Published in final edited form as:

Psychopharmacology (Berl). 2020 February ; 237(2): 345–361. doi:10.1007/s00213-019-05367-0.

mGlu5 in GABAergic neurons modulates spontaneous and psychostimulant-induced locomotor activity

Chia-Shan Wu^{1,2,3,*}, Christopher P. Jew^{1,2,4,*}, Hao Sun^{1,2}, Carlos J. Ballester Rosado^{1,2,4}, Hui-Chen Lu^{1,2,4,5}

¹The Cain Foundation Laboratories, Baylor College of Medicine, Houston, TX 77030

²Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Baylor College of Medicine, Houston, TX 77030

³Department of Nutrition and Food Science, Texas A&M University, College Station, TX 77843

⁴Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030,

⁵Linda and Jack Gill Center, Department of Psychological and Brain Sciences, Indiana University, Bloomington, Indiana 47405.

Abstract

Rationale—A role of group I metabotropic glutamate receptor 5 (mGlu5) in regulating spontaneous locomotion and psychostimulant-induced hyperactivity has been proposed.

Objectives—This study aims to determine if mGlu5 in GABAergic neurons regulates spontaneous or psychostimulant-induced locomotion.

Methods—We generated mice specifically lacking mGlu5 in forebrain GABAergic neuron by crossing DLX-Cre mice with mGlu5^{flox/flox} mice to generate DLX-mGlu5 KO mice. The locomotion of adult mice was examined in the open field assay (OFA) and home cage setting. The effects of the mGlu5 antagonist 6-methyl-2-(phenylethynyl)pyridine (MPEP), cocaine and methylphenidate on acute motor behaviors in DLX-mGlu5 KO and littermate control mice were assessed in OFA. Striatal synaptic plasticity of these mice was examined with field potential electrophysiological recordings.

Results—Deleting mGlu5 from forebrain GABAergic neurons results in failure to induce long-term depression (LTD) in the dorsal striatum and absence of habituated locomotion in both novel and familiar settings. In a familiar environment (home cage), DLX-mGlu5 KO mice were hyperactive. In the OFA, DLX-mGlu5 KO mice exhibited initial hypo-activity, and then gradually increased their locomotion with time, resulting in no habituation response. DLX-mGlu5 KO mice

Corresponding Authors: Hui-Chen Lu, Ph.D., 1101 E. 10th Street, Bloomington, IN 47405, Tel: - 812-856-9663, hclu@indiana.edu, Chia-Shan Wu, Ph.D., 123 Cater-Mattil, 2253 TAMU, College Station, TX 77843., Tel: -979-862-9144, cjwu@tamu.edu.

*These authors contributed equally to this work.

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Conflict of Interest

On behalf of all authors, the corresponding authors state that there is no conflict of interest.

exhibited almost no locomotor response to MPEP (40 mg/kg), while the same dose elicited hyperlocomotion in control mice. The DLX-mGlu5 KO mice also showed reduced hyperactivity response to cocaine, while they retained normal hyperactivity response to methylphenidate, albeit with delayed onset.

Conclusion—mGlu5 in forebrain GABAergic neurons is critical to trigger habituation upon the initiation of locomotion as well as to mediate MPEP-induced hyperlocomotion and modulate psychostimulant-induced hyperactivity.

Keywords

Metabotropic receptors; GABAergic; locomotion; habituation; hyperactivity; psychostimulant

Introduction

Glutamatergic dysfunction has been proposed to be a major factor in the etiology of many psychiatric diseases (Del Arco and Mora 2009; Howes et al. 2017; Tsai and Coyle 2002; Vinson and Conn 2012). The group 1 metabotropic glutamate receptor subtype 5 (mGlu5) is a G protein-coupled receptor that primarily signals through $G\alpha_{q/11}$ and modulates various kinases, ion channels, and intracellular calcium stores through DAG and IP3 signaling (reviewed in (Conn and Pin 1997; Niswender and Conn 2010). Genetic studies have found associations of the mGlu5 gene (*GRM5*) with both schizophrenia (Kirov et al. 2012; Matosin and Newell 2012; Niswender and Conn 2010) and attention-deficit/hyperactivity disorder (ADHD) (Elia et al. 2012b). Accumulating evidence suggests mGlu5 as a new target for the treatment of schizophrenia and improving cognitive function in multiple brain disorders (Foster and Conn 2017; Nicoletti et al. 2015).

mGlu5 has been proposed to play important roles in several behaviors including locomotor reactivity to novel environments, sensorimotor gating, anxiety, cognition, and drug-conditioned behaviors (Ballard et al. 2005; Balschun et al. 2006; Barker et al. 2006; Bordi et al. 1996; Pietraszek et al. 2005). Acute application of the noncompetitive mGlu5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) induces hyperlocomotion (Kachroo et al. 2005). In addition, MPEP treatment antagonizes catalepsy, hypo-locomotion and muscle rigidity in rodent models of Parkinson's disease (Ossowska et al. 2001; Spooren et al. 2000). Global mGlu5 KO mice are hyperactive (Bird et al. 2010; Gray et al. 2009; Olsen et al. 2010) and are defective in prepulse inhibition of the acoustic startle reflex, as well as in learning various tasks (Brody et al. 2004; Conn et al. 2009; Kinney et al. 2003; Lu et al. 1997; Olsen et al. 2010). Glutamate-mediated neurotransmission is also involved in psychostimulant-induced hyperlocomotion (Vanderschuren and Kalivas 2000; Verma and Moghaddam 1998; Vezina and Kim 1999), and mGlu5 KO mice do not exhibit cocaine-induced hyperlocomotion and fail to acquire self-administration of cocaine (Chiamulera et al. 2001). However, despite the abundance of mGlu5 in glutamatergic neurons, deleting mGlu5 only in cortical glutamatergic neurons (Glu-mGlu5 KO) resulted in a slight increase in novelty-induced locomotion but no change in sensorimotor gating, anxiety, and motor function (Jew et al. 2013). Furthermore, hyperlocomotion induced by the psychostimulant methylphenidate is enhanced in Glu-mGlu5 KO mice (Jew et al. 2013).

Besides glutamatergic neurons (Ballester-Rosado et al. 2010), mGlu5 is also abundantly expressed in brain areas enriched with GABAergic neurons, including the striatum (Testa et al. 1994) and the globus pallidus (Hanson and Smith 2002). Here we explored the role of mGlu5 in forebrain GABAergic neurons by generating a forebrain GABAergic-specific mGlu5 conditional KO mouse using floxed mGlu5 (Xu et al. 2009) and DLX 5/6-Cre transgenic mice (Monory et al. 2006). Both immunofluorescence staining and biochemical assays were used to evaluate tissue-specific removal of mGlu5 expression. We assessed the role of mGlu5 signaling in GABAergic neurons in striatal plasticity, in locomotion in the open field assay (OFA) and home cage settings, and tested locomotor responses to MPEP, cocaine, and methylphenidate.

Materials and Methods

Animals

DLX (distal-less homeobox) 5/6-Cre (DLX-Cre) transgenic mice line (Tg(dlx6a-cre)1Mekk/J; stock number 008199, Jackson Laboratory, Bar Harbor, ME, USA) were generated by using an expression cassette containing a 1.4 kb EcoRI-XhoI fragment of the Zebrafish *dlx5a/dlx6a* locus and the Cre coding sequence, downstream of a 3.5 kb fragment from the immediate 5'-flanking region of zebrafish *dlx6a*, including part of the 5'UTR (Monory et al. 2006) and backcrossed to C57BL/6 mice for 3 generations. In these mice, Cre activity is restricted to the GABAergic neurons located in the striatum and forebrain, with activity appearing from as early as embryonic day (E) 12.5 (Chao et al. 2010; Monory et al. 2006). DLX-mGlu5 KO mice were generated by crossing DLX^{Cre/+} with mGlu5 floxed (mGlu5^{f/f}) mice in C57BL/6 background (Jew et al. 2013) to generate DLX^{Cre/+};mGlu5^{f/f} (DLX-mGlu5 KO) and mGlu5^{f/f} (control) mice (Ballester-Rosado et al. 2016).

Double fluorescence reporter mice (membrane-Tomato/membrane-Green; referred to as Rosa-mT/mG mice in the text) were purchased from the Jackson Laboratories (stock number 007676) (Muzumdar et al. 2007). The Rosa-mT/mG reporter line expresses red fluorescence prior to, and green fluorescence following, Cre-mediated recombination events, allowing direct visualization of both recombined and non-recombined cells. The Tau^{mGFP} reporter mice (Tau-Lox-STOP-LoxmGFP-IRES-NLS-LacZ) contain a floxed 'stop transcription' sequence in front of membrane-anchored green fluorescent protein (mGFP) and an IRES-NLS-lacZ gene inserted into exon 2 of the Tau locus (Hippenmeyer et al. 2005). Tissue specific expression of Cre leads to the deletion of the stop signal and allows the expression of both mGFP and nuclear β -galactosidase. DLX-reporter mice were generated by crossing DLX^{Cre/+} with Rosa-mT/mG (Muzumdar et al. 2007) or Tau^{mGFP} reporter mice.

Mouse colonies were maintained as groups in a pathogen-free environment with a 14–10 hour light:dark cycle (lights on at 6:00 AM) with access to food and water *ad libitum*. Animals were treated in compliance with the U.S. Department of Health and Human Services and Baylor College of Medicine guidelines. The Baylor institutional Animal Care and Use Committee (IACUC) approved this study.

Genotyping

Genotyping was conducted as previously described (Ballester-Rosado et al. 2010). For the mGlu5 floxed allele, PCRs were conducted with a mixture of primers MGF-1 and MGF-2. The PCR products were 200 bp for the wt mGlu5 allele and 250 bp for the loxP-flanked allele. The primer sequences were as follows: MGF-1: 5'-AGA TGT CCC ACT TAC CTG ATG T-3'; MGF-2: 5'-AGT TCC GTG TCT TTA TTC TTA GC-3'. For DLX-Cre, PCRs were conducted with a mixture of primers Cre-F, Cre-R. The PCR products were 500 bp for the DLX-Cre allele. The primer sequences were as follows: Cre-F: 5'-GAT CTC CGG TAT TGA AAC TCC AGC-3'. Cre-R: 5'-GCT AAA CAT GCT TCA TCG TCG G-3'. For Tau^{mGFP} mice, PCR reactions were conducted as described in Wu *et al.* (Wu et al. 2010).

Tissue preparation and immunohistochemistry

Mice were deeply anesthetized with an intraperitoneal injection (3 ml/kg) of a rodent anesthetic cocktail containing ketamine 37.6 mg/ml, xylazine 1.92 mg/ml and acepromazine 0.38 mg/ml. Following establishment of anesthesia, mice were transcardially perfused with phosphate-buffered saline (PBS), pH 7.4, followed by 4% paraformaldehyde in PBS, pH 7.4. The brains were then post-fixed in the same fixative overnight at 4°C, washed with PBS, and serially sectioned into 100 µm thick sections in the coronal plane using a Leica VT-1000 vibrating microtome (Leica Microsystems, Bannockburn, IL). For immunofluorescence staining, free-floating sections were washed with PBS/0.01% Triton X-100 (PBST) and permeabilized with 0.3% Triton X-100 in PBS at room temperature for 20 minutes. Free-floating sections were blocked for one hour with 3% normal goat serum in PBST at room temperature, and then incubated with primary antibodies diluted in PBST/1% normal goat serum/2% BSA at 4°C overnight. The next day, sections were washed with PBST, and incubated with Alexa 488- or Alexa 594-conjugated goat anti-rabbit or anti-mouse antibodies (all at 1:500, Invitrogen, Grand Island, NY, USA). Sections were mounted with Vectashield containing DAPI for counterstaining (Vector Labs, Burlingame, CA, USA). The following primary antibodies were used: rabbit anti-mGlu5 (Millipore, Temecula, CA, USA; 1:2000), rabbit anti-β-galactosidase (Millipore, Billerica, MA, USA; 1:1000), and mouse anti-parvalbumin (Sigma, St. Louis, MO, USA; 1:1000).

Imaging

Fluorescent images were taken with a Zeiss AxioImager M1 system (Oberkochen, Germany) with 5x/0.16 Zeiss objectives, using AxioVision software. Confocal images were obtained using a Zeiss 710 system with 10x/0.3, 20x/0.5, 40x/1.3 (oil) objective lens. Alexa 488, Alexa 594, DAPI and fluorophores were excited with lasers of appropriate excitation wavelength sequentially (488 nm, 561 nm, or 405 nm) and scanned with emission filters selected to optimally separate fluorescence. All corresponding images were acquired from the same setting and processed as a whole in Adobe Photoshop CS2 (Adobe Systems, San Jose, CA, USA) for brightness/contrast, orientation, and background correction to better illustrate the staining patterns.

Western blot analysis

Brain stem, cortices, spinal cord and striatum were harvested from 6-month-old mice and homogenized in buffer (0.32 M sucrose, 1 mM EDTA, 5 mM HEPES, pH 7.4) with protease and phosphatase inhibitor cocktails (Roche Applied Science, Indianapolis, IN, USA). Protein concentrations were determined using the Bradford assay (Bio-Rad), and 25 µg of total protein was electrophoretically separated on 4–15% gradient SDS-PAGE gels and transferred onto nitrocellulose membranes (Criterion system from Bio-Rad, Hercules, CA, USA). Membranes were probed with rabbit anti-β-actin (Sigma; 1:2000), rabbit anti-mGlu1 (Millipore; 1:1000), rabbit anti-mGlu5 antibodies (Millipore; 1:2000). Immunoreactivity was detected with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA, USA; 1:5000), and visualized with WEST PICO chemiluminescence reagent (Pierce) and an ImageQuant LAS 4000 (GE Healthcare, Little Chalfont, UK). Densitometric quantification was performed using ImageJ software (NIH, Bethesda, MA, USA). The expression levels of each protein of interest were first normalized to that of β-actin, and then normalized to the mean value of the corresponding littermate control for each brain region examined.

Electrophysiology

Brain slices were prepared from P17–P26 mice. Coronal brain slices (400 µm) were cut in ice-cold modified artificial cerebrospinal fluid (ACSF) containing the following (in mM): 94 sucrose, 30 NaCl, 4.5 KCl, 1 MgCl₂, 26 NaHCO₃, 1.2 NaH₂PO₄, and 10 D-glucose and adjusted to pH 7.4 by bubbling with 95% O₂/5% CO₂. A hemislice containing the cortex and the anterior striatum was immediately transferred to a holding chamber and submerged in oxygenated ACSF consisting of the following (in mM): 124 NaCl, 5 KCl, 1.25 NaH₂PO₄, 1.3 MgSO₄, 2 CaCl₂, 26 NaHCO₃, and 11 glucose. Slices were recovered for 1 h at 37°C and slowly cooled down to room temperature before transferring to a recording chamber. ACSF or ACSF containing 50 µM D (–)-2-amino-5-phosphonopentanoic acid (APV, Sigma-Aldrich) at 32°C were perfused into slice chambers with ~2 ml/min flow rate regulated by minipuls3 peristaltic pump (Gilson, Middleton, WI, USA).

Field potential recordings were used to record neural responses in the dorsomedial or dorsolateral striatum to cortico-striatal connections using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). Recording pipettes (0.5–2 MΩ tip resistance) made from borosilicate glass were filled with 0.9% NaCl solution. Stimuli (0.1 ms duration) were applied through bipolar sharpened and insulated stainless steel microelectrodes (FHC, Bowdoin, ME, USA) placed into the white matter area dorsal to the recording area. Data were collected with 10 kHz sampling rate filtered by 4 kHz using pClamp 10.3 (Molecular Devices) and data were analyzed with Clampfit. The stimulus intensity ranged from 100 µA to 1 mA was set to the level at which an evoked population spike (PS) was approximately one half the amplitude of the maximal obtainable response. The majority of the slices were also subjected to maximal stimulation at the end of an experiment to ensure that no significant deterioration of the slices occurred during the experiment.

LTP in the dorsomedial striatum and LTD in dorsolateral striatum, respectively, were induced by a high frequency stimulation paradigm (HFS), consisted of four 1-s duration

with 100-Hz trains separated by ten seconds inter-stimulus interval. LTD experiments were conducted in the continuous presence of 50 μ M APV. The magnitude of LTD or LTP was defined as the ratio of the average response from 21–40 min following HFS to the average response amplitude during a 20 min baseline. All summary data are presented as means $\pm$ SEM.

Behavioral testing

Male and female mice at 2–4 months of age were subjected to selected tests from the test battery originally described by Crawley and Paylor (Crawley and Paylor 1997). Mice were rested in their home cages for at least one week before being tested in the next assay (McIlwain et al. 2001; Paylor et al. 2006). For pharmacological treatment for the open field assay, mice were rested for at least two days. Information on the number of animals used for each behavioral paradigm can be found in the Tables. Each mouse was subjected to each behavior paradigm once, i.e. naïve to each test, except for the 2-day open-field assay (OFA) and pharmacology experiments where injection and OFA were repeated for each mouse (less than five times total).

Prior to any behavioral testing, mice were allowed to acclimatize to the testing room for at least 30 minutes. Testing was performed under standard room conditions, approximately 750–800 lux of illumination (Spencer et al. 2011; Spencer et al. 2006). 55 dB of white noise was used in OFA, where anxiety could be a confounder, to reduce audio interference from the experimenter or environment (Spencer et al. 2011). Behavioral testing was performed between 10:00 AM and 4:00 PM (mid phase of the light cycle). Experimenters were blind to the genotypes of the mice.

Open Field Assay (OFA)

Each mouse was placed into the center of a clear Plexiglas chamber (40 cm $\times$ 40 cm $\times$ 30 cm) with photo beams to record its horizontal and vertical movements in an OFA. Activity was recorded using a computer-operated VersaMax Animal Activity Monitor System (Accuscan Instruments, Columbus, OH). Before all open field experiments, mice were individually weighed, tails marked, and introduced into the testing room to acclimate for at least 30 minutes before testing. Data were collected in 10-minute interval bins for 60 min, and the assay was repeated on the following day.

Home cage

Mice were individually placed in standard polypropylene cages (16.5 $\times$ 27 cm) similar in size to their regular housing cages, and their horizontal locomotor activities were assessed by an automated recording system (San Diego Instruments, San Diego, CA) as described (Moretti et al. 2005) over 3 days. The home cage testing room followed the same 14–10 hour light:dark cycle (lights on at 6:00 AM) used in the mouse colony holding rooms. The cages were placed into frames equipped with five infrared photocell beams (5 cm apart). Locomotion was measured as the number of sequential breaks in two adjacent beams. Total locomotion was calculated for the first 6 hours of each phase (light phase 6:00 AM–12:00 PM; dark phase 8:00 PM–2:00AM) and averaged over each day of the experiment.

Pharmacology

2-methyl-6-(phenylethynyl)pyridine (MPEP) hydrochloride was obtained from Tocris (San Diego, CA, USA) and dissolved in 0.9% saline at 4 mg/ml prior to testing. Methylphenidate (threo-methyl α -phenyl- α -(2-piperidyl)acetate hydrochloride) was obtained from Sigma and dissolved in 0.9% saline at 1 mg/ml prior to testing. (–)-Cocaine hydrochloride (Sigma) was dissolved in 0.9% saline at 5 mg/ml. MPEP was injected at a dose of 40 mg/kg i.p. Methylphenidate was injected at doses of 4 or 8 mg/kg i.p. Cocaine was administered at a 15 mg/kg dose i.p.

For MPEP, methylphenidate, and cocaine experiments, each mouse was tested with drug and corresponding vehicle in separate OFAs. Mice were randomly assigned to treatment groups and treatments were reversed with greater than 2 days between experiments. The mice were first placed inside a Plexiglas chamber and were recorded for 30 (for cocaine) or 60 minutes (for MPEP and methylphenidate). After the habituation period, the mice were returned to their home cages with access to food and water, while their Plexiglas chambers were cleaned. Each mouse was then given a single i.p. injection and immediately placed back into its original Plexiglas chamber for a 60-minute recording period. For data presentation, the last 10 minutes of the pre-injection habituation recording is included with the post-injection recording period for MPEP, methylphenidate, and cocaine experiments. A green arrow indicates the relative time point of injection. Data presented in a line graph represent aggregate scores over each 10-minute bin. For WT and KO comparisons, pooled data represent the combined scores over the entire 60-minute period.

Statistical Analyses

Statistical analysis was conducted using SPSS (SPSS, Chicago, IL, USA), or Prism 3.0 (Graph-Pad Software). All data were first analyzed by two-way ANOVA (gender $\times$ genotype) or (genotype $\times$ treatment), three-way ANOVA (gender $\times$ genotype $\times$ treatment) with repeated measures. Whenever data failed Mauchly's Test of Sphericity, the Greenhouse-Geisser results are reported for degrees of freedom. When there was no genotype $\times$ gender or treatment $\times$ gender interaction, gender data were combined for statistical comparisons. When there was significant genotype $\times$ time interaction effect but no significant gender $\times$ genotype effects, gender data were pooled and data analyzed with Student's t-test for each time point. All data were presented as mean $\pm$ SEM. The level of statistical significance was set at $p < 0.05$.

Results

Deleting mGlu5 from forebrain GABAergic neurons

GABAergic neuron-specific mGlu5 KO mice were generated by crossing mGlu5 floxed (mGlu5^{f/f}) mice (Xu et al. 2009) with DLX-Cre transgenic mice (Chao et al. 2010; Monory et al. 2006). To confirm the expression pattern of Cre-activity, we crossed DLX-Cre mice with two different reporter lines, the Rosa-mT/mG (Muzumdar et al. 2007) and Tau^{mGFP} reporter mice (Hippenmeyer et al. 2005; Muzumdar et al. 2007). Similar to previous reports, DLX-Cre mediated recombination was most evident in the striatum and the reticular nuclei where GABAergic neurons are enriched (Fig. 1A, green immune-positive signals). With

Tau^{mGFP} reporter mice, where Cre-mediated recombination could be detected by both membrane-targeted GFP and β -galactosidase localized in the nuclei, we found that DLX-Cre activity was prominent in the reticular thalamic nucleus and striatum, as indicated by a high-density of β -galactosidase immuno-positive cells in these regions (Fig. 1B, red immune-positive signals). Modest numbers of β -galactosidase and GFP positive cells were found in the cortex (Fig. 1C). A fraction of β -galactosidase positive cells was labeled by parvalbumin (PV), a marker for fast-spiking inhibitory neurons, suggesting that DLX-Cre targets PV-GABAergic neurons (Fig. 1C2, C3).

To evaluate knockout efficiency, mGlu5 immunostaining was conducted with DLX-mGlu5 KO mice and their littermate controls. High-levels of mGlu5 expression were observed in the striatum, cortex, and thalamus of wild-type mice (Fig. 1D, E). In DLX-mGlu5 KO mice, mGlu5 immuno-reactivity was substantially reduced in the striatum and reticular thalamic nucleus, while no obvious change was evident in the cortex and the remainder of the thalamus (Fig. 1F, G). To quantify the extent of reduction of mGlu5 and possible alteration of mGlu1 levels in DLX-mGlu5 KO mice, we performed western blot analysis to quantify mGlu5 and mGlu1 protein levels in the cortex, striatum, brainstem, and cerebellum of DLX-mGlu5 KO and littermate control mice (Fig. 2A–B). In control mice, mGlu5 was very abundant in the striatum and cortex but was almost non-detectable in the cerebellum, while mGlu1 was expressed at similar levels across the different brain regions (Fig. 2A). In DLX-mGlu5 KO brains, mGlu5 but not mGlu1 levels were significantly reduced in the striatum ($p < 0.001$) but normal in the cortex and brain stem (Fig. 2A, C). mGlu1 levels were comparable between DLX-mGlu5 KO and control mice in all regions measured. We previously reported that in cortical glutamatergic mGlu5 KO mice, mGlu5 levels were greatly reduced in the cortex but not in the striatum (Jew et al. 2013). Taken together, cortical mGlu5 expression is mainly present in the glutamatergic neurons while striatal mGlu5 is primarily present in GABAergic neurons.

Impaired synaptic plasticity in DLX-mGlu5 KO dorsal striatum

In the striatum, mGlu5 receptors are distributed on the dendritic spines of medium spiny neurons (Conn et al. 2005) and can modulate dopamine transporter activity (Chiamulera et al. 2001; Page et al. 2001). Dopamine controls various forms of long-term synaptic plasticity in distinct striatal sub-regions (Centonze et al. 2001; Shen et al. 2008). A high frequency stimulation (HFS) paradigm that triggers long-term potentiation (LTP) in the anterior dorsomedial striatum induces long-term depression (LTD) in the anterior dorsolateral striatum during postnatal days 15–34 (P15–34) (Partridge et al. 2000). To investigate whether these two forms of dopamine-dependent corticostriatal plasticity require mGlu5, field potential recordings in the dorsolateral and dorsomedial striatum were conducted with P19–26 DLX-mGlu5 KO mice and their wildtype littermates (Fig. 3). HFS stimulation triggered LTP (Fig. 3A–C; $142.8\% \pm 9.1\%$ of baseline level; $n = 14$ slices from 9 mice) or LTD (Fig. 3D–F; $71.7\% \pm 6.0\%$ of baseline level; $n = 11$ slices from 6 mice) formation in the wildtype dorsomedial and dorsolateral striatum, respectively. In DLX-mGlu5 KO dorsomedial striatum, a stable and long-lasting potentiation of fEPSP (> 40 min) was also induced by HFS ($149.9\% \pm 14.3\%$; $n = 11$ slices from 7 mice) similar to wildtype (Fig. 3B, C). By contrast, the HFS paradigm failed to induce LTD in DLX-mGlu5 KO dorsolateral

striatum ($96.0\% \pm 6.0\%$ of baseline; $n = 8$ slices from 5 mice) (Fig. 3E, F). Taken together, our results showed that mGlu5 in GABAergic neurons is required for LTD formation in dorsolateral-striatum.

DLX-mGlu5 mutant mice are hyperactive and defective in habituation

Dopaminergic signaling plays key roles in the formation of synaptic plasticity in the dorsal striatum and regulates locomotor activity (Beninger 1983; Centonze et al. 2001). The defective striatal LTD formation in DLX-mGlu5 KO mice together with the hyperactive locomotor phenotype observed in global mGlu5 KO mice (Gray et al. 2009; Olsen et al. 2010) prompted us to determine the roles of GABAergic mGlu5 in locomotor behaviors. Both naïve male and female DLX-mGlu5 KO and their littermate control mice were subject to the OFA (Crawley and Paylor 1997). Locomotor activity was measured as total distance traveled, total movement time, and vertical activity (rearing), and analyzed using two-way ANOVA (gender $\times$ genotype; Table 1). Mice were re-exposed to the same field for the following day. Both male and female naïve DLX-mGlu5 KO mice on Day 1 showed significantly higher locomotor activity compared to control mice (Table 1; representative activity plots were shown in Fig. 4A). Since there were no significant gender and genotype $\times$ gender effects, gender data were combined and presented in Fig. 4B–C (total distance traveled: $F_{1,31} = 17.6$, $p < 0.001$; total movement time: $F_{1,31} = 39.1$, $p < 0.001$). Interestingly, the hyperactive phenotype in DLX-mGlu5 KO mice persisted upon re-exposure to the same open field arena (Fig. 4B–C). On both Day 1 and Day 2, DLX-mGlu5 KO mice were slightly less active compared to their littermates during the first 10 minutes of testing but subsequently became more active. While control mice reduced their locomotion with time, DLX-mGlu5 KO mice steadily increased their locomotor activity throughout the one hour testing period (Fig. 4A–C). Analysis of open field data by 10 minutes time bins, using two-way ANOVA (genotype $\times$ time interval) with repeated measures, revealed significant genotype $\times$ interval interaction effect for total distance ($F_{2,643, 47.579} = 4.480$, $p = 0.01$) and for movement time ($F_{2,332, 41.970} = 8.017$, $p = 0.001$). Despite the hyperactivity phenotype, anxiety levels in DLX-mGlu5 KO weren't significantly altered compared to littermate control mice, albeit there was a trend for increased time spent in center zone in the KO mice (Table 1). Consistently, DLX-mGlu5 KO mice exhibited more center crossings during the hour-long open field test (Fig. 4A). In summary, DLX-mGlu5 KO mice exhibited significantly decreased habituation response, and this habituation deficit might account for their overall hyperactivity.

To dissociate anxiety as a factor contributing to the observed hyperactivity in DLX-mGlu5 KO mice, we next examined whether deleting mGlu5 in GABAergic neurons also results in hyperactivity in a familiar environment. The diurnal activity levels of DLX-mGlu5 KO and their littermate control mice were assessed in home cage-like environment across multiple days. Both genotypes exhibited more activity during the dark phase, and female mice were significantly more active than male mice (gender: $F_{1,31} = 21.594$, $p < 0.001$; genotype $\times$ gender: $F_{1,31} = 6.539$, $p = 0.016$). DLX-mGlu5 KO mice were extremely hyperactive during the dark phase compared to their littermate controls (Fig. 4D–E and Table 1; genotype: $F_{1,31} = 57.586$, $p < 0.001$). During the light phase, DLX-mGlu5 KO mice exhibited normal

activity levels. Taken together, our data suggest that deleting mGlu5 function in GABAergic neurons results in hyperactivity and defective habituation behavior.

mGlu5 expression in GABAergic neurons is required for the hyper-kinetic effects of the mGlu5 antagonist MPEP

Acute MPEP application induces hyperlocomotion (Kachroo et al. 2005; Thomas et al. 2011). To examine whether mGlu5 signaling in GABAergic neurons mediates the hyper-kinetic effect of MPEP, we quantified the impact of acute 40 mg/kg MPEP administration on the locomotion of DLX-mGlu5 KO mice and their littermate controls using the OFA (Fig. 5). As expected, MPEP treatment increased locomotion compared to vehicle treatment in control mice (Fig 5, blue line and bars). The MPEP-induced hyperlocomotion was abolished in DLX-mGlu5 KO mice (pink line and bars). Two-way ANOVA (genotype $\times$ treatment) confirmed a significant difference in genotypes for the effects evoked by MPEP treatment in distance traveled ($F_{1,36} = 9.576$, $p = 0.004$), and in movement time ($F_{1,36} = 17.283$, $p < 0.001$). In summary, administering MPEP to DLX-mGlu5 KO mice did not elicit the hyperactivity phenotype characteristic of control mice. These data suggest that mGlu5 in GABAergic neurons mediates the hyper-kinetic effects of MPEP.

Cocaine induced locomotor hyperactivity is reduced in DLX-mGlu5 KO mice

Global mGlu5 KO mice do not exhibit cocaine-induced hyper-locomotion and fail to acquire self-administration of cocaine (Chiamulera et al. 2001). To assess whether mGlu5 in GABAergic neurons is required for cocaine-induced hyper-locomotion, we tested the impact of cocaine on male DLX-mGlu5 KO and their littermate control mice. Control mice responded to cocaine treatment with hyperactivity twenty minutes after injections and their hyperactivity peaked around 40 min post-injection (Fig. 6). DLX-mGlu5 KO mice responded to cocaine with a substantial reduction in locomotion during the first 30 minutes post injection, followed by a gradual recovery back to normal locomotion speed (Fig. 6A–D). ANOVA (treatment $\times$ genotype) analysis of total movement time over 1 hour post-injection showed a significant treatment effect ($F_{1,64} = 6.111$, $p = 0.016$). Comparison of treatment effect compared to control within each genotype showed a significant difference in WT ($t_{11} = -5.430$, $p < 0.001$), but not in DLX-mGlu5 KO mice ($t_{20} = 0.169$, $p = 0.868$, paired Student's t-test). In summary, we found that cocaine responsiveness was significantly reduced in DLX-mGlu5 KO mice.

DLX-mGlu5 mutant mice exhibit a delayed response to methylphenidate

mGlu5 deletion and single nucleotide polymorphisms have been found in ADHD patients (Elia et al. 2010; Elia et al. 2012a; Hinney et al. 2011). Methylphenidate, also known as Ritalin, is a commonly used psychostimulant to treat ADHD patients (Greenhill et al. 2006; Kratochvil et al. 2002; Sunohara et al. 1999; Swanson et al. 1995). Hyperactivity in ADHD animal models is typically normalized by treatment with psychostimulants like methylphenidate (e.g. (Avalle et al. 2004; Ishisaka et al. 2012; Zhu et al. 2012)). To evaluate whether mGlu5 deletion in GABAergic neurons alters the response to methylphenidate, we examined its impact on locomotion of WT and DLX-mGlu5 KO animals. Following injection of 8 mg/kg methylphenidate, both DLX-mGlu5 KO mice and controls exhibited a significant increase in walking distance, albeit with different kinetics (Fig. 6E–F). DLX-

mGlu5 KO mice responded to methylphenidate with a delay. Control mice reached a maximum response 11–20 minutes post injection, while DLX-mGlu5 KO mice reached their maximum response at 31–40 minutes post injection. Comparisons of the total distance traveled for the 11–20 minutes post injection showed a significant treatment effect in control mice, but not in DLX-mGlu5 KO mice (Fig. 6F). Analysis of total distance traveled over the 1 hour period by two-way ANOVA (genotype $\times$ treatment) showed a significant treatment effect ($F_{1,28} = 12.622$, $p = 0.001$), but no significant genotype ($F_{1,28} = 0.011$, $p = 0.918$) or genotype $\times$ treatment interaction effects ($F_{1,28} = 0.112$, $p = 0.740$). This observation suggests that DLX-mGlu5 KO mice have a delayed response to methylphenidate but eventually the locomotor response reaches a similar intensity as observed in wildtype mice.

When DLX-mGlu5 KO mice were treated with 4 mg/kg of methylphenidate, they also exhibited hyperactivity (data not shown). Both control and KO mice experienced a 2–3 fold enhancement of horizontal activity after methylphenidate with no genotype or interaction effect (treatment: $F_{1,34} = 4.769$, $p < 0.05$; genotype: $F_{1,34} = 0.004$, $p = 0.950$; genotype $\times$ treatment: $F_{1,34} = 0.072$, $p = 0.790$). Again, despite the delay in responding to methylphenidate, no difference was observed between the maximum increase of total distance traveled in 31–40 minutes post-injections for control mice and the distance for 41–50 minutes post-injections for DLX-mGlu5 KO mice ($t_{17} = -0.149$, $p = 0.884$). Taken together, DLX-mGlu5 KO mice responded to methylphenidate with about a 10–20 minutes delay.

Discussion

The basal ganglia are an interconnected group of subcortical nuclei that are involved in the control of motor behavior, reward, and motivational processes (Graybiel 2005). Studies indicate that mGlu5 receptors are expressed throughout the basal ganglia, where they have several important functions in regulating neuronal excitability and synaptic transmission, and have emerged as a prominent drug target for therapies to treat psychological disorders and addiction (Conn et al. 2005; Gubellini et al. 2004). Here we employed a genetic approach to demonstrate that mGlu5 in the forebrain GABAergic neurons modulates corticostriatal synaptic plasticity, spontaneous locomotor activities and habituation responses, as well as the locomotor response to cocaine, methylphenidate and MPEP.

As the primary basal ganglia input nucleus, the striatum integrates and processes glutamatergic inputs from the cortex and thalamus, as well as receiving dopaminergic inputs from the *Substantia Nigra Compacta* (SNc) (Conn et al. 2005; Graybiel 2005). In the striatum, 95% of the striatal neurons are parvalbumin (PV)-negative medium spiny neurons (MSNs) (Chuhma et al. 2011; Durieux et al. 2011). In the striatum, mGlu5 are expressed by medium spiny GABA neurons, as well as by PV-containing GABA interneurons and neuropeptide Y/somatostatin-positive GABA interneurons (Tallaksen-Greene et al. 1998; Testa et al. 1994). In the current study, we employed DLX-cre to target GABAergic neurons (Ghanem et al. 2008). Previous studies have used this Cre line to delete the gene-of-interest in GABAergic neurons, and reported DLX-Cre recombination occurring in GABAergic neurons enriched basal forebrain, including the striatum and the thalamic reticular nucleus (Chao et al. 2010; Monory et al. 2006). As expected, we also found that DLX-cre

recombination occurred mainly in the striatum and the thalamic reticular nucleus (Figs. 1A and 1B). In DLX-mGlu5 KO mice, mGlu5 was mostly eliminated in the striatum and in the thalamic reticular nucleus (Fig. 1F and 1G). The extent of mGlu5 deletion in the striatum suggests that mGlu5 is deleted in both dopamine receptor D1 (D1) and D2-expressing MSNs.

Here we report that removing mGlu5 from GABAergic neurons led to an enhancement of locomotion that didn't habituate with time. The enhanced locomotion and reduced habituation was particularly evident during the dark phase in the home cage setting. Interestingly, a previous report showed that deleting mGlu5 in PV-subtype GABAergic interneurons (PV-mGlu5 KO) has minimal impact on locomotion or habituation (Barnes et al. 2015). The discrepancies are likely due to the temporal and spatial differences in Cre expressions between DLX- and PV-Cre mouse lines. DLX-Cre is expressed from embryonic stages in most GABAergic neurons, while PV-Cre is postnatally expressed and limited to PV-subtype neurons (Taniguchi et al. 2011). Furthermore, only 0.7% of striatal neurons are PV-positive (Tepper et al. 2010). Taken together the pivotal role of MSNs in motor control (Graybiel et al. 1994; Yin and Knowlton 2006) and the differential behavior phenotypes between DLX- and PV-mGlu5 KO mice in locomotion, our data implicate mGlu5 in striatal MSNs as having a critical role in mediating spontaneous locomotion and habituation.

In addition to the impaired habituation response, DLX-mGlu5 KO mice also exhibited hypo-locomotion in the first 10 minutes in a novel environment. This is not likely due to enhanced anxiety levels since DLX-mGlu5 KO mice showed reduced anxiety; however, the reduced anxiety may contribute to the overall hyperactivity observed in DLX-mGlu5 KO mice in the hour-long open field test. The complex pattern of motor behaviors suggests that mGlu5 receptors in GABAergic neurons are required for the initiation of movement, and for the subsequent switch to suppression of motor function and habituation response. The prevailing model of basal ganglia function proposes that two projection circuits originate from distinct populations of MSNs in the striatum, referred to as direct and indirect pathways (Bateup et al. 2010; Gerfen and Surmeier 2011). These two pathways have opposing effects on movement: activity of the D1-expressing MSNs in the direct-pathway facilitates movement, while activity of the D2-expressing MSNs in the indirect-pathway inhibits movement (Bateup et al. 2010). On the other hand, a more recent study using *in vivo* photometry challenged this classical view, demonstrating that increases in neural activity occurred concurrently in both direct (D1) and indirect (D2) MSNs in the dorsal striatum when animals initiated actions, but not when they were inactive (Cui et al. 2013). Interestingly, a mild reduction of activity in the novel environment has been reported in D1-specific mGlu5 knockdown mice, with no change in the distance traveled in familiar environment; the authors suggest that mGlu5 in D1-expressing neurons are important for novelty-seeking behaviors (Parkitna et al. 2013). Furthermore, dorsolateral striatum has been shown to be important in habit formation (Malvaez and Wassum 2018; Yin et al. 2004). Taken together, while our current genetic model cannot distinguish the contribution of mGlu5 in D1 and D2 pathways in the complex motor behavior pattern observed, we speculate that absence of mGlu5 in D1-MSNs contribute to the observed hypokinesia initially when DLX-mGlu5 KO mice were placed in novel environment. The absence of mGlu5 in D2-MSNs may contribute

to the impaired habituation and enhanced locomotion after getting familiar with the environment.

Striatal plasticity has been hypothesized to be a key underlying cellular mechanism mediating motor activity and motor learning (Gittis and Kreitzer 2012; Mathur and Lovinger 2012). Striatopallidal LTD is the main form of synaptic plasticity in the dorsolateral striatum and requires activation of mGlu5 receptors (Kreitzer and Malenka 2007; Lovinger 2010; Partridge et al. 2000; Yin and Knowlton 2006). Here we found that mGlu5 deletion from MSNs resulted in impairment of LTD formation in the dorsolateral region of rostral striatum. This is consistent with a previous study using pharmacological blockade (Sung et al. 2001). This form of LTD formation depends on endocannabinoid and dopamine signaling (Gerdeman et al. 2002). The dorsal striatum is the gateway to the basal ganglia and receives glutamatergic inputs from the cortex and thalamus to regulate motor function (Graybiel et al. 1994; Kreitzer and Malenka 2008). In the striatum, mGlu5 is distributed on the dendritic spines of medium spiny neurons and in close proximity to diacylglycerol lipase- α , a synthesizing enzyme for the endocannabinoid, 2-arachidonoyl-glycerol (2AG) (Uchigashima et al. 2007). 2AG is an endocannabinoid that facilitates the formation of LTD in the dorsolateral striatum (Kreitzer and Malenka 2007). Our data support the hypothesis that mGlu5 activity in the dorsal striatum engages endocannabinoid system to form corticostriatal LTD (Sung et al. 2001). Further studies are needed to determine whether LTD deficit in the dorsolateral striatum of DLX-mGlu5 KO mice disrupts endocannabinoid signaling and contributes to habituation impairment. Normal LTP formation in the anterior dorsomedial striatum of DLX-mGlu5 mice suggests that this type of NMDAR-dependent plasticity (Partridge et al. 2000) does not require functional mGlu5.

In the current study, we found that MPEP increased locomotor activity in WT mice, and this hyperactivity was diminished in DLX-mGlu5 KO mice. The MPEP-induced hyperlocomotion differed from previous reports. Global deletion of mGlu5 did not affect locomotor activity (Chiamulera et al. 2001), while pharmacological studies using negative allosteric modulators (NAMs) of mGlu5 largely showed no effects on locomotion; these include systemic administration of MPEP (Sethna and Wang 2016) and CTEP (Areal et al. 2019), and intra-dorsal striatum and intra-nucleus accumbens infusion of MTEP (Knackstedt et al. 2014). In contrast, another study showed that MPEP infusion (25 nmol/0.5 μ l) into dorsal striatum resulted in increased locomotor activity, whereas MPEP infusion into ventral striatum or motor cortex resulted in hypokinesia (Guimaraes et al. 2015). The reasons for the differing effects of mGlu5 antagonism could be due to different doses administered, different site of administration, and/or different receptor occupancy, specificity and efficacy due to different NAMs used. Previous pharmacokinetic study showed that MPEP is highly brain-penetrant and able to occupy brain mGlu5 receptors at relatively low doses (Anderson et al. 2002). Maximal inhibition of binding occurred at 30 mg/kg, at which the radio-ligand binding was decreased by 92%. A 50 mg/kg dose did not reduce binding beyond that of the 30 mg/kg dose. Thus 40 mg/kg ip, the dose we used, is above maximal inhibition, hence the possibility of non-specific effects at NMDA receptors cannot be excluded (O'Leary et al. 2000). It is possible that non-specific MPEP action at 40 mg/kg induced the hyperactivity observed in WT mice.

Peculiarly, vehicle injection triggered locomotion was abolished by MPEP treatment in DLX-mGlu5 KO mice. A possible mechanism is through blockage of mGlu5 in dopaminergic neurons in the ventral tegmental area (VTA). Ferrada et al. (Ferrada et al. 2017) reported that intra-VTA infusion of the selective mGlu5 agonist CHPG increased release of dopamine, suggesting the possibility that MPEP, acting at mGlu5 receptors in dopaminergic neurons in VTA, could reduce dopaminergic inputs to the striatum, counteracting the reduced corticostriatal glutamatergic inputs into striatum in DLX-mGlu5 KO mice, thus resulting in overall hypo-locomotion.

In the present study, we further probed the role of mGlu5 in GABAergic neurons in the basal ganglia by examining cocaine- and methylphenidate-induced hyper-locomotion in our genetic model. The role of mGlu5 in mediating cocaine-induced behavioral changes has been suggested from both genetic (Chiamulera et al. 2001) and pharmacological studies (Kenny et al. 2005; McGeehan and Olive 2003; Tessari et al. 2004; Wang et al. 2013). Here, we found that absence of mGlu5 in GABAergic neurons suppressed the extent and kinetic of hyper-locomotion response to cocaine, and delayed the hyper-locomotion response triggered by methylphenidate. The psycho-motor stimulant properties of cocaine and methylphenidate are generally attributed to their enhancement of dopamine neurotransmission in mesolimbic regions of the brain (Faraone 2018; Johanson and Fischman 1989). A previous study has compared the pharmacokinetics and distribution of cocaine and methylphenidate in the human brain, to assess whether they compete for the same binding sites, using positron emission tomography to measure the temporal and spatial distribution of carbon 11 [11C]-labeled methylphenidate and [11C]cocaine (Volkow et al. 1995). The uptake of [11C]methylphenidate in the brain was high (mean $\pm$ SD, 7.5% $\pm$ 1.5%), with maximal concentration occurring in the striatum. Furthermore, the regional distribution of [11C]methylphenidate was identical to that of [11C]cocaine, and they competed with each other for the same binding sites. Interestingly, a triple mutation in the second transmembrane domain of mouse dopamine transporter (DAT) has been identified that markedly decreases sensitivity to cocaine and methylphenidate, but not to amphetamine or methamphetamine; this suggests that cocaine and methylphenidate share same binding sites to DAT (Chen et al. 2005). Taken together, while differential effects were observed with cocaine and methylphenidate in the current study, we cannot rule out the possibility that a higher dose of cocaine (15 mg/kg dose were used here) may produce a similar response curve as methylphenidate in the DLX-mGlu5 KO mice, affecting the kinetic but not the extent of hyper-locomotion response.

Cocaine induces fast dopamine increases in the striatum, which are recognized to underlie its rewarding effects (Phillips et al. 2003). Since the kinetics in cocaine-induced responses in the striatum have been better characterized, the altered kinetics in locomotor response to cocaine in DLX-mGlu5 KO mice will be discussed in more detail here. Both D1 and D2 receptors are involved in cocaine's rewarding effects; activation of striatal D1-MSNs (direct-pathway) enhances cocaine reward, whereas activation of D2-MSNs suppresses it (indirect-pathway) (Lobo et al. 2010). Cocaine-induced phosphorylation of extracellular signal-regulated kinase (ERK) occurred exclusively in D1-expressing neurons in the dorsal striatum and NAc core and shell, 15 minutes after cocaine injection, whereas no staining was detected in D2-expressing neurons at this time point (Bertran-Gonzalez et al. 2008).

Furthermore, using *in vivo* microprobe optical imaging, Luo et. al (Luo et al. 2011) demonstrated the dynamic changes in intracellular calcium responses (used as marker of neuronal activation) to acute cocaine for D1-versus D2-expressing MSNs in striatum. Acute cocaine (8 mg/kg, i.p.) rapidly activated D1-MSNs in striatum within 8.3 ± 2.3 minutes after cocaine administration, after which the increases in intracellular calcium plateaued. In contrast, cocaine induced progressive decreases in intracellular calcium in D2-MSNs continuously throughout the 30 minutes that followed cocaine administration. Hence, cocaine's effects involve both its fast stimulation of D1 (resulting in rapid activation of direct-pathway neurons) and a slower stimulation of D2 (resulting in longer-lasting deactivation of indirect-pathway neurons). Different from a previous report showing that global mGlu5 deletion completely abolished the locomotor stimulant effects of cocaine (from 10 to 40 mg/kg doses) (Chiamulera et al. 2001), we found that deleting mGlu5 expression only in GABAergic neurons altered the kinetics of cocaine-induced locomotion. Cocaine administration substantially suppressed locomotion in the first 10 min but then resume the locomotor stimulant effects at later time point (15 mg/kg dose; Fig. 5A–D). These observations suggest that mGlu5 in GABAergic neurons is required for the first phase of cocaine-induced locomotion. How mGlu5 deletion altered dopamine triggered responses differently in D1- vs D2-MSNs remains to be elucidated.

The neurobiological mechanisms underlying the rise in locomotor activities during the latter phase after cocaine or methylphenidate administration in the DLX-mGlu5 KO mice is less clear. The data are in contrast to previous genetic (Chiamulera et al. 2001) and pharmacological studies showing that mGlu5 blockade suppresses cocaine-induced behavioral changes (Kenny et al. 2005; Li et al. 2018; McGeehan and Olive 2003; Tessari et al. 2004; Wang et al. 2013). Interestingly, overexpression of Regulator of G-protein Signaling 4 (RGS4), a negative modulator of mGlu5, in dorsal striatum augmented amphetamine-induced horizontal activity over 90 min (Schwendt et al. 2012). Similarly, intra-striatal MTEP augmented amphetamine-induced horizontal activity (Schwendt et al. 2012). While the reasons for the discrepancies in the pharmacological data are not known, the conflicting data emphasize the need for site-specific and cell-type specific manipulations to dissect out the regulation of striatal circuitries under normal and psycho-stimulant conditions. Overall, our current data suggest that absence of mGlu5 in GABAergic neurons in the striatum counteracted cocaine- and methylphenidate-induced activation of dopamine signals in the early phase, but did not affect dopamine signals in the latter phase, in terms of locomotor activities.

In summary, we found that mGlu5 in striatal GABAergic neurons is required for one form of striatal synaptic plasticity and for regulating locomotion and habituation responses. We propose that mGlu5 is required for action initiation in novel environment by activating the direct pathway, while mGlu5 in the indirect pathway is required to inhibit locomotion and initiate habituation response. In addition, mGlu5 in striatal GABAergic neurons is required for the rapid activation of direct-pathway neurons induced by cocaine and methylphenidate. Future studies employing drug-seeking, sensitizing and relapse paradigms in the current or cell-type specific genetic models could provide further insights into the striatal circuitry in basal ganglia function.

Acknowledgments

This work was supported by the NIH NS048884 to HCL. We would like to acknowledge the helpful comments from Dr. Corinne Marie Spencer and the contributions of the Confocal Imaging and Mouse Neurobehavior cores located in Baylor and TCH supported by BCM IDDRC 5P30HD024064 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health & Human Development or the National Institutes of Health.

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Figure 1. mGlu5 expression is reduced in a tissue-specific manner in DLX5/6-mGlu5 knockout mice.

(A) Representative image shows the distribution of GFP and tdTomato fluorescence in DLX-Cre;Rosa-mT/mG brain (n=3; coronal plane). (B) β -galactosidase staining of DLX-Cre;Tau^{mGFP} thalamocortical brain slice reveals the distribution of Cre-positive cells (n=3). (C1–3) β -galactosidase, GFP and parvalbumin immunostaining images show that many Cre-positive cells in the primary somatosensory cortex of DLX-Cre;Tau^{mGFP} mice express parvalbumin. (D–G) mGlu5 immunostaining images of DLX-mGlu5 KO (n=5) and control brains (n=5). Abbreviations: cx, cortex; st, striatum; cp, choroid plexus; hi, hippocampus; VPM, ventral posteromedial thalamic nucleus; VPL, ventral posterolateral thalamic nucleus; Rt, reticular thalamic nucleus; DLG, dorsolateral geniculate nucleus; VLG, ventral lateral geniculate nucleus; Po, posterior thalamic nuclear group; th, thalamus; VM, ventromedial thalamic nucleus; wm: white matter.

Figure 2. Western analysis mGlu5 and mGlu1 levels in various brain regions of DLX-mGlu5 KO and control mice.

(A) Examples of the Western blots used to quantify the abundance of mGlu5, mGlu1 and β -actin in the cortex, striatum, brainstem and cerebellum of the DLX-mGlu5 KO mice ($n = 4$) and wildtype littermates ($n = 4$). (B, C) Summaries for the levels of mGlu5 (B) and mGlu1 (C) normalized to β -actin from four pairs of animals. In DLX-mGlu5 KO brains, mGlu5 levels were significantly reduced in the striatum (WT = $100 \pm 3.2\%$, KO = $8.0 \pm 2.8\%$; $p < 0.001$, Student's t-test), but normal in the cortex, cerebellum, and brain stem (cortex: WT =

$100 \pm 3.7\%$, KO = $105 \pm 3.2\%$; cerebellum: WT = $100 \pm 3.8\%$, KO = $89.3 \pm 3.7\%$; brain stem: WT = $100 \pm 19.4\%$, KO = $80.9 \pm 14.9\%$). Unlike mGlu5, the levels of mGlu1 in DLX-mGlu5 KO brains were similar to control (cortex: WT = $100.0 \pm 7.2\%$, KO = $102.7 \pm 8.9\%$; striatum: WT = $100.0 \pm 1.5\%$, KO = $100.9 \pm 1.0\%$; brain stem: WT = $100 \pm 11.2\%$, KO = $100.1 \pm 7.8\%$; cerebellum: WT = $100 \pm 3.5\%$, KO = $109.2 \pm 5.0\%$). ***, $p < 0.001$.

Figure 3. Normal LTP in dorsomedial (DM) striatum and impaired LTD in dorsolateral (DL) striatum in DLX-mGlu5 KO mice.

(A) Schematic diagram shows the placement of the stimulating electrode in the white matter and the recording electrode in DM striatum for LTP induction. (B) Sample plot of normalized EPSP amplitudes during an experiment examining the effect of HFS in DM striatum. (C) Summary shows similar levels of LTP in DLX-mGlu5 KO mice and their littermates. (D) Schematic diagram shows the placement of the stimulating electrode in the white matter and the recording electrode in DL striatum. (E) Example recordings of normalized EPSP amplitudes during an experiment examining the effect of HFS in DL striatum. Inset traces are representative of averaged fEPSP's (from six recordings) recorded before (a) and after (b) HFS induction. (F) Summary shows LTD deficits in DLX-mGlu5 KO mice.

Figure 4. DLX-mGlu5 KO mice are hyperactive in novel (A-C) and familiar environments (D-E). (A-D) Locomotor activity in DLX-mGlu5 KO and control mice was monitored in an open field arena. No significant gender $\times$ genotype interaction effects for total distance traveled, total movement time, or average time in the center zone were observed, thus gender data were pooled and analyzed in 10 min time bins for exploration reactivity to the novel environment and within-session habituation behavior. (A) Examples of movement paths from control and DLX-mGlu5 KO mice show their movement history in the open field arena during the 60-minute testing period. Different colors label movement paths at different times. (B-C) Summary of the distance traveled (B) and movement time (C) measured in the open field assay. (D, E) Locomotor activity in DLX-mGlu5 KO or control mice was monitored in their home cage environment. (E-F) Summary for the home cage activity binned per hour for 3 days. Both genotypes exhibited more activity during the dark phase, and female mice were significantly more active than male mice (gender: $F_{1,31} = 21.594$, $p < 0.001$; genotype $\times$ gender: $F_{1,31} = 6.539$, $p = 0.016$). Both male and female DLX-mGlu5 KO mice exhibit a significant increase in locomotion compared to their littermate controls during the dark phase ($p < 0.001$). (F) Graph shows the average activity level during the first six

hours of Day 1 in the light and dark phases for the two genotypes. * $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; Student's t-test.

Figure 5. The hyper-kinetic effect of MPEP is absent in DLX-mGlu5 KO mice.

The mGlu5 antagonist MPEP induces hyperactivity in control but not DLX-mGlu5 KO mice. No gender $\times$ genotype or gender $\times$ treatment interactions for any of the measures were found, thus gender data were combined and analyzed by two-way ANOVA for genotype $\times$ treatment interactions. (A-C). Several measures of activity levels were monitored: total distance traveled (A, B) and movement time (C). Control mice responded to MPEP treatment with significant increases in distance traveled, movement time, vertical activity, and center distance ratio. In contrast, MPEP treatment had no significant impact on the locomotor activity of DLX-mGlu5 KO mice. *, $p < 0.05$; ***, $p < 0.001$ compared with vehicle control for each genotype, paired Student's t-test.

Figure 6. DLX-mGlu5 KO mice exhibit abnormal responses to cocaine and methylphenidate. (A-D) Cocaine-induced locomotion changes measured with the open field assay. (A) Summary for vehicle or cocaine-induced movement time changes. (B) Bar graphs show the total movement time 11–60 minutes post-injection (green arrows). (C) Summary for cocaine-induced changes in walking distance. (D) Bar graphs show the total distance traveled during 11–60 minutes post-injection. ANOVA (treatment $\times$ genotype) analysis of total distance traveled over 1 hour post-injection showed that a 15 mg/kg injection of cocaine positively enhanced distance traveled (treatment: $F_{1,64} = 4.587$, $p = 0.036$). Again, comparison of treatment effect within each genotype only showed a significant difference between vehicle and cocaine-treated littermate control mice ($t_{11} = -2.601$, $p = 0.025$), but not for DLX-mGlu5 KO mice ($t_{20} = -0.999$, $p = 0.330$, paired Student's t-test). (E-F) DLX-mGlu5 KO mice respond to methylphenidate with a delay. (E) Summary for 8 mg/kg methylphenidate-induced walking distance changes. (F) Bar graphs show the total distance traveled by DLX-mGlu5 KO and control mice during 11–20 and 11–60 minutes after vehicle or methylphenidate injections (green arrows). Control mice reached a maximum response to methylphenidate 11–20 minutes post injection, while DLX-mGlu5 KO mice reached their maximum response at 31–40 minutes post injection. However, comparisons of the total distance traveled for the 11–60 minutes post injection revealed significant treatment effect in

both control and DLX-mGlu5 KO mice. There was no difference in the maximum distance traveled within a 10 minute time bin in response to methylphenidate between control mice at 11–20 minutes after injection and DLX-mGlu5 KO mice at 31–40 minutes after injection ($t_{14} = -0.084$, $p = 0.934$). *, $p < 0.05$, **, $p < 0.01$; ***, $p < 0.001$ compared with vehicle control for each genotype, paired Student's t-test.

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Table 1.

Behavioral data summary for DLX-mGlu5 mice.

Values are listed as mean $\pm$ SEM (number of animals analyzed).

Assay	Measurement	male		female		genotype effect p value	Gender effect p value	genotype $\times$ gender effect p value
		WT	KO	WT	KO			
Body Weight	weight (g)	22.65 $\pm$ 0.78 (28)	21.28 $\pm$ 0.76 (36)	17.79 $\pm$ 0.64 (23)	17.80 $\pm$ 0.45 (22)	0.36	<0.001 *	0.354
	total distance traveled (cm) DAY 1	3399.64 $\pm$ 705.98 (8)	6573.68 $\pm$ 915.99 (12)	2472.16 $\pm$ 301.76 (5)	7081.18 $\pm$ 878.66 (10)	0.000 *	0.822	0.445
Open Field Assay	total distance traveled (cm) DAY 2	3318.09 $\pm$ 595.73 (8)	5441.09 $\pm$ 1038.81 (12)	3108.44 $\pm$ 497.75 (5)	5976.89 $\pm$ 968.10 (10)	0.020 *	0.873	0.716
	movement time (s) DAY 1	1735.09 $\pm$ 111.35 (8)	2379.93 $\pm$ 127.45 (12)	1186.86 $\pm$ 111.23 (5)	2172.05 $\pm$ 108.46 (10)	0.000 *	0.007 *	0.201
	movement time (s) DAY 2	1597.36 $\pm$ 134.77 (8)	2063.63 $\pm$ 202.35 (12)	1291.52 $\pm$ 117.68 (5)	1911.63 $\pm$ 146.53 (10)	0.007 *	0.233	0.685
	vertical activity (# beam breaks) DAY 1	713.75 $\pm$ 154.83 (8)	453.92 $\pm$ 87.04 (12)	345.20 $\pm$ 59.54 (5)	405.70 $\pm$ 84.87 (10)	0.456	0.055	0.128
	vertical activity (# beam breaks) DAY 2	752.78 $\pm$ 137.18 (8)	293.08 $\pm$ 67.07 (12)	407.20 $\pm$ 114.01 (5)	366.10 $\pm$ 97.58 (10)	0.025 *	0.211	0.059
	time spent in center zone (s) DAY 1	317.94 $\pm$ 144.08 (8)	644.46 $\pm$ 220.17 (12)	67.20 $\pm$ 18.41 (5)	326.41 $\pm$ 46.71 (10)	0.111	0.121	0.852
Home Cage Activity	time spent in center zone (s) DAY 2	190.38 $\pm$ 65.21 (8)	516.65 $\pm$ 155.96 (12)	37.06 $\pm$ 5.43 (5)	207.75 $\pm$ 52.60 (10)	0.052	0.07	0.532
	average activity in dark phase (# beam breaks)	2183.45 $\pm$ 243.03 (10)	7633.43 $\pm$ 1155.42 (7)	4450.10 $\pm$ 652.48 (10)	15446.88 $\pm$ 1946.04 (8)	<0.001 *	<0.001 *	0.016 *
	average activity in light phase (# beam breaks)	794.35 $\pm$ 57.98 (10)	1144.14 $\pm$ 154.25 (7)	1198.95 $\pm$ 119.37 (10)	1328.31 $\pm$ 249.58 (8)	0.124	0.061	0.472

Abbreviations: A.U., arbitrary unit;

*, $p < 0.05$.