mGluR long-term depression regulates GluA2 association with COPII vesicles and exit from the endoplasmic reticulum

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Abstract

mGluR long-term depression (mGluR-LTD) is a form of synaptic plasticity induced at excitatory synapses by metabotropic glutamate receptors (mGluRs). mGluR-LTD reduces synaptic strength and is relevant to learning and memory, autism, and sensitization to cocaine; however, the mechanism is not known. Here we show that activation of Group I mGluRs in medium spiny neurons induces trafficking of GluA2 from the endoplasmic reticulum (ER) to the synapse by enhancing GluA2 binding to essential COPII vesicle proteins, Sec23 and Sec13. GluA2 exit from the ER further depends on IP₃ and Ryanodine receptor-controlled Ca²⁺ release as well as active translation. Synaptic insertion of GluA2 is coupled to removal of high-conducting Ca²⁺-permeable AMPA receptors from synapses, resulting in synaptic depression. This work demonstrates a novel mechanism in which mGluR signals release AMPA receptors rapidly from the ER and couple ER release to GluA2 synaptic insertion and GluA1 removal.

Keywords calcium permeable AMPA receptor; COPII; endoplasmic reticulum; GluA2; mGluR-LTD

Subject Categories Neuroscience

DOI 10.15252/embj.201694526 | Received 11 April 2016 | Revised 9 October 2016 | Accepted 11 October 2016 | Published online 17 November 2016

The EMBO Journal (2017) 36: 232–244

Introduction

AMPA receptors (AMPARs) are ionotropic glutamate receptors responsible for the fast, excitatory, synaptic transmission in the nervous system. AMPAR trafficking at the postsynaptic membrane contributes much of the plasticity that underlies long-term potentiation (LTP) and long-term depression (LTD). AMPARs are four-subunit receptors comprising a combination of GluA1–4 subunits, and their trafficking pathways depend on the composition of their subunits (Barry & Ziff, 2002). Pharmacological activation of NMDA receptors or low-frequency stimulation can destabilize synaptic

AMPARs leading to endocytosis and LTD (Xia *et al*, 2000; Chung *et al*, 2003; Ahmadian *et al*, 2004).

An alternate form of LTD is induced by activation of Group I metabotropic glutamate receptors (mGluR-LTD). mGluR-LTD has important roles in memory and behavioral flexibility, as well as in addiction and autism (Lüscher & Huber, 2010). Hippocampal studies demonstrate a role for protein synthesis and AMPAR endocytosis (Lüscher & Huber, 2010). Group I mGluRs are Gq-coupled receptors whose activation drives PIP₂ hydrolysis and IP₃ generation leading to release of Ca²⁺ from the ER via IP₃ receptors (Gladding *et al*, 2009; Goto & Mikoshiba, 2011). ER Ca²⁺ release plays a crucial role in mGluR-LTD, although the molecular targets and the link to AMPAR trafficking remain unclear (Miyata *et al*, 2000; Jo *et al*, 2008; Holbro *et al*, 2009).

A unique form of mGluR-LTD has been identified in the medium spiny neurons of the NAc. After extended withdrawal from cocaine, an uncommon form of AMPAR that lacks GluA2 subunits is highly expressed in the NAc. These AMPARs are higher-conducting and Ca²⁺-permeable (CP-AMPARs), and they are associated with the incubation of cocaine craving (Conrad *et al*, 2008). After induction of mGluR-LTD in the NAc, CP-AMPARs are removed and GluA2-containing AMPARs that are lower-conducting and Ca²⁺-impermeable (CI-AMPAR) are inserted at the synapse. This mGluR-LTD-mediated AMPAR exchange alleviates the incubation of cocaine craving (McCutcheon *et al*, 2011; Loweth *et al*, 2013). mGluR-LTD induces a similar AMPAR exchange in the VTA during development as well as after a single injection of cocaine (Mameli *et al*, 2007; Bellone *et al*, 2011). Similarly, stimulation of cerebellar stellate cells induces this AMPAR exchange and displays a requirement for Ca²⁺ and PICK1 in regulation of GluA2-containing AMPAR insertion at the plasma membrane (Liu & Cull-Candy, 2000; Gardner *et al*, 2005). However, the mechanism that underlies these instances of AMPAR exchange is not known.

Recent studies from our laboratory have demonstrated a role for basal ER Ca²⁺ release in driving GluA2 exit from the ER by means of a PICK1-CaMKII-dependent mechanism (Greger *et al*, 2002; Lu *et al*, 2014). These studies suggest a regulatory role for ER Ca²⁺ release and ER exit in GluA2-containing AMPAR trafficking.

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In order for transmembrane proteins like AMPARs to reach the plasma membrane, they must progress through the secretory pathway. The initial step in the secretory pathway is exit from the ER in COPII secretory vesicles (Budnik & Stephens, 2009). Although the composition of COPII vesicles is well defined, little is known about the regulation of glutamate receptor trafficking at this stage (Cho *et al*, 2014; Mignogna *et al*, 2015).

Since the mGluR-LTD-mediated AMPAR exchange in medium spiny neurons (MSNs) ameliorates the incubation of cocaine craving, we used cultured striatal MSNs as a model to examine the mechanism underlying this type of mGluR-LTD. We reveal a novel regulatory step of AMPAR trafficking from the ER after mGluR activation. Specifically, we demonstrate that activation of mGluRs initiates a complex program that differently regulates GluA1 and GluA2 trafficking as a means to effect synaptic plasticity. While mGluR activation drives the removal of GluA1 from the synapse, GluA2 is inserted by means of regulation of its exit from the ER via an ER Ca^{2+} release-dependent mechanism. Furthermore, mGluR activation selectively induces GluA2 exit from the ER by mediating GluA2 association with Sec23 and Sec13, essential proteins of the COPII export vesicles. Finally, we provide evidence that ER Ca^{2+} release and the differential regulation of AMPARs plays a role in the exchange of CP-AMPA with CI-AMPA that is known to underlie various forms of mGluR-LTD.

Results

Ca^{2+} release from the ER is required for DHPG-mediated increase in surface GluA2 levels

Because mGluR-LTD inserts CI-AMPA at the synapse in MSNs (McCutcheon *et al*, 2011), we examined surface GluA2 levels after mGluR activation. Since MSNs are inhibitory and do not release glutamate, we utilized a system of co-cultured striatal MSNs with excitatory cortical pyramidal neurons for immunofluorescence and electrophysiology experiments. MSNs were identified by staining neurons with a DARPP32 antibody that strongly marks both D1 and D2 MSNs (Penrod *et al*, 2011). For biochemistry experiments, where co-cultures were not feasible, we used monocultured striatal MSNs.

We first verified that our surface staining protocol was indeed only staining surface GluA2 and not internal GluA2. Surface labeling cells with antibodies against both GluA2 and DARPP32 only revealed GluA2 staining, while internal staining with both GluA2 and DARPP32 revealed dual staining, thus confirming that our surface staining protocol only labels surface proteins (Fig 1A). Co-cultured neurons were treated with the Group I mGluR agonist, DHPG (50 μM), and surface expression levels of AMPAR subunits were examined using live cell immunostaining. Consistent with previous studies of mGluR-LTD (Snyder *et al*, 2001; Huang *et al*, 2004; Moulton *et al*, 2006; Waung *et al*, 2008), we detected reduced levels of surface GluA1 after 30 min of DHPG treatment (Fig 1C and D, DHPG, 0.82 ± 0.06). Surface levels of GluA2, however, increased after 30 min of DHPG treatment (Fig 1E and F, DHPG, 1.21 ± 0.07). Virally expressed, myc-tagged GluA2 (myc-GluA2) also demonstrated a similar increase in surface levels compared with total myc after 30 min of DHPG treatment (Fig 1G and H, Ctrl, 0.34 ± 0.02 ; DHPG, 0.44 ± 0.02).

We recently showed that GluA2 exit from the ER is dependent on ER Ca^{2+} release (Lu *et al*, 2014). Since activation of Group I mGluRs generates IP3 and increases release of Ca^{2+} from the ER, we examined surface levels of GluA2 upon DHPG treatment after blocking ER Ca^{2+} channels. Cells were treated with inhibitors of the IP3 receptor (2APB, 50 μM) and the Ryanodine receptor (dantrolene, 25 μM) for 20 min prior to DHPG exposure. These inhibitors had no effect on surface GluA2 levels in control cells (Fig 1B, 2APB/dantrolene, 1.02 ± 0.14). Similarly, this manipulation had no effect on the DHPG-mediated reduction of surface GluA1 (Fig 1C and D, 2APB/dantrolene + DHPG, 0.63 ± 0.1). However, it prevented the increase of surface GluA2 (Fig 1E and F, 2APB/dantrolene + DHPG, 0.88 ± 0.06) and surface myc-GluA2 levels (Fig 1G and H, 2APB/dantrolene + DHPG, 0.39 ± 0.02), suggesting that Ca^{2+} release from the ER contributes to DHPG-mediated GluA2 trafficking.

To verify the contribution of both IP3Rs and RyRs, a second set of structurally unrelated inhibitors was used to examine the role of IP3Rs and RyRs. Cells were pretreated with both RyR inhibitor, S107 (5 μM) for 2 h (Andersson *et al*, 2011; Liu *et al*, 2012), and IP3R inhibitor, xestospongin C (XeC, 20 μM) for 20 min (Saleem *et al*, 2014). Subsequent treatment with DHPG led to reduced surface GluA2 levels compared with cells treated with DHPG alone (Fig 2A, XeC/S107 + DHPG, 0.888 ± 0.006). Previous work from our laboratory has shown that only the treatment of both 2APB and dantrolene together had an effect on GluA2 trafficking, but not either inhibitor alone (Lu *et al*, 2014). Similarly, individually pretreating cells with either S107 or XeC prior to DHPG treatment had no effect on surface GluA2 levels (Fig 2B, S107 + DHPG, 1.03 ± 0.03 , XeC + DHPG, 0.99 ± 0.02). These results confirm our previous findings with 2APB and dantrolene. Together this suggests that mGluR activation delivers GluA2-containing AMPARs to the cell surface in an ER Ca^{2+} release-dependent manner, involving both IP3Rs and RyRs.

To further establish the source of the Ca^{2+} , we examined DHPG-induced surface GluA2 levels after depleting Ca^{2+} from ER stores or after chelating extracellular Ca^{2+} . 1 μM of thapsigargin (TG) was used to deplete ER stores. Given that depletion of ER Ca^{2+} itself had no effect on surface levels of GluA2 (Fig 2C, 10 min TG + DHPG, 0.93 ± 0.1), we subsequently treated cells with TG followed by DHPG. Under these conditions, DHPG failed to increase surface GluA2 (Fig 2D, 15 min TG + DHPG, 1.03 ± 0.08) consistent with a dependence of GluA2 trafficking on Ca^{2+} release from ER stores. On the other hand, when co-cultures were pretreated with the cell-impermeable Ca^{2+} chelator, BAPTA (2 mM, 5 min), subsequent treatment of these cells with DHPG still induced increased GluA2 accumulation at the cell surface (Fig 2E, BAPTA + DHPG, 1.23 ± 0.02). Pretreatment with the cell-permeable Ca^{2+} chelator BAPTA-AM (10 mM, 5 min), however, prevented the DHPG-mediated GluA2 increase at the cell surface (Fig 2F, BAPTA-AM + DHPG, 1.00 ± 0.03). These experiments support the notion that ER Ca^{2+} stores are the source of the Ca^{2+} required for the DHPG-mediated surface insertion of GluA2.

DHPG mediates increase of surface GluA2 by regulating GluA2 exit from the ER

To directly test whether activation of mGluRs regulates GluA2 trafficking at the level of the ER, we used GluA2 sensitivity to endoglycosidase H (endo H) as a measure of the maturity of GluA2.

Figure 1. DHPG treatment reduces surface GluA1 levels and increases surface GluA2 levels in an ER Ca^{2+} -dependent manner.

A Labeling of non-permeabilized (left) and permeabilized (right) cells demonstrates that surface labeling does not label internal proteins. GluA2 labeling (green) is visible both on the surface and internally, while DARPP32 labeling (red) is only visible internally.

B Top: Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures with either no treatment or 20-min 2APB and dantrolene. Bottom: Quantified expression of GluA2 under both conditions, $n = 9$ replicates of 10 cells each, Student's t -test.

C Representative images of surface GluA1 and DARPP32 expression in striatal neurons from co-cultures with either no treatment, 30-min DHPG, or 2APB and dantrolene pretreatment followed by DHPG.

D Quantified expression of GluA1 under each condition. For 30-min DHPG, $n = 9$ replicates of 10 cells each; for 30-min DHPG + 2APB/dan, $n = 5$ replicates of 10 cells each, $*P < 0.05$, $***P < 0.001$, ANOVA.

E Representative images of surface GluA2 and DARPP32 expression in striatal neurons under the same conditions as in (C).

F Quantified expression of GluA2 under each condition. For 30-min DHPG, $n = 12$ replicates of 10 cells each; for 30-min DHPG + 2APB/dan, $n = 10$ replicates of 10 cells each, $*P < 0.05$, ANOVA.

G Representative images of surface myc-GluA2, internal myc-GluA2, and DARPP32 expression in striatal neurons from co-cultures under the same conditions as in (C).

H Quantified expression of surface myc: total myc under each condition, $n = 4$ replicates of 10 cells each, $*P < 0.05$, ANOVA.

Data information: All error bars represent s.e.m. A1 = GluA1, A2 = GluA2, D = DARPP32, s-myc = surface myc, i-myc = internal myc, m = merge.

Endo H selectively cleaves immature glycosylation residues from ER-retained proteins, but not mature proteins that have progressed to the Golgi (Greger *et al*, 2002). Monocultured striatal neurons were pulsed with ^{35}S for 60 min and chased for 4 h before GluA2 was immunoprecipitated and treated with endo H. Treatment with DHPG for 30 min after the chase period increased the ratio of mature (endo H insensitive) GluA2 to total GluA2, compared with untreated samples (Fig 3A, DHPG, 1.18 ± 0.07). Consistent with our surface labeling experiments, this increase was prevented when ER Ca^{2+} release was blocked during the final 20 min of the chase period immediately

before DHPG was added (Fig 3A, 2APB/dantrolene + DHPG, 0.93 ± 0.04). When similar conditions were used to examine how DHPG affects maturation of GluA1, we did not detect any change in the ratio of mature GluA1 to total GluA1 after either DHPG (Fig 3B, DHPG, 1.04 ± 0.03), or DHPG with 2APB/dantrolene pretreatment (Fig 3B, 2APB/dantrolene + DHPG, 1.08 ± 0.05). This supports the notion that mGluR activation mediates regulation of GluA2, but not GluA1, exit from the ER via release of Ca^{2+} from ER stores.

To demonstrate that the DHPG-mediated increase of surface GluA2 is due to enhanced GluA2 exit from the ER and is not a

Figure 2. Blocking Ca^{2+} release from the ER but not extracellular Ca^{2+} entry into the cell prevents the DHPG-mediated increase of surface GluA2 levels.

- A** Left: Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures treated either for 30 min with DHPG (DHPG) or for 30 min with DHPG and pretreated with xestospongin C and S107 (XeC/S107 + DHPG). Right: Quantified expression of surface GluA2 under each condition, $n = 3$ replicates of 10 or 20 cells each, **** $P < 0.0001$, Student's t -test.
- B** Left: Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures treated either for 30 min with DHPG (DHPG), or for 30 min with DHPG and pretreated with S107 (S107 + DHPG), or for 30 min with DHPG and pretreated with xestospongin C (XeC + DHPG). Right: Quantified expression of surface GluA2 under each condition, $n = 4$ replicates of 10 cells each, ANOVA.
- C** Top: Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures under control or 10-min thapsigargin (TG) treatment. Bottom: Quantified expression of surface GluA2 under each condition, $n = 3$ replicates of 10 cells each, Student's t -test.
- D** Top: Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures treated with either no DHPG or with DHPG for 30 min, after 15 min of thapsigargin. Bottom: Quantified expression of surface GluA2 under each condition, $n = 4$ replicates of 10 cells each, Student's t -test.
- E** Top: Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures treated with either no DHPG or with DHPG for 30 min, after 5 min of BAPTA. Bottom: Quantified expression of GluA2 under each condition, $n = 3$ replicates of 10 cells each, *** $P < 0.001$, Student's t -test.
- F** Top: Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures with either no DHPG or with DHPG for 30 min after 5 min of BAPTA-AM. Bottom: Quantified expression of GluA2 under each condition, $n = 3$ replicates of 10 cells each, Student's t -test.

Data information: All error bars represent s.e.m.

result of DHPG-mediated recycling of surface GluA2, we treated co-cultured MSNs with brefeldin A (5 μM , 15 min). Brefeldin A prevents vesicles from exiting the ER and results in a collapsed Golgi apparatus. Incubation with DHPG after pretreatment with brefeldin A did not increase surface levels of GluA2 (Fig 3C and D, BFA + DHPG, 1.01 ± 0.04) supporting the idea that DHPG increases surface GluA2 levels by regulating GluA2 exit from the ER.

DHPG increases GluA2 association with COPII ER exit vesicle proteins

Secreted proteins and transmembrane receptors exit the ER via COPII vesicles in the early secretory pathway (Lippincott-Schwartz *et al*, 2000; Ramírez & Couve, 2011). To examine whether the increased GluA2 exit from the ER is due to enhanced insertion into COPII vesicles, we measured the association between GluA2 and

Figure 3. DHPG regulates GluA2, but not GluA1, exit from the ER in an ER Ca^{2+} -dependent manner.

- A** Top: Representative image of a radiograph of mature and immature bands of GluA2 after no treatment, DHPG, or 2APB/dan followed by DHPG and incubated with endo H. Bottom: Quantified graph of the ratio of the mature, endo H-insensitive GluA2 band to the total GluA2 for each condition. For DHPG, $n = 16$; for DHPG + 2APB/dan, $n = 11$; $*P < 0.05$, ANOVA.
- B** Top: Representative image of a radiograph of mature and immature bands of GluA1 after no treatment, DHPG, or 2APB/dan followed by DHPG and incubated with endo H. Bottom: Quantified graph of the ratio of the mature, endo H-insensitive GluA1 band to the total GluA1 for each condition. For DHPG, $n = 4$; for DHPG + 2APB/dan, $n = 4$; ANOVA.
- C** Representative images of surface GluA2 and DARPP32 expression in striatal neurons from co-cultures with either no DHPG or 30-min DHPG after 15 min of brefeldin A treatment.
- D** Quantified expression of surface GluA2 under each condition, $n = 3$ replicates of 10 cells each, Student's t -test.

Data information: All error bars represent s.e.m.

Source data are available online for this figure.

Sec23, an essential COPII protein, using co-immunoprecipitation (coIP) studies. Upon coIP of GluA2 from striatal neurons in mono-cultures, we detected increased Sec23 associated with GluA2 after incubation with DHPG for 10 min (Fig 4A and B, 10 min DHPG, 1.75 ± 0.11) and 30 min (Fig 4C and D, 30 min DHPG, 1.57 ± 0.23).

To confirm that DHPG induces a specific GluA2-Sec23 interaction, we performed a coIP from both untreated neurons and neurons treated with DHPG for 30 min using a GluA2 antibody or an IgG control. After correcting for non-specific complexes, we observed a significantly higher level of Sec23 interacting with GluA2 in the DHPG-treated neurons relative to the untreated control (Fig 4E, Ctrl, 0.17 ± 0.15 ; 30 min DHPG, 1.5 ± 0.04), supporting the enhanced induction of a specific GluA2-Sec23 complex by DHPG. To determine whether this complex existed in the unstimulated state, we repeated the experiment in unstimulated cells under more stringent conditions. We observed Sec23 in the GluA2 coIP, but not in the IgG control, confirming the presence of a Sec23-GluA2 interaction in the

unstimulated state (Fig 4F). We conclude that even in the basal state, GluA2 is found in complexes with Sec23 and that the level of this complex increases strongly with DHPG treatment.

After establishing the DHPG-mediated increased association between GluA2 and Sec23, we next examined whether ER Ca^{2+} release contributed to this enhanced interaction. Interestingly, blocking ER Ca^{2+} release before DHPG treatment had no effect on the increased association between GluA2 and Sec23 (Fig 4A and C, 10 min DHPG + 2APB/dan, 1.73 ± 0.47 ; 30 min DHPG + 2APB/dan, 1.63 ± 0.23). Since mGluR-LTD induces protein translation (Mameli *et al*, 2007; Scheyer *et al*, 2014), we blocked protein synthesis with cycloheximide (CHX, 50 μM) for 5 min before DHPG treatment. This treatment reduced the enhanced association between GluA2 and Sec23 (Fig 4B and D, 10 min DHPG, 1.70 ± 0.16 ; 10 min DHPG + CHX, 1.3 ± 0.28 ; 30 min DHPG, 1.50 ± 0.18 ; 30 min DHPG + CHX, 0.93 ± 0.10). These experiments suggest that mGluR activation regulates GluA2 exit from the ER by driving GluA2 into COPII-coated ER exit vesicles in a protein

Figure 4. DHPG drives increased association between GluA2 and the COPII vesicle protein Sec23.

- A** Top: Representative immunoblots from GluA2 co-immunoprecipitations probed for Sec23 and GluA2 after no treatment, 10-min DHPG, and 2APB/dantrolene followed by 10-min DHPG. Bottom: Quantification of Sec23 normalized to GluA2 under each of these conditions. For 10-min DHPG, $n = 8$; for 10 min DHPG + 2APB/dan, $n = 6$; $*P < 0.05$, ANOVA.
- B** Top: Representative immunoblots from GluA2 co-immunoprecipitations probed for Sec23 and GluA2 after no treatment, 10-min DHPG, and cycloheximide followed by 10 min DHPG. Bottom: Quantification of Sec23 normalized to GluA2 under each of these conditions. For 10 min DHPG, $n = 8$; for 10 min DHPG + CHX, $n = 5$; $*P < 0.05$, ANOVA.
- C** Top: Representative immunoblots from GluA2 co-IPs probed for Sec23 and GluA2 after no treatment, 30-min DHPG, and 2APB/dantrolene followed by 30-min DHPG. Bottom: Quantification of Sec23 normalized to GluA2 under each of these conditions. For 30-min DHPG, $n = 17$; for 30-min DHPG + 2APB/dan, $n = 14$; $*P < 0.05$, ANOVA.
- D** Top: Representative immunoblots from GluA2 co-immunoprecipitations probed for Sec23 and GluA2 after no treatment, 30-min DHPG, and cycloheximide followed by 30-min DHPG. Bottom: Quantification of Sec23 normalized to GluA2 under each of these conditions. For 30-min DHPG, $n = 12$; for 30-min DHPG + CHX, $n = 11$; $*P < 0.05$, ANOVA.
- E** Top: Representative immunoblot demonstrating IgG and GluA2 coIP after no treatment and 30-min DHPG. Bottom: Quantification of Sec23 after subtracting the non-specific signal, $n = 3$; $**P < 0.01$, Student's t -test.
- F** Representative immunoblot of a control coIP that demonstrates the specificity of the GluA2 coIP.

Data information: All error bars represent s.e.m.

Source data are available online for this figure.

translation-dependent manner. Furthermore, while Ca^{2+} release from the ER is required for GluA2 exit from the ER, it has no role in the initial association of GluA2 with the COPII protein, Sec23.

In order to assess how the kinetics of GluA2 association with Sec23 change, we further examined the association between GluA2 and Sec23 at longer time points. While 10 and 30 min of treatment with DHPG led to increased GluA2 association with Sec23, this association returned to baseline after 60 min of DHPG treatment (Fig 5A and B, 10 min DHPG, 1.68 ± 0.10 ; 30 min DHPG, 1.59 ± 0.17 , 60 min DHPG, 1.00 ± 0.12). To further examine the enhanced interaction of GluA2 with COPII vesicles, we assayed the interaction between GluA2 and a component of the outer layer of the COPII vesicle, Sec13. We detected a small, but significant, increase in the

association of GluA2 and Sec13 after 30 min of DHPG treatment (Fig 5A and B, 10 min DHPG, 1.15 ± 0.10 ; 30 min DHPG, 1.23 ± 0.08 , 60 min DHPG, 0.91 ± 0.09), supporting the idea that DHPG facilitates GluA2 ER exit via entry into COPII vesicles. In addition, total expression levels of Sec23 and Sec13 were not affected by DHPG treatment at any of the time points examined, ruling out contributions from such changes (Fig 5C).

Ca^{2+} release from the ER is required for CP-AMPA to CI-AMPA exchange at synapses

To examine whether the release of ER Ca^{2+} is required for the mGluR-induced exchange of AMPARs, we used whole-cell patch-clamp electrophysiology in co-cultured striatal and cortical neurons. The presence of synaptic CP-AMPA was detected by measuring the change in amplitude and frequency of mini excitatory postsynaptic currents (mEPSCs) after the application of naspm, a polyamine that specifically blocks CP-AMPA (Kim & Ziff, 2014). Since few synaptic CP-AMPA are expressed under basal conditions, we incubated co-cultures with TTX ($2 \mu\text{M}$) for 48 h, a treatment known to increase synaptic CP-AMPA (Thiagarajan *et al*, 2005; Sutton *et al*, 2006; Kim & Ziff, 2014). We detected sensitivity to naspm in both the mEPSC amplitude (Fig 6A–C, TTX, 15.28 ± 0.50 ; TTX + naspm, 12.84 ± 0.47) and frequency (Fig 6A and D, TTX 1.84 ± 0.20 ; TTX + naspm, 0.56 ± 0.16) of MSNs after 48 h of TTX treatment. The decrease in frequency is likely due to the silencing of synapses containing only CP-AMPA by naspm (Lee *et al*, 2013a; Morita *et al*, 2014).

DHPG treatment for at least 30 min after 48 h of TTX treatment eliminated the sensitivity to naspm both in the mEPSC amplitude (Fig 6A–C, TTX + DHPG, 14.79 ± 0.63 ; TTX + DHPG + naspm, 15.11 ± 0.60) and in mEPSC frequency (Fig 6A and D, TTX + DHPG, 1.04 ± 0.17 ; TTX + DHPG + naspm, 1.14 ± 0.27), suggesting that mGluR activation leads to the replacement of synaptic CP-AMPA by GluA2-containing AMPARs. Interestingly, although DHPG itself had no effect on the amplitude of mEPSCs after DHPG treatment alone (compare white columns in Fig 6C), there was a significant decrease in the frequency of the DHPG-treated cells (compare white columns in Fig 6D). One interpretation is that the mGluR-LTD induced in these cells is generated by removing CP-AMPA from synapses only containing CP-AMPA, thus silencing them, while CI-AMPA are inserted into other synapses primarily containing CI-AMPA (Lee *et al*, 2013a; Morita *et al*, 2014).

To test whether Ca^{2+} release from the ER is required for this AMPAR exchange to occur, we blocked ER Ca^{2+} channels before adding DHPG. In this case, DHPG-treated cells still retained their sensitivity to naspm indicating the presence of CP-AMPA even after DHPG treatment (Fig 6A–C, TTX + DHPG + 2APB/dan, 14.79 ± 0.55 ; TTX + DHPG + 2APB/dan + naspm, 13.04 ± 0.46). Furthermore, the mEPSC frequency was significantly reduced in the presence of naspm compared with the already reduced frequency after DHPG treatment (Fig 6A,D and F, TTX + DHPG + 2APB/dan, 0.94 ± 0.22 ; TTX + DHPG + 2APB/dan + naspm, 0.55 ± 0.13). This suggests that ER Ca^{2+} release is required for this DHPG-mediated exchange of AMPARs.

Examining the mEPSC amplitude and mEPSC frequency in the presence of naspm can serve as an alternate indicator of GluA2 trafficking (Fig 6E and F). By eliminating the contribution of

Figure 5. The kinetics of GluA2 association with Sec23 after DHPG treatment.

A Representative immunoblots from GluA2 coIP probed for Sec23, Sec13, and GluA2 after no DHPG, 10, 30, and 60 min of treatment with DHPG.

B Graph depicting the kinetics of the association between GluA2 and Sec23 as well as GluA2 and Sec13 after treatment with DHPG for the same points in time as in (A). For Sec23, 10-min DHPG, $n = 16$; 30-min DHPG, $n = 15$; 60-min DHPG, $n = 7$; *** $P < 0.001$, ANOVA. For Sec13, 10-min DHPG, $n = 7$; 30-min DHPG, $n = 14$; 60-min DHPG, $n = 6$; * $P < 0.05$, ANOVA. Error bars represent s.e.m.

C Representative images of total expression levels of Sec23 (top) and Sec13 (bottom). Expression levels do not change in the presence of DHPG.

Source data are available online for this figure.

Figure 6. ER Ca^{2+} release is required for DHPG-mediated exchange of CP-AMPA receptors with CI-AMPA receptors.

A Individual traces of mEPSCs of both control and naspm-treated neurons after TTX, TTX + DHPG, and TTX + DHPG + 2APB/dan treatments.
 B Averaged mEPSCs and an enlarged time scale from cells under the same conditions as in (A). Traces from cells that were not treated with naspm are in black and naspm-treated traces are in gray.
 C Average amplitude of mEPSCs (pA) for both control (white bars) and naspm-treated (black bars) neurons under each of the conditions, TTX, TTX + DHPG, and TTX + DHPG + 2APB/dantrolene.
 D Average frequency of mEPSC (Hz) for both control (white bars) and naspm-treated (black bars) neurons under each of the conditions, TTX, TTX + DHPG, and TTX + DHPG + 2APB/dantrolene.
 E Average amplitude of mEPSCs (pA) for only naspm-treated neurons under each of the conditions, TTX, TTX + DHPG, and TTX + DHPG + 2APB/dantrolene.
 F Average frequency of mEPSC (Hz) for only naspm-treated neurons under each of the conditions, TTX, TTX + DHPG, and TTX + DHPG + 2APB/dantrolene.
 G Average rise time of mEPSC (ms) for both control (white bars) and naspm-treated (black bars) neurons under each of the conditions, TTX, TTX + DHPG, and TTX + DHPG + 2APB/dantrolene.
 H Average decay time of mEPSC (ms) for both control (white bars) and naspm-treated (black bars) neurons under each of the conditions, TTX, TTX + DHPG, and TTX + DHPG + 2APB/dantrolene.

Data information: All error bars represent s.e.m. Number of replicates in (C, E, G, and H): TTX, $n = 26$; TTX + naspm, $n = 17$; TTX + DHPG, $n = 18$; TTX + DHPG + naspm, $n = 23$; TTX + DHPG + 2APB/dan, $n = 32$; TTX + DHPG + 2APB/dan + naspm, $n = 35$; $*P < 0.05$, $**P < 0.01$, ANOVA. Number of replicates in (D and F): TTX, $n = 26$; TTX + naspm, $n = 18$; TTX + DHPG, $n = 18$; TTX + DHPG + naspm, $n = 23$; TTX + DHPG + 2APB/dan, $n = 32$; TTX + DHPG + 2APB/dan + naspm, $n = 30$; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$, ANOVA.

GluA2-lacking, CP-AMPA receptors the presence of GluA2-containing, CI-AMPA receptors can be observed. Incubation with DHPG and induction of mGluR-LTD lead to an increase in mEPSC amplitude and frequency, while pretreatment with 2APB and dantrolene prevents this DHPG-mediated rise in CI-AMPA receptors (Fig 6E and F). This supports our hypothesis that DHPG drives the synaptic insertion of GluA2-containing AMPARs, which is prevented by blocking Ca^{2+} release from the ER.

Interestingly, we did not detect any change in rise or decay time constants in the presence of nasp (Fig 6G and H). This is consistent with previous studies of CP-AMPA receptors in stellate cells which do not observe any effect on decay times upon the expression of CP-AMPA receptors (Liu & Cull-Candy, 2000, 2002).

Discussion

mGluR-LTD has been most extensively studied in the hippocampus where it induces AMPAR endocytosis in a protein translation-dependent manner (Hou *et al*, 2006; Waung *et al*, 2008; Lüscher & Huber, 2010). Although Group 1 mGluRs are Gq-coupled and their activation leads to enhanced Ca^{2+} release via increased IP3 expression, hippocampal studies have placed little emphasis on this canonical downstream signaling pathway of Gq, instead focusing on the role of protein translation in the removal of synaptic AMPARs during mGluR-LTD (Huber *et al*, 2000; Hou & Klann, 2004; Hou *et al*, 2006; Waung & Huber, 2009). Furthermore, although it is generally believed that Ca^{2+} is not required for hippocampal mGluR-LTD (Fitzjohn *et al*, 2001; Gladding *et al*, 2009), several recent studies have challenged this assumption (Jo *et al*, 2008; Holbro *et al*, 2009; Lüscher & Huber, 2010). Under certain conditions mGluR-LTD in medium spiny neurons will induce an exchange of CP-AMPA receptors with GluA2-containing CI-AMPA receptors (McCutcheon *et al*, 2011). This mGluR-induced exchange of AMPARs has been demonstrated in several different brain regions including the ventral tegmental area (VTA) and the nucleus accumbens (Bellone & Lüscher, 2005; Mameli *et al*, 2007; McCutcheon *et al*, 2011; Loweth *et al*, 2013). As with hippocampal mGluR-LTD, protein synthesis has been implicated in these studies (Mameli *et al*, 2007; Scheyer *et al*, 2014), but the mechanism for this AMPAR exchange is not well understood.

We have provided evidence that activating mGluR-LTD in MSNs regulates the trafficking of AMPARs at the level of exit from the ER and ultimately leads to the insertion of GluA2 at the plasma membrane in a Ca^{2+} -dependent manner. We have shown that activation of Group I mGluRs leads to decreased surface GluA1 and increased surface GluA2 levels. The increased GluA2 is due to regulation of ER exit by the release of Ca^{2+} from ER stores. We have further demonstrated that mGluR activation increases the association of GluA2 with Sec23, and to a lesser degree with Sec13, essential proteins of the COPII transport vesicles. Finally, we have shown that the release of ER Ca^{2+} regulates the insertion of GluA2-containing AMPARs during the mGluR-induced exchange of synaptic AMPARs, suggesting that this mechanism may be important in a more physiological context as well. These DHPG-induced biochemical and cell biological changes are consistent with comparable electrophysiological studies examining mGluR-LTD (Snyder *et al*, 2001; Mameli *et al*, 2007).

Although some studies have demonstrated that inducing mGluR-LTD by treating cells with DHPG results in a decrease of both surface GluA1 and surface GluA2, many of these studies were done in hippocampal neurons which use different mechanisms for inducing mGluR-LTD (Snyder *et al*, 2001; Waung *et al*, 2008). In addition, some studies labeled surface receptors before treating cells with DHPG, thus examining what happens to the AMPARs that are at the surface prior to DHPG treatment (Snyder *et al*, 2001). Our study, on the other hand, examined surface AMPARs after cells were treated with DHPG, thus analyzing the net expression of AMPAR subunits at the surface. It is likely that both GluA1 and GluA2 subunits are removed from the cell surface upon DHPG treatment in the form of a GluA1/2 heterotetramer, but our study shows that in medium spiny neurons, there is a net increase in the surface levels of GluA2, despite the fact that some GluA2 is likely internalized.

Our immunostaining experiments suggest that while blocking ER Ca^{2+} release affects DHPG-mediated insertion of surface GluA2, it does not affect the removal of surface GluA1 (Fig 1). A careful examination of our mEPSC data is consistent with this finding, as DHPG treatment reduced mEPSC frequency both in the presence and in absence of 2APB/dantrolene pretreatment (compare white columns in Fig 6D). This is likely due, at least in part, to removal of CP-AMPA receptors, suggesting that the DHPG-mediated removal of synaptic GluA1 was not affected by 2APB and dantrolene.

Based on our data, we hypothesize that a multiple-step mechanism is involved in this process. Upon the initial induction of mGluR-LTD, rapid protein synthesis occurs which is required for the enhanced association between GluA2 and COPII vesicles (Fig 4). While the identity of the individual protein(s) remains unclear, novel synthesis of GluA2 itself is a likely possibility as several other studies have demonstrated that DHPG can induce a rapid synthesis of the GluA2 subunit, and this synthesis is required for mGluR-LTD in the VTA (Kacharmina *et al*, 2000; Ju *et al*, 2004; Mameli *et al*, 2007; Waung & Huber, 2009). This is consistent with our findings that show a rapid increased association between GluA2 and Sec23 in a protein synthesis-dependent manner 10 min after DHPG treatment and extending to 30 min (Figs 4 and 5).

The next step in this mechanism would require the release of Ca^{2+} from ER stores, which would regulate GluA2 trafficking to the Golgi either by releasing the vesicles with GluA2-containing AMPARs from the ER, or by inducing their fusion with the Golgi (Fig 3). Since ER Ca^{2+} release does not affect GluA1, we propose that these GluA2-containing AMPARs are GluA2/3 heteromers. The final step would be the insertion of the GluA2/3 heteromers into the synapse (Figs 1 and 6), either in a constitutive manner or in a yet unidentified regulated step, to replace synaptic CP-AMPA receptors, resulting in a form of LTD.

Although the precise role for ER Ca^{2+} is unclear, our findings that thapsigargin had no effect on GluA2 surface levels (Fig 2), in addition to the fact that Ca^{2+} release did not affect GluA2 association with Sec23 (Fig 4), argue against a passive mechanism for ER Ca^{2+} where the role is to regulate the release from Ca^{2+} -dependent chaperones. A recent study from our laboratory has demonstrated that basal ER Ca^{2+} release activates a CaMKII-PICK1 interaction that is involved in GluA2 exit from the ER and insertion at the cell surface (Lu *et al*, 2014). Moreover, we showed that a mutant GluA2 that cannot bind PICK1 is prevented from exiting the ER (Greger *et al*, 2002). In addition, PICK1 is known to interact with both the

extreme C terminus of GluA2 via its PDZ domain (Greger *et al*, 2002) and with negatively charged membranes like ER exit sites via its BAR domain (Jin *et al*, 2006), making it possible that PICK1 contributes to the Ca^{2+} -dependent regulation of GluA2 exit from the ER.

Though much is known about the basic mechanisms of the secretory pathway, little is known about the regulation of glutamate receptor trafficking from the ER or the regulation of the early secretory pathway in neurons in general. Nonetheless, several recent reports have begun to highlight the importance of this regulatory step in neurons by demonstrating that proteins such as RAB39B and LRRK2, whose mutations are associated with cognitive impairment and intellectual disability in Parkinson's disease and autism, are important in mediating the ER exit and trafficking of glutamate receptors in the early secretory pathway (Cho *et al*, 2014; Mignogna *et al*, 2015). We extend these findings by demonstrating a role for activity-dependent regulation of AMPAR trafficking from the ER during mGluR-LTD as well as providing a mechanism for the mGluR-mediated exchange of AMPARs at the synapse.

The mGluR-mediated AMPAR exchange may have a more extensive role in other brain regions and under different conditions. While most AMPARs contain GluA2 subunits, many cell types acutely express the higher-conducting CP-AMPA receptors under specific conditions. During the initial phases of LTP (Plant *et al*, 2006), NMDAR-LTD (Sanderson *et al*, 2012), fear memory formation (Hong *et al*, 2013) or development (Ho *et al*, 2007), synaptic CP-AMPA receptors are expressed and subsequently replaced by GluA2-containing CI-AMPA receptors. Emerging evidence demonstrates that chronic synaptic expression of CP-AMPA receptors is associated with debilitating disorders such as the development of PTSD (Lee *et al*, 2013b; Park *et al*, 2014), the incubation of cocaine craving (Conrad *et al*, 2008), and ischemia (Liu *et al*, 2004; Liu & Zukin, 2007). In all these conditions, release of Ca^{2+} that can elicit the exit of GluA2-containing AMPARs from the ER and induce an AMPAR exchange may be a novel target for developing therapeutic treatments for these pathologies.

Materials and Methods

Primary neuronal monocultures and co-cultures

Coverslips and 6-cm petri dishes were coated with poly-L-lysine in boric acid buffer and incubated overnight at 37°C prior to dissection. Immediately before plating, coverslips and plates were washed twice with sterile PBS.

Striatal medium spiny neurons were obtained from E19 Sprague Dawley rat embryos (Taconic) and cultured as monocultures for biochemistry experiments, and striatal and prefrontal cortical cells (PFC) were co-cultured for electrophysiology and immunofluorescence studies. Striatal and PFC cells were harvested as described (Penrod *et al*, 2011) and processed as described previously (Lu *et al*, 2014). Briefly, striatal cells were trypsinized for 7–8 min while PFC cells were trypsinized for 15 min at 37°C. Cells were washed three times in PHG buffer (1× PBS, 10 mM HEPES, 0.6% glucose pH 7.35) and resuspended in 5 ml of plating media (minimal essential medium, 10% horse serum, 0.45% glucose, 1 mM pyruvate, 1× penicillin/streptomycin). Cells were plated on coverslips at a density

of 150,000 cells per coverslip with a ratio of 2:3 striatal: cortical cells for co-cultures and a density of 3×10^6 striatal cells on 6-cm plates for monocultures. After incubation for 30–60 min in plating media, plating media was aspirated and replaced with neurobasal media. Monocultures were used at 8 DIV, and co-cultures were used at 9–18 DIV and were fed with fresh neurobasal media every 7 days.

Live cell immunostaining

Cells were initially treated with drugs depending on the experiment (DHPG, Tocris 0805; 2APB, Calbiochem 100065; dantrolene, Calbiochem 251680; BAPTA, Tocris 2786; BAPTA-AM, Sigma A1076; xestospingon C, Abcam ab120914; S107, Calbiochem 500469). After drug treatment, 14 – 17 DIV co-cultures were stained live with a GluA1 or GluA2 antibody (GluA1 Calbiochem PC246, 1:20; GluA2 Millipore MAB397, 1:50) for 15 min at 37°C in a humidified chamber, followed by three quick washes in PBS and fixed for 10 min at room temperature in a 4% PFA, 0.12M sucrose, PBS solution. Cells were then permeabilized for 5 min in 0.2% Triton X-100 and washed three times with PBS for 5 min at room temperature. Cells were blocked in 10% BSA in PBS for 1 h at room temperature and then incubated with a DARPP32 antibody diluted in 3% BSA (DARPP32 mouse 1:200, BD Biosciences or DARPP32 rabbit 1:500, Millipore) for 60 min at room temperature. Cells were washed three times in PBS with 5 min washes and incubated with secondary antibody diluted in 3% BSA for 60 min at room temperature bound to fluorophores (488 or 568) either from molecular probes (1:1,000) or from Jackson (1:300). Cells were then washed three times with PBS for 5 min, mounted on coverslips, and stored. Virally infected cells were infected 14 h before treatment with drugs with a sindbis virus expressing myc-tagged GluA2 as described previously (Osten *et al*, 2000).

Image analysis and quantitation

Immunofluorescence images were acquired on either a Nikon PCM 2000 confocal microscope or a Zeiss LSM 800 confocal microscope under 60× objective. All images were acquired using the same settings for one experiment. Images were analyzed using ImageJ software. The areas of three dendrites on each image were manually defined by outlining the DARPP32 signal, and the ratio of the fluorescence of surface GluA1 or GluA2 within the outlined dendrites to the total area was calculated for each dendrite.

Whole-cell recordings

Miniature EPSCs (mEPSCs) were recorded from medium spiny neurons in cultured striatal-PFC co-cultures at DIV 9–15. MSNs were distinguished from PFCs both by morphology (soma size) and by membrane capacitance. Neurons were voltage-clamped with the whole-cell ruptured technique as previously described (Kim & Ziff, 2014). The bath solution contained (in mM) 119 NaCl, 5 KCl, 2.5 CaCl_2 , 1.5 MgCl_2 , 30 glucose 20 HEPES (Life Technologies), and 0.001 glycine (Sigma), pH 7.4. Patch electrodes (5–8 MΩ) were filled with (in mM) 120 K-gluconate (Sigma), 9 NaCl, 1 MgCl_2 , 10 HEPES, 0.2 EGTA (Sigma), 2 Mg-ATP (Sigma), and 0.2 GTP (Sigma). We added 0.5 μM TTX (Tocris Biosciences) to the bath to inhibit action potentials. mEPSCs were recorded at –60 mV with a

Warner amplifier (PC-501A) and filtered at 1 kHz. Recordings were digitized (Digidata 1440, Molecular Devices) and analyzed using the Mini Analysis software (Synaptosoft). The axial resistance ($R_a < 25 \text{ M}\Omega$) was monitored during recording to eliminate artifacts. To induce synaptic scaling, cells were treated with TTX (2 μM) for 48 h prior to recordings. For experiments using DHPG (50 μM , Tocris), cells were incubated with DHPG for 30 min at 37°C prior to recording. For experiments using 2APB (50 μM , Calbiochem) and dantrolene (25 μM , Calbiochem), or DHPG, cells were incubated with 2APB/dantrolene at 37°C for 20 min, followed by DHPG treatment at 37°C for 30 min prior to starting recordings. For experiments using naspm, 20 μM naspm (Tocris) was added to the bath solution and mEPSCs were recorded for up to 1 h after naspm was added. For all experiments, drugs that were used to incubate cells prior to recording remained in the bath solution throughout the duration of the experiment. mEPSC amplitude and frequency were analyzed by ANOVA followed by Fisher's LSD *post hoc* test.

Pulse-chase experiments

Experiments were performed as described previously (Greger *et al*, 2002) with some modifications. Monocultures of striatal neurons DIV 8 were washed in DMEM lacking met/cys (containing 0.5 mM glutamine and 1× B27; Gibco). Cells were met-depleted in this medium for 20 min and pulsed with 100 mCi/ml of Easy Tag Express- ^{35}S (1175 Ci/mmol; NEN) for 1 h. Cells were then washed with neurobasal medium (NB) and chased in 50:50 conditioned NB: fresh NB for 4 h. When 2APB/dantrolene was used, it was added 20 min before the end of the chase period, and DHPG was added 20 min later, immediately after the 4-h chase period. Cells were lysed in lysis buffer (in mM) [150 NaCl, 20 Hepes, 2 EDTA, 1% Triton, 0.1% SDS, and 1× PI (pH 7.4)] while rocking for 60 min at 4°C. Lysates were cleared for 20 min at 16,000 × g and used for IPs. Lysates were incubated with 2–3 μg of GluA2 antibodies (polyclonal, C terminus; Chemicon) for 1 h. Twenty-five microliters of Protein A-agarose beads (Santa Cruz) was then added for 50 min; pellets were washed five times with lysis buffer. Pellets were digested with 15 mU of endo H as described previously (Greger *et al*, 2002). Samples were separated on 6–7% SDS–PAGEs, dried, and fluorographed (Amersham).

Co-immunoprecipitation

CoIPs were performed following the Pierce Co-immunoprecipitation kit (26149). Columns were bound to GluA2 antibody. DIV 8 cells are treated with drugs and immediately placed on ice. They were washed twice with ice-cold PBS and either frozen at –20°C or continued with coIP as described in the kit. Briefly, cells were scraped in lysis buffer, incubated for 5 min while rocking at 4 degrees followed by a 10-min centrifugation at 13,000 × g. About 1/20 of lysis was removed and used as whole lysate, and the remainder was incubated overnight at 4°C with GluA2 columns. Lysate was washed and eluted according to the instructions in the Pierce Co-Immunoprecipitation Kit and stored at –20°C until run on an immunoblot. For the more stringent washes, lysate was washed six times in the wash buffer with the addition of 1% Tween.

Western blots

Immunoblots were performed as described previously and antibodies were used as follows GluA1 (Millipore 1:1,000, mouse), GluA2/3 (Millipore 1:200, rabbit), Sec23 (Sigma 1:1,000, rabbit), and Sec13 (Bethyl 1:1,000, Rabbit) and developed using ECL Plus (PerkinElmer). Immunoblots were quantified using ImageJ software.

Expanded View for this article is available online.

Acknowledgements

We thank Ingo Greger, Seonil Kim, and the rest of the Ziff laboratory for their helpful discussions and comments. This work was supported by NIH grants 5R01MH067229 (EBZ) and T32 DA007254 (JEP). MS was a fellow of the Brazilian Governmental Agency, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Author contributions

JEP and EBZ designed the experiments. JEP, LK, and MFS performed the experiments. JEP and EBZ wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Ahmadian G, Ju W, Liu L, Wyszynski M, Lee SH, Dunah AW, Taghibiglou C, Wang Y, Lu J, Wong TP, Sheng M, Wang YT (2004) Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. *EMBO J* 23: 1040–1050
- Andersson DC, Betzenhauser MJ, Reiken S, Meli AC, Umanskaya A, Xie W, Shiomi T, Zalk R, Lacampagne A, Marks AR (2011) Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. *Cell Metab* 14: 196–207
- Barry MF, Ziff EB (2002) Receptor trafficking and the plasticity of excitatory synapses. *Curr Opin Neurobiol* 12: 279–286
- Bellone C, Lüscher C (2005) mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors. *Addict Biol* 21: 1280–1288
- Bellone C, Mameli M, Lüscher C (2011) *In utero* exposure to cocaine delays postnatal synaptic maturation of glutamatergic transmission in the VTA. *Nat Neurosci* 14: 1439–1446
- Budnik A, Stephens DJ (2009) ER exit sites – localization and control of COPII vesicle formation. *FEBS Lett* 583: 3796–3803
- Cho HJ, Yu J, Xie C, Rudrabhatla P, Chen X, Wu J, Parisiadou L, Liu G, Sun L, Ma B, Ding J, Liu Z, Cai H (2014) Leucine-rich repeat kinase 2 regulates Sec16A at ER exit sites to allow ER-Golgi export. *EMBO J* 33: 2314–2331
- Chung HJ, Steinberg JP, Huganir RL, Linden DJ (2003) Requirement of AMPA receptor GluR2 phosphorylation for cerebellar long-term depression. *Science* 300: 1751–1755
- Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng L-J, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature* 454: 118–121
- Fitzjohn SM, Palmer MJ, May JER, Neeson A, Morris SAC, Collingridge GL (2001) A characterisation of long-term depression induced by

- metabotropic glutamate receptor activation in the rat hippocampus *in vitro*. *J Physiol* 537: 421–430
- Gardner SM, Takamiya K, Xia J, Suh J-G, Johnson R, Yu S, Huganir RL (2005) Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. *Neuron* 45: 903–915
- Gladding CM, Fitzjohn SM, Molnar E (2009) Metabotropic glutamate receptor-mediated long-term depression: molecular mechanisms. *Pharmacol Rev* 61: 395–412
- Goto J-I, Mikoshiba K (2011) Inositol 1,4,5-trisphosphate receptor-mediated calcium release in Purkinje cells: from molecular mechanism to behavior. *Cerebellum* 10: 820–833
- Greger IH, Khatri L, Ziff EB (2002) RNA editing at Arg607 controls AMPA receptor exit from the endoplasmic reticulum. *Neuron* 34: 759–772
- Ho MTW, Pelkey KA, Topolnik L, Petralia RS, Takamiya K, Xia J, Huganir RL, Lacaille J-C, McBain CJ (2007) Developmental expression of Ca^{2+} -permeable AMPA receptors underlies depolarization-induced long-term depression at mossy fiber CA3 pyramid synapses. *J Neurosci* 27: 11651–11662
- Holbro N, Grunditz Å, Oertner TG (2009) Differential distribution of endoplasmic reticulum controls metabotropic signaling and plasticity at hippocampal synapses. *Proc Natl Acad Sci USA* 106: 15055–15060
- Hong I, Kim J, Kim J, Lee S, Ko H-G, Nader K, Kaang B-K, Tsien RW, Choi S (2013) AMPA receptor exchange underlies transient memory destabilization on retrieval. *Proc Natl Acad Sci USA* 110: 8218–8223
- Hou L, Klann E (2004) Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. *J Neurosci* 24: 6352–6361
- Hou L, Antion MD, Hu D, Spencer CM, Paylor R, Klann E (2006) Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. *Neuron* 51: 441–454
- Huang C-C, You J-L, Wu M-Y, Hsu K-S (2004) Rap1-induced p38 mitogen-activated protein kinase activation facilitates AMPA receptor trafficking via the GDI.Rab5 complex. Potential role in (S)-3,5-dihydroxyphenylglycine-induced long term depression. *J Biol Chem* 279: 12286–12292
- Huber KM, Kayser MS, Bear MF (2000) Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* 288: 1254–1257
- Jin W, Ge W-P, Xu J, Cao M, Peng L, Yung W, Liao D, Duan S, Zhang M, Xia J (2006) Lipid binding regulates synaptic targeting of PICK1, AMPA receptor trafficking, and synaptic plasticity. *J Neurosci* 26: 2380–2390
- Jo J, Heon S, Kim MJ, Son GH, Park Y, Henley JM, Weiss JL, Sheng M, Collingridge GL, Cho K (2008) Metabotropic glutamate receptor-mediated LTD involves two interacting Ca^{2+} sensors, NCS-1 and PICK1. *Neuron* 60: 1095–1111
- Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, Adams SR, Garner CC, Tsien RY, Ellisman MH, Malenka RC (2004) Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci* 7: 244–253
- Kacharmina JE, Job C, Crino P, Eberwine J (2000) Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *Proc Natl Acad Sci USA* 97: 11545–11550
- Kim S, Ziff EB (2014) Calcineurin mediates synaptic scaling via synaptic trafficking of Ca^{2+} -permeable AMPA receptors. *PLoS Biol* 12: e1001900
- Lee BR, Ma Y-Y, Huang YH, Wang X, Otaka M, Ishikawa M, Neumann PA, Graziane NM, Brown TE, Suska A, Guo C, Lobo MK, Sesack SR, Wolf ME, Nestler EJ, Shaham Y, Schlüter OM, Dong Y (2013a) Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nat Neurosci* 16: 1644–1651
- Lee S, Song B, Kim J, Park K, Hong I, An B, Song S, Lee J, Park S, Kim J, Park D, Lee CJ, Kim K, Shin KS, Tsien RW, Choi S (2013b) GluA1 phosphorylation at serine 831 in the lateral amygdala is required for fear renewal. *Nat Neurosci* 16: 1436–1444
- Lippincott-Schwartz J, Roberts TH, Hirschberg K (2000) Secretory protein trafficking and organelle dynamics in living cells. *Annu Rev Cell Dev Biol* 16: 557–589
- Liu SQ, Cull-Candy SG (2000) Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* 405: 454–458
- Liu SJ, Cull-Candy SG (2002) Activity-dependent change in AMPA receptor properties in cerebellar stellate cells. *J Neurosci* 22: 3881–3889
- Liu S, Lau L, Wei J, Zhu D, Zou S, Sun H-S, Fu Y, Liu F, Lu Y (2004) Expression of Ca^{2+} -permeable AMPA receptor channels primes cell death in transient forebrain ischemia. *Neuron* 43: 43–55
- Liu SJ, Zukin RS (2007) Ca^{2+} -permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci* 30: 126–134
- Liu X, Betzenhauser MJ, Reiken S, Meli AC, Xie W, Chen B-X, Arancio O, Marks AR (2012) Role of leaky neuronal ryanodine receptors in stress-induced cognitive dysfunction. *Cell* 150: 1055–1067
- Loweth JA, Scheyer AF, Milovanovic M, LaCrosse AL, Flores-Barrera E, Werner CT, Li X, Ford KA, Le T, Olive MF, Szumlanski KK, Tseng KY, Wolf ME (2013) Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving. *Nat Neurosci* 17: 73–80
- Lu W, Khatri L, Ziff EB (2014) Trafficking of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor subunit GluA2 from the endoplasmic reticulum is stimulated by a complex containing Ca^{2+} /calmodulin-activated kinase II (CaMKII) and PICK1 protein and by release of Ca^{2+} from internal stores. *J Biol Chem* 289: 19218–19230
- Lüscher C, Huber KM (2010) Group I mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. *Neuron* 65: 445–459
- Mameli M, Balland B, Lujan R, Lüscher C (2007) Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science* 317: 530–533
- McCutcheon JE, Loweth JA, Ford KA, Marinelli M, Wolf ME, Tseng KY (2011) Group I mGluR activation reverses cocaine-induced accumulation of calcium-permeable AMPA receptors in nucleus accumbens synapses via a protein kinase C-dependent mechanism. *J Neurosci* 31: 14536–14541
- Mignogna ML, Giannandrea M, Gurgone A, Fanelli F, Raimondi F, Mapelli L, Bassani S, Fang H, Van Anken E, Alessio M, Passafaro M, Gatti S, Esteban JA, Huganir R, D'Adamo P (2015) The intellectual disability protein RAB39B selectively regulates GluA2 trafficking to determine synaptic AMPAR composition. *Nat Comms* 6: 6504
- Miyata M, Finch EA, Khiroug L, Hashimoto K, Hayasaka S, Oda S-I, Inouye M, Takagishi Y, Augustine GJ, Kano M (2000) Local calcium release in dendritic spines required for long-term synaptic depression. *Neuron* 28: 233–244
- Morita D, Rah JC, Isaac JTR (2014) Incorporation of inwardly rectifying AMPA receptors at silent synapses during hippocampal long-term potentiation. *Philos Trans R Soc Biol Sci* 369: 20130156
- Moult PR, Gladding CM, Sanderson TM, Fitzjohn SM, Bashir ZI, Molnar E, Collingridge GL (2006) Tyrosine phosphatases regulate AMPA receptor trafficking during metabotropic glutamate receptor-mediated long-term depression. *J Neurosci* 26: 2544–2554

- Osten P, Khatri L, Perez JL, Köhr G, Giese G, Daly C, Schulz TW, Wensky A, Lee LM, Ziff EB (2000) Mutagenesis reveals a role for ABP/GRIP binding to GluR2 in synaptic surface accumulation of the AMPA receptor. *Neuron* 27: 313–325
- Park K, Song B, Kim J, Hong I, Song S, Lee J, Park S, Kim J, An B, Lee HW, Lee S, Kim H, Lee JC, Lee S, Choi S (2014) ABA renewal involves enhancements in both GluA2-lacking AMPA receptor activity and GluA1 phosphorylation in the lateral amygdala. *PLoS ONE* 9: e100108
- Penrod RD, Kourrich S, Kearney E, Thomas MJ, Lanier LM (2011) An embryonic culture system for the investigation of striatal medium spiny neuron dendritic spine development and plasticity. *J Neurosci Methods* 200: 1–13
- Plant K, Pelkey KA, Bortolotto ZA, Morita D, Terashima A, McBain CJ, Collingridge GL, Isaac JTR (2006) Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. *Nat Neurosci* 9: 602–604
- Ramírez OA, Couve A (2011) The endoplasmic reticulum and protein trafficking in dendrites and axons. *Trends Cell Biol* 21: 219–227
- Saleem H, Tovey SC, Molinski TF, Taylor CW (2014) Interactions of antagonists with subtypes of inositol 1,4,5-trisphosphate (IP3) receptor. *Br J Pharmacol* 171: 3298–3312
- Sanderson JL, Gorski JA, Gibson ES, Lam P, Freund RK, Chick WS, Dell'Acqua ML (2012) AKAP150-anchored calcineurin regulates synaptic plasticity by limiting synaptic incorporation of Ca²⁺-permeable AMPA receptors. *J Neurosci* 32: 15036–15052
- Scheyder AF, Wolf ME, Tseng KY (2014) A protein synthesis-dependent mechanism sustains calcium-permeable AMPA receptor transmission in nucleus accumbens synapses during withdrawal from cocaine self-administration. *J Neurosci* 34: 3095–3100
- Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF (2001) Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat Neurosci* 4: 1079–1085
- Sutton MA, Ito HT, Cressy P, Kempf C, Woo JC, Schuman EM (2006) Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* 125: 785–799
- Thiagarajan TC, Lindskog M, Tsien RW (2005) Adaptation to synaptic inactivity in hippocampal neurons. *Neuron* 47: 725–737
- Wang MW, Pfeiffer BE, Nosyreva ED, Ronesi JA, Huber KM (2008) Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. *Neuron* 59: 84–97
- Wang MW, Huber KM (2009) Protein translation in synaptic plasticity: mGluR-LTD, Fragile X. *Curr Opin Neurobiol* 19: 319–326
- Xia J, Chung HJ, Wihler C, Huganir RL, Linden DJ (2000) Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. *Neuron* 28: 499–510