDB has been recently involved in locomotion speed-correlated input through glutamatergic synaptic integration and glutamatergic projections toward the medial entorhinal cortex (Justus et al., 2017). This could represent alternative options to consider for future work.

Dysfunction of the cholinergic system has been associated with the pathophysiology of schizophrenia. Postmortem and neuroimaging studies suggest that cholinergic receptor signaling is reduced in the cortex and subcortical regions (e.g., hippocampus and striatum) in individuals with schizophrenia (Crook et al., 2000; Dean et al., 2002; Raedler et al., 2003). As a result, targeting the cholinergic system has been a promising strategy to more effectively treat cognitive deficits for quite some time now. Here, we provide the evidence that dysfunction of cholinergic neurons in the MSDB specifically lead to behavioral deficits related to sensorimotor gating, further supporting the relevance of the cholinergic system for treating psychiatric conditions involving perturbances of the sensory gating system such as schizophrenia.

Although our results presented here are clearly indicating a role of ChAT-neurons, the results regarding the PV-positive GABAergic neurons and the glutamatergic neurons might require further validation. Based on the literature, one might have expected that PV-positive interneurons could also have a role in the described phenomena (Barnes et al., 2015; Bygrave et al., 2016). Discrepancies may be attributable to the animal models used including differences in their genetic background and their purity (Paylor and Crawley, 1997). The three mouse lines used in the present study had the same genetic background and were backcrossed similarly.

So far, positive allosteric modulators of mGluR5 have shown promise in preclinical studies using animal models of schizophrenia (Lindsley and Stauffer, 2013; Matosin et al., 2017). Although positive allosteric modulators of mGluR5 have not yet reached FDA approval, it is anticipated that mGluR5-based therapeutics might provide a viable alternative option for the treatment of schizophrenia, alone or in combination (Lindsley et al., 2004; Kinney et al., 2005; Stefani and Moghaddam, 2010; Rook et al., 2015; Maksymetz et al., 2017). Because deficits in PPI are rather common in a large but specific subset of psychiatric diseases, without being able to distinguish diagnostic overlap from comorbidities (Geyer, 2006), targeting the “component” PPI rather than the entire disease, might be beneficial for schizophrenia and for any pathology involving gating disturbances.

Conclusion

The present study using conditional mGluR5 KO mice demonstrates that a specific neuronal cell type, the cholinergic neurons of the medial septum, seems important for the development of phenotypes related to psychiatric disorders, for sensorimotor

gating specifically. We believe that identifying the precise cell type(s) involved in pathological behaviors is crucial to better understand specific components involved in complex diseases and disorders, and ultimately to design targeted therapeutic strategies for these components rather than the entire syndromes.

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