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Metabotropic glutamate receptor 5 upregulates surface NMDA receptor expression in striatal neurons via CaMKII

Dao-Zhong Jin¹, Bing Xue¹, Li-Min Mao¹, and John Q. Wang^{1,2,*}

¹Department of Basic Medical Science, School of Medicine, University of Missouri-Kansas City, MO 64108, USA

²Department of Anesthesiology, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA

Abstract

Metabotropic and ionotropic glutamate receptors are closely clustered in postsynaptic membranes and are believed to interact actively with each other to control excitatory synaptic transmission. Metabotropic glutamate receptor 5 (mGluR5), for example, has been well documented to potentiate ionotropic NMDA receptor activity, although underlying mechanisms are poorly understood. In this study, we investigated the role of mGluR5 in regulating trafficking and subcellular distribution of NMDA receptors in adult rat striatal neurons. We found that the mGluR1/5 agonist DHPG concentration-dependently increased NMDA receptor GluN1 and GluN2B subunit expression in the surface membrane. Meanwhile, DHPG reduced GluN1 and GluN2B levels in the intracellular compartment. The effect of DHPG was blocked by an mGluR5 selective antagonist MTEP but not by an mGluR1 selective antagonist 3-MATIDA. Pretreatment with an inhibitor or a specific inhibitory peptide for synapse-enriched Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) also blocked the DHPG-stimulated redistribution of GluN1 and GluN2B. In addition, DHPG enhanced CaMKII α activity and elevated GluN2B phosphorylation at a CaMKII-sensitive site (serine 1303). These results demonstrate that mGluR5 regulates trafficking of NMDA receptors in striatal neurons. Activation of mGluR5 appears to induce rapid trafficking of GluN1 and GluN2B to surface membranes through a signaling pathway involving CaMKII.

Keywords

mGluR; striatum; GluN1; GluN2B; NR2B; G protein-coupled receptor; phosphorylation; DHPG

*Corresponding author: Dr. John Q. Wang, Department of Basic Medical Science, University of Missouri-Kansas City, School of Medicine, 2411 Holmes Street, Kansas City, Missouri 64108, USA, Tel: (816) 235-1786; Fax: (816) 235-5574, wangjq@umkc.edu.

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Conflict of interest

The authors declare that they have no conflict of interest.

1. Introduction

Glutamate plays a key role in synaptic transmission in the mammalian brain. This transmitter once released from presynaptic terminals activates postsynaptic glutamate receptors to regulate neuronal activity (Niswende and Conn, 2010; Traynelis et al., 2010). A major glutamate receptor is ionotropic *N*-methyl-D-aspartate receptors (NMDAR), which are usually present as tetramers composed by obligatory GluN1 and modulatory GluN2 subunits (Traynelis et al., 2010). GluN2 subunits are noticeable for their long intracellular C-termini (CT), which form key zones for major protein-protein interactions and posttranslational modifications (Bard and Groc, 2011; Mao et al., 2011). A well characterized example is the binding and phosphorylation of GluN2B (formerly known as NR2B) CT by Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII). This synapse-enriched serine/threonine kinase (Kelly et al., 1984) is activated by cytoplasmic Ca^{2+} rises (Colbran and Brown, 2004). Active CaMKII then binds to GluN2B CT to phosphorylate serine 1303 (S1303) and regulate NMDAR distribution and function (Omikumar et al., 1996; Strack and Colbran, 1998; Leonard et al., 1999).

Another subclass of glutamate receptors is G protein-coupled metabotropic glutamate receptors (mGluR). Among eight mGluR subtypes (mGluR1–8) that are classified into three functional groups, mGluR1/5 subtypes (group I) have been most thoroughly studied (Niswende and Conn, 2010). Activation of G α_q -coupled mGluR1/5 stimulates the phospholipase C β 1 (PLC)-associated pathway to yield inositol-1,4,5-triphosphate (IP_3) and diacylglycerol. The former releases Ca^{2+} from internal stores and activates various Ca^{2+} -sensitive effectors, including CaMKII (Jin et al., 2013b). Of note, mGluR1/5 are predominantly postsynaptic (Niswende and Conn, 2010). In the striatum, mGluR5 is mostly enriched and is abundant in medium spiny neurons, a cell type consisting of 90–95% of the neurons within this region (Testa et al., 1994; Tallaksen-Greene et al., 1998).

Glutamate receptors within the postsynaptic density microdomain are believed to crosstalk closely with each other to control the efficacy of synapses. An established model is that mGluR5 potentiates NMDAR activity based on early evidence that mGluR5 activation consistently enhanced NMDAR-mediated currents in many types of cells (Huang and van den Pol 2007; Rosenbrock et al., 2010). NMDARs reside in both synaptic and extrasynaptic locations, and GluN1/GluN2B heteromers are a principal subtype at extrasynaptic sites (Petralia, 2012). Similarly, mGluR5 is peri- and extrasynaptic (Lujan et al., 1996; Kuwajima et al., 2004). Thus, mGluR5 and adjacent GluN1/GluN2B may intimately interact with each other to regulate synaptic transmission. However, at present, little is known about molecular mechanisms underlying the mGluR5-NMDAR coupling.

NMDARs are membrane-bound and are normally expressed in surface and to a lesser extent intracellular membranes. These receptors traffic between the two compartments to control the exact number of functional receptors in the surface location (Groc and Choquet, 2006; Kennedy and Ehler, 2006; Lau and Zukin, 2007). Since NMDAR trafficking is a sensitive step subjected to the regulation by changing synaptic inputs, we investigated the role of mGluR5 in regulating surface expression of NMDARs in rat striatal neurons as a possible mechanism for the mGluR5 potentiation of NMDAR activity. A CaMKII-dependent

pathway was also analyzed to evaluate the importance of CaMKII in linking mGluR5 to NMDARs.

2. Results

2.1. DHPG enhances surface GluN1/GluN2B expression

BS³-linked and -unlinked receptor proteins in rat striatal slices were readily distinguished. As shown in Fig. 1A, GluN1 and GluN2B each displayed a BS³-linked high molecular weight band which contained subunit aggregates linked by cell impermeable BS³. This band represents the surface-expressed receptor. In addition, a BS³-unlinked low molecular weight band was seen for GluN1 (115 kDa) and GluN2B (180 kDa), which reflects the receptor monomer in the intracellular pool. In BS³-treated tissue, these monomeric bands were lower in their density as compared to control tissue which contained both surface and intracellular monomers. The selectivity of BS³ in linking surface receptors was confirmed by its inability to link an intracellular protein, α -actinin (100 kDa) (Fig. 1A). To test whether activation of mGluR1/5 alters surface expression of NMDAR subunits, we incubated striatal slices with an orthosteric mGluR1/5 agonist DHPG (0.5 or 50 μ M for 5 min). We then performed BS³ crosslinking assays with these slices to measure the amount of receptors in specific surface and intracellular compartments. We found that DHPG at a lower concentration (0.5 μ M) did not alter the amount of BS³-linked and BS³-unlinked GluN1 proteins in surface and intracellular membranes, respectively (Fig. 1B). Remarkably, DHPG at a higher concentration (50 μ M) significantly elevated surface levels of GluN1 and concurrently reduced intracellular levels of GluN1. As shown in Fig. 1C, DHPG concentration-dependently induced a parallel increase and decrease in GluN2B abundance in surface and intracellular pools, respectively. DHPG did not change the level of α -actinin, a control of BS³ surface crosslinking activity (Fig. 1D). These results reveal that the mGluR1/5 agonist DHPG can trigger a rapid redistribution of GluN1 and GluN2B in striatal neurons, which results in an addition of surface receptors and a simultaneous loss of intracellular receptors.

2.2. mGluR5 selectively regulates GluN1/GluN2B expression

DHPG activates both mGluR1 and mGluR5. To evaluate the relative importance of mGluR1 and mGluR5 in mediating the DHPG effect, we investigated GluN1/GluN2B responses to DHPG in the presence of an mGluR1 or mGluR5 antagonist. In rat striatal slices, adding the mGluR1 antagonist 3-MATIDA (30 min prior to DHPG) at 10 μ M, a concentration at which 3-MATIDA partially blocked DHPG-stimulated IP₃ formation (Jin et al., 2013a), had a minimal impact on the GluN1 responses to DHPG (50 μ M, 5 min) in terms of its increased surface and decreased intracellular expression (Fig. 2A). In contrast, the mGluR5 antagonist MTEP (10 μ M, 30 min prior to DHPG) was largely effective. In the presence of MTEP, the DHPG-stimulated increase in surface GluN1 expression was reversed, while the decline in intracellular GluN1 expression was no longer statistically significant (Fig. 2A). In assays with GluN2B, 3-MATIDA remained ineffective (Fig. 2B). MTEP, however, significantly blocked the upregulated surface and downregulated intracellular expression of GluN2B (Fig. 2B). Neither 3-MATIDA nor MTEP altered α -actinin expression in the presence or absence of DHPG (Fig. 2C). The two antagonists alone did not change basal levels of surface and intracellular expression of GluN1 and GluN2B (Fig. 2A and 2B). These data demonstrate a

subtype specific role of mGluR5 in regulating surface and intracellular GluN1/GluN2B receptor expression in striatal neurons.

2.3. CaMKII links mGluR5 to NMDARs

DHPG or mGluR5 positive allosteric modulators readily activated CaMKII α in rat hippocampal, cortical and striatal neurons (Choe and Wang, 2001; Mockett et al., 2011; Uslaner et al., 2009; Jin et al., 2013b). Moreover, mGluR5 but not mGluR1 activity-dependently recruited active CaMKII α to adjacent GluN2B subunits, enabling CaMKII α to phosphorylate GluN2B at a CaMKII α -sensitive site (S1303), based on a stepwise model recently established in striatal neurons (Jin et al., 2013b). It is likely that mGluR5 activity-dependently mobilizes local CaMKII to regulate NMDAR distribution. To clarify this, we investigated the effect of a CaMKII inhibitor on the GluN1/GluN2B responses to DHPG. In rat striatal slices, the CaMKII inhibitor KN93 (20 μ M, 30 min prior to DHPG) significantly blocked the DHPG-stimulated changes in surface and intracellular expression of GluN1, while its inactive analog KN92 did not (Fig. 3A). Similarly, KN93 lowered the impact of DHPG on GluN2B expression in surface and intracellular fractions. In the presence of KN93, DHPG produced a significantly less change in GluN2B levels in the two locations (Fig. 3B). Neither KN93 nor KN92 had an effect on basal levels of GluN1 and GluN2B in surface and intracellular pools. Expression of α -actinin was insensitive to all drug treatments (Fig. 3C). These data reveal a critical role of CaMKII in linking mGluR5 activation to activity-regulated NMDAR redistribution. This bridging role of CaMKII was further supported by subsequent pharmacological studies with a highly selective CaMKII inhibitory peptide, Tat-CaMKIINtide (Chang et al., 1998; Vest et al., 2007). CaMKIINtide was rendered cell permeability by fusing it to a cell membrane transduction domain (Tat). Adding this peptide (2 μ M, 45 min before DHPG) blocked the DHPG-stimulated surface expression of GluN1 and GluN2B and subtraction of these subunits in intracellular fractions (Fig. 4A and 4B), while the peptide did not alter α -actinin expression (Fig. 4C). Thus, activation of CaMKII is required for a signaling pathway linking mGluR5 to NMDARs.

2.4. DHPG increases CaMKII α and GluN2B phosphorylation

In addition to crosslinking assays that detected subcellular expression of GluN1 and GluN2B, we added an experiment to assess total cellular GluN1 and GluN2B proteins in their responses to DHPG. To this end, we added DHPG (50 μ M, 5 min) to rat striatal slices. We then lysed slices to probe changes in total GluN1 and GluN2B protein levels following DHPG application. DHPG did not alter basal levels of total GluN1 and GluN2B levels (Fig. 5A). Thus, DHPG seems to regulate the trafficking process of these proteins, while leaving their expression of total proteins unchanged.

To determine whether DHPG activates CaMKII α in our experimental setting, we analyzed phosphorylation of CaMKII α at T286 in response to DHPG. DHPG applied to striatal slices (50 μ M, 5 min) evidently enhanced CaMKII α T286 phosphorylation (Fig. 5B), indicating activation of CaMKII α after DHPG stimulation. This increase in T286 phosphorylation was blocked by KN93 but not KN92 (Fig. 5B). It was also blocked by pretreatment with Tat-CaMKIINtide (Fig. 5C). CaMKII α levels were not altered in these pharmacological experiments (data not shown). Given that active CaMKII α phosphorylates GluN2B CT at a

specific serine site (S1303) (Omkumar et al., 1996; Strack et al., 2000), we monitored GluN2B S1303 phosphorylation in the same DHPG-treated samples. DHPG significantly increased the amount of pGluN2B-S1303 proteins (Fig. 5D). Thus, DHPG can induce concurrent increases in CaMKII α T286 and GluN2B S1303 phosphorylation in striatal neurons.

3. Discussion

We set forth to evaluate the role of group I mGluRs in the regulation of subcellular expression of NMDARs in striatal neurons. We found that the group I mGluR agonist DHPG increased surface expression of GluN1 and GluN2B subunits. Meanwhile, DHPG reduced intracellular expression of these subunits, indicating a redistribution of these proteins in response to DHPG. The mGluR5 antagonist but not the mGluR1 antagonist blocked the DHPG-stimulated redistribution of GluN1 and GluN2B. Inhibition of CaMKII with an inhibitor or an inhibitory peptide also reduced the DHPG effect. Finally, DHPG increased phosphorylation of CaMKII α at T286 and GluN2B at S1303. These results demonstrate an important role of mGluR5 in regulating NMDAR trafficking in striatal neurons. CaMKII participates in the signaling pathway linking mGluR5 to NMDARs.

NMDAR trafficking is a step sensitive to the activity-dependent regulation. In early studies, dopamine has been demonstrated to regulate NMDAR trafficking in striatal neurons. Adding a dopamine D1 receptor agonist SKF82958 to rat or mouse striatal slices for 5 or 10 min increased GluN1, GluN2A and GluN2B expression in the synaptosomal membrane (LP1) isolated through a subcellular fractionation procedure and meanwhile reduced expression of these subunits in the intracellular membrane pool (LP2 and P3) (Dunah and Standaert, 2001; Dunah et al., 2004). In cultured rat striatal neurons, SKF82958 mainly induced rapid trafficking of GluN2B (Hallett et al., 2006). Similarly, using BS³-crosslinking assays, it was found that the D1 agonist SKF81297 (5 min) rapidly enhanced surface expression of BS³-linked GluN1 and GluN2B but not GluN2A in rat cultured prefrontal cortical neurons (Gao and Wolf, 2008). Intracellular levels of the three subunits (BS³-unlinked) remained stable in response to SKF81297. Thus, D1 signals are thought to stabilize GluN1/GluN2B receptors at the surface membrane and decrease their internalization and degradation (Gao and Wolf, 2008). In addition to dopamine receptors, mGluRs could act as an essential regulator of subcellular NMDAR expression. Indeed, in the current study, we found that the group I mGluR agonist DHPG concentration-dependently elevated surface NMDAR expression in striatal neurons. Notably, mGluR5 rather than mGluR1 exerted the subtype-specific regulation of NMDAR redistribution, consistent with many early electrophysiological studies showing that mGluR5 but not mGluR1 potentiates NMDAR activity (see below). The DHPG-stimulated surface expression of GluN1 and GluN2B was accompanied by a loss of the two subunits in the intracellular fraction. This suggests that intracellular receptors are, at least in part, a source for increased surface GluN1 and GluN2B expression. In contrast to GluN2B, DHPG appeared to slightly reduce the surface level of GluN2A in rat striatal neurons (Jin et al., unpublished observations). A separate future study will investigate the effect of DHPG on surface GluN2A expression in details.

mGluR5 has been well documented to potentiate NMDARs. A short activation of group I mGluRs potentiated NMDAR currents in neurons from multiple brain regions (Pisani et al., 1997; 2000; 2001; Awad et al., 2000; Doherty et al., 2000; Attucci et al., 2001; Mannaioni et al., 2001; Benquet et al., 2002; Kotecha et al., 2003; Grishin et al., 2004; Huang and van den Pol, 2007; Yang et al., 2011). Since the mGluR5 antagonist MPEP but not the mGluR1 antagonist LY367385 or CPCCOEt prevented DHPG from potentiating NMDARs in various neurons (Awad et al., 2000; Attucci et al., 2001; Mannaioni et al., 2001; Pisani et al., 2001; Huang and van den Pol, 2007; but Heidinger et al., 2002), mGluR5 is likely the subtype carrying out the potentiation (Bonsi et al., 2008). In support, DHPG potentiated striatal NMDAR currents in mGluR1 knockout mice, while DHPG did not in mice deficient in mGluR5 (Pisani et al., 2001). And the mGluR5 agonist CHPG and the mGluR5 positive allosteric modulator ADX-47273 augmented striatal and hippocampal NMDA responses (Rosenbrock et al., 2010; Pisani et al., 2001). The mGluR5-NMDAR crosstalk is believed to occur within the postsynaptic density (Huang and van den Pol, 2007), although underlying mechanisms are unclear. In this study, we found that DHPG (5 min) induced rapid trafficking of NMDARs to surface membranes. Of note, this event was mGluR5 selective. Thus, mGluR5 augments NMDARs likely through a mechanism involving redistribution of NMDARs. Of note, DHPG (50 μ M; 15 min) reduced GluN1 surface expression in rat cultured hippocampal neurons (Snyder et al., 2001). Thus, DHPG may induce an early rise of surface expression of NMDARs, followed by a late decrease in their surface expression. Future studies are needed to clarify if the increased surface expression of NMDARs in response to DHPG constitutes a metaplastic basis for the mGluR5-dependent potentiation of NMDAR function. Additionally, whether and how the mGluR5-NMDAR interplay shifts striatal synaptic plasticity and overall striatal functions is another intriguing topic worthwhile investigation in future studies.

Another important finding in this study is the role of CaMKII in linking mGluR5 to NMDARs. Based on a stepwise model we described previously (Jin et al., 2013b), inactive CaMKII binds to mGluR5 CT, which contributes to accumulating the kinase at perisynaptic sites under the basal condition. In response to cytoplasmic Ca^{2+} rises or DHPG, CaMKII becomes activated and thereby dissociates from mGluR5 as opposed to mGluR1 CT which CaMKII once activated binds to at a higher level (Jin et al., 2013a). The dissociated CaMKII binds to adjacent GluN2B to regulate NMDARs. We now add an important step into this model. After binding to GluN2B, CaMKII promotes a rapid redistribution of the receptors, leading to a larger number of receptors in the surface fraction, which probably accounts for the potentiation of NMDAR function. This CaMKII-dependent mGluR5-NMDAR coupling is in accordance with the observations that 1) DHPG activated CaMKII in striatal and hippocampal neurons (Choe and Wang, 2001; Mockett et al., 2011; Jin et al., 2013b; this study), 2) Ca^{2+} or DHPG readily recruited active CaMKII to GluN2B (Leonard et al., 1999; Colbran, 2004; Bayer et al., 2006; Jin et al., 2013b), and 3) mGluR5 signals enhanced phosphorylation of GluN2B at S1303 (Jin et al., 2013b; this study), a CaMKII-sensitive site where phosphorylation augmented NMDAR currents (Liao et al., 2001). Of note, protein kinase C (PKC), an effector downstream to the mGluR5-diacylglycerol pathway, also phosphorylates NMDAR subunits at multiple sites including S1303 (Omkumar et al., 1996; Tingley et al., 1997; Liao et al., 2001) and contributes to the mGluR5 potentiation of

NMDAR activity (Pisani et al., 1997; Calabresi et al., 1999). It is therefore likely that CaMKII and PKC act in concert to connect mGluR5 to NMDARs to control synaptic transmission.

4. Experimental procedures

4.1 Animals

Young adult male Wistar rats weighting 225–350 g (Charles River, New York, NY) were used. Animals were individually housed at 23°C and humidity of 50 ± 10% with food and water available *ad libitum*. The animal room was on a 12/12 h light/dark cycle with lights on at 0700. All animal use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee. The ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines have been followed.

4.2 Striatal slice preparation

Rat striatal slices were prepared as described previously (Liu et al., 2009; Jin et al., 2013a). Prepared slices were preincubated in artificial cerebrospinal fluid (ACSF) at 30°C for 60 min. The solution was replaced with fresh ACSF for an additional preincubation (10–20 min). Pharmacological agents were then added and incubated at 30°C. After drug treatment, slices were proceeded to surface receptor crosslinking assays (see below) or lysed in RIPA (radioimmunoprecipitation assay) buffer for following Western blot analysis of changes in levels of proteins of interest.

4.3. Surface receptor crosslinking assays

We performed surface receptor crosslinking assays using striatal slices to measure an amount of native GluN1 and GluN2B proteins in the confined surface and intracellular membrane pools. These crosslinking assays were conducted according to our previous work (Mao et al., 2009; Van Dolah et al., 2011). Briefly, after drug incubation, striatal slices were washed and incubated in ice-cold ACSF containing 2 mM of the membrane-impermeable crosslinking reagent bis(sulfosuccinimidyl) suberate (BS³) for 30 min. The BS³ crosslinking reaction was terminated by quenching with glycine (20 mM) for 10 min at 4°C. The slices were then washed four times in cold ACSF (10 min each). The slices were homogenized to obtain total protein homogenate and analyzed directly by SDS polyacrylamide gel electrophoresis (4–12% Tris-glycine gels, Invitrogen, Carlsbad, CA).

4.4. Western blot analysis

We performed Western blot as described previously (Jin et al., 2013a). Briefly, SDS 4–12% Tris-glycine gels or SDS NuPAGE 4–12% Bis-Tris gels were used to separate proteins (Invitrogen, Carlsbad, CA). Separated proteins on gels were transferred to polyvinylidene fluoride membranes. Membranes were incubated with primary antibodies overnight at 4°C. The primary antibodies used in this study include a rabbit antibody against CaMKII α (Santa Cruz Biotechnology, Santa Cruz, CA; 1:1,000), phospho-CaMKII α -T286 (pCaMKII α , Cell Signaling, Danvers, MA; 1:1,000), GluN2B (Millipore, Billerica, MA; 1:1,000), phospho-GluN2B-S1303 (pGluN2B, Millipore; 1:1,000), or actin (Santa Cruz, 1:2,000), or a mouse

antibody against GluN1 (Millipore, 1:2,000) or α -actinin (Millipore, 1:5,000). After primary antibody incubation, membranes were incubated with a secondary antibody (1:2,000). Immunoblots were developed with the enhanced chemiluminescence reagent (GE Healthcare Life Sciences, Piscataway, NJ). Immunoblots were measured using NIH gel analysis software.

4.5. Pharmacological agents

(*RS*)-3,5-dihydroxyphenylglycine (DHPG), 3-methyl-aminothiophene dicarboxylic acid (3-MATIDA), and 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride (MTEP) were purchased from Tocris Cookson Inc. (Ballwin, MO). KN93 and KN92 were purchased from Sigma. A CaMKII inhibitory peptide, CaMKIINtide (KRPPKLGQIGRSKRVVIEDDR) derived from the CaMKII inhibitory protein CaMKIIN (Chang et al., 1998), was used. CaMKIINtide was synthesized to contain a Tat domain (YGRKKRRQRRR) to render the Tat-fusion peptide (Tat-CaMKIINtide) cell permeability. All drugs were freshly prepared at the day of experiments.

4.6. Statistics

The data are presented as means $\pm$ SEM and were evaluated using Student's *t* test or one-way analysis of variance (ANOVA), as appropriate, followed by a Bonferroni (Dunn) comparison of groups. Probability levels of < 0.05 were considered statistically significant.

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Highlights

1. Activation of mGluR5 enhances surface expression of NMDA receptors.
2. Activation of mGluR1 has no impact on surface NMDA receptor expression.
3. Synapse-enriched CaMKII links mGluR5 to NMDA receptors.

Figure 1. Effects of DHPG on surface and intracellular expression of NMDAR subunits in rat striatal neurons

A, Representative immunoblots showing BS³-linked and -unlinked subunit proteins in BS³-treated tissue versus control (Con) tissue. Note that intracellular α-actinin proteins showed no cross-linking with BS³. **B** and **C**, Effects of DHPG on surface and intracellular expression of GluN1 (**B**) and GluN2B (**C**) in striatal neurons. Note that DHPG concentration-dependently increased surface expression of GluN1 and GluN2B, while DHPG reduced their expression in the intracellular pool. **D**, Effects of DHPG on expression

of α -actinin in striatal neurons. Representative immunoblots are shown to the left of the quantified data (B–D). Striatal slices were incubated with vehicle (0 μ M) or DHPG (0.5 or 50 μ M) for 5 min (B–D). Slices were then collected for BS³ crosslinking assays to analyze changes in surface (BS³-linked) versus intracellular (BS³-unlinked) expression of NMDAR subunits. Data were analyzed with one-way ANOVA: surface and intracellular GluN1 for $F(2,18) = 7.4$ and $F(2,18) = 5.9$, respectively; $p < 0.05$, and GluN2B for $F(2,18) = 17.1$ and $F(2,18) = 10.6$, respectively; $p < 0.05$. Data are presented as means $\pm$ SEM (n = 7 per group). * $p < 0.05$ versus vehicle.

Figure 2. Effects of DHPG on surface and intracellular expression of NMDAR subunits in the presence of an mGluR1 or mGluR5 antagonist in rat striatal neurons

A and B. Effects of the mGluR1/5 antagonists on the DHPG-induced changes in surface and intracellular expression of GluN1 (A) and GluN2B (B) in striatal neurons. Note that the mGluR5 antagonist MTEP but not the mGluR1 antagonist 3-MATIDA reduced the DHPG-induced changes in subcellular expression of GluN1 and GluN2B. **C.** Effects of the mGluR1/5 antagonists on α -actinin expression in striatal neurons. Representative immunoblots are shown to the left of the quantified data. Striatal slices were incubated with 3-MATIDA (10 μ M) or MTEP (10 μ M) 30 min prior to and during DHPG incubation (50 μ M, 5 min). Slices were then collected for BS³ crosslinking assays to analyze changes in surface (BS³-linked) versus intracellular (BS³-unlinked) expression of GluN1 and GluN2B. Data were analyzed with one-way ANOVA: surface and intracellular GluN1 for $F(5,30) = 17.4$ and $F(5,30) = 37.9$, respectively; $p < 0.05$, and GluN2B for $F(5,24) = 9.0$ and $F(5,24) = 40.9$, respectively; $p < 0.05$. Data are presented as means $\pm$ SEM ($n = 5-6$ per group). * $p < 0.05$ versus vehicle (veh) + vehicle. + $p < 0.05$ versus vehicle + DHPG.

Figure 3. Effects of KN93 on the DHPG-induced changes in surface and intracellular expression of NMDAR subunits in rat striatal neurons

A and B. Effects of KN93 on the DHPG-induced changes in surface and intracellular expression of GluN1 (A) and GluN2B (B) in striatal neurons. Note that KN93 but not KN92 reduced the DHPG-induced changes in subcellular expression of GluN1 and GluN2B. **C.** Effects of KN93 on α -actinin expression in striatal neurons. Representative immunoblots are shown to the left of the quantified data. Striatal slices were incubated with KN93 (20 μ M) or KN92 (20 μ M) 30 min prior to and during DHPG incubation (50 μ M, 5 min). Slices were then collected for BS³ crosslinking assays to analyze changes in surface (BS³-linked) versus intracellular (BS³-unlinked) expression of GluN1 and GluN2B. Data were analyzed with one-way ANOVA: surface and intracellular GluN1 for $F(5,42) = 9.0$ and $F(5,42) = 17.1$, respectively; $p < 0.05$, and GluN2B for $F(5,30) = 18.3$ and $F(5,30) = 16.8$, respectively; $p < 0.05$. Data are presented as means $\pm$ SEM ($n = 6-8$ per group). * $p < 0.05$ versus vehicle (veh) + vehicle. + $p < 0.05$ versus vehicle + DHPG.

Figure 4. Effects of Tat-CaMKIINtide on the DHPG-induced changes in surface and intracellular expression of NMDAR subunits in rat striatal neurons

A and B. Effects of Tat-CaMKIINtide on the DHPG-induced changes in surface and intracellular expression of GluN1 (A) and GluN2B (B) in striatal neurons. **C.** Effects of Tat-CaMKIINtide on α -actinin expression in striatal neurons. Representative immunoblots are shown to the left of the quantified data. Striatal slices were incubated with Tat-CaMKIINtide (2 μ M) 45 min prior to and during DHPG incubation (50 μ M, 5 min). Slices were then collected for BS³ crosslinking assays to analyze changes in surface (BS³-linked) versus intracellular (BS³-unlinked) expression of GluN1 and GluN2B. Data were analyzed with one-way ANOVA: surface and intracellular GluN1 for $F(3,24) = 19.7$ and $F(3,24) = 10.6$, respectively; $p < 0.05$, and GluN2B for $F(3,32) = 7.4$ and $F(3,32) = 10.1$, respectively;

$p < 0.05$. Data are presented as means $\pm$ SEM (n = 6–9 per group). * $p < 0.05$ versus vehicle (veh) + vehicle. + $p < 0.05$ versus vehicle + DHPG.

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Figure 5. Effects of DHPG on total GluN1 and GluN2B proteins and phosphorylation of CaMKIIα and GluN2B in rat striatal neurons

A, Effects of DHPG on total GluN1 and GluN2B protein levels. **B** and **C**, Effects of KN93 (B) and Tat-CaMKIINtide (C) on the DHPG-stimulated phosphorylation of CaMKIIα. **D**, Effects of DHPG on GluN2B S1303 phosphorylation. Representative immunoblots are shown to the left of the quantified data. Striatal slices were incubated with DHPG (50 μM, 5 min) and then collected to prepare lysates for Western blot analysis (A and D). In separate experiments (B and C), striatal slices were incubated with KN93 or KN92 (20 μM) 30 min or Tat-CaMKIINtide (2 μM) 45 min prior to and during DHPG incubation (50 μM, 5 min). Data are presented as means ± SEM (n = 3–6 per group). *p < 0.05 versus vehicle (veh) + vehicle and +P < 0.05 versus vehicle + DHPG (Student's *t* test for A and D, one-way ANOVA for B and C).