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mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors

Camilla Bellone[1] and Christian Lüscher[1,2]
[1]Department of Basic Neurosciences, University Geneva, 1 Michel Servet, 1211 Geneva, Switzerland
[2]Department of Clinic of Neurology, University of Geneva, Geneva, Switzerland

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Abstract

Excitatory glutamatergic synapses on dopamine (DA) neurons of the ventral tegmental area (VTA) undergo long-lasting changes during conditioning of natural rewards and in response to drug exposure. It has been suggested that the ensuing context-dependent behavioural changes are associated with an increased efficacy of synaptic afferents determined by the balance of long-term potentiation (LTP) and long-term depression (LTD). However, the molecular nature of the forms of LTP/LTD involved remains elusive. Here, using acute rat brain slices, we describe a form of long-term depression (LTD) that was engaged by synaptic activity or exogenous agonists activating group I metabotropic glutamate receptors (mGluR) and was sensitive to mGluR1 antagonists. Prior to mGluR-LTD, AMPAR mediated excitatory postsynaptic currents (EPSCs) showed strong rectification at positive potentials and were sensitive to Joro spider toxin (JST), a selective blocker of GluR2-lacking AMPARs. After mGluR-LTD, AMPAR EPSCs had linear current-voltage relations and became insensitive to JST. We conclude that activation of mGluR1s triggers a redistribution exchanging native receptors for GluR2 containing AMPARs, ultimately causing LTD that may oppose pathological neuroadaptation.

Introduction

Recent work has considerably increased our understanding of synaptic plasticity, and has emphasized the importance of AMPA receptors trafficking (Lüscher *et al*., 2000; Lüscher & Frerking, 2001). To explore how such mechanisms might contribute to normal learning about reward and its usurpation by drugs, we have studied synaptic plasticity in dopamine neurons of the VTA. DA neurons are important in this context because their activity codes for the prediction error of reward under physiological conditions (Schultz *et al*., 1997), and mediates the incentive salience of addictive drugs (Robbins & Everitt, 1999). During classical conditioning of natural rewards and after the exposure to addictive drugs, experience-dependent plasticity may change the firing behaviour of dopamine neurons in the VTA. For example, the association of a food reward with a conditioned stimulus will shift the activation of the dopamine neurons to the time of conditioned stimulus presentation (Schultz *et al*., 1997). Similarly, contextual cues preceding drug reinstatement also activate the VTA (McFarland & Kalivas, 2001) and intermittent stimulation of excitatory afferents of DA neurons induces behavioural sensitization (Schenk & Snow, 1994). These results strongly suggest that associative plasticity of glutamatergic afferents to DA neurons contributes to the profound pathological changes that occur with repeated use of addictive drugs.

Behavioural sensitization, conditioned locomotor response and conditioned place preference are drug-associated phenomena that are context- and NMDAR-dependent, which suggests the involvement of LTP (Hyman & Malenka, 2001). In fact, a single dose of a drug of abuse may lead to changes of the AMPA/NMDA ratio that occludes subsequent LTP induction (Ungless *et al*., 2001; Saal *et al*., 2003). If LTP is related to the induction of behavioural changes then the mirror phenomenon LTD would constitute a break to potentiation and represent a protective mechanism (Kauer, 2004). Interestingly, the induction of LTD in the VTA is blocked by amphetamines (Jones *et al*., 2000).

LTD is expressed at many synapses and can be induced with various protocols activating distinct pathways. In the VTA, low frequency stimulation (LFS) leads to a NMDAR independent LTD through voltage-gated calcium channels (Jones *et al*., 2000; Thomas *et al*., 2000) and protein kinase A (PKA) activation (Gutlerner *et al*., 2002). In addition, at many synapses, burst-like synaptic activity may trigger a LTD by activating perisynaptic mGluRs (Anwyl, 1999). In the context of behavioural sensitization, mGluR-LTD is of particular interest because mGluR antagonists directly applied into the VTA block sensitization (Kim & Vezina, 1998). Subsequent studies revealed that group I mGluRs have opposing effects; while mGluR5 knockout mice do not express sensitization to cocaine (Chiamulera *et al*., 2001), manipulations disrupting the mGluR1 receptor complex cause sensitization in the drug-naïve animals (Ghasemzadeh *et al*., 2003; Kalivas *et al*., 2003; Szumlinski *et al*., 2003).

Here we provide the initial description of mGluR-LTD of afferent excitatory transmission onto DA neurons of the VTA. We show that the induction of this form of LTD depends on the activation of mGluR1s and that its expression involves a switch of AMPAR subunits.

Correspondence: Dr Christian Lüscher, [1]Department of Basic Neurosciences, as above.
E-mail: Christian.Luscher@medecine.unige.ch

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Materials and methods

Electrophysiology in acute slices

Animals were killed by decapitation according to the guidelines of the Canton of Geneva Veterinary Office Horizontal slices (300 μm) of the brain stem were prepared from P15–P21 Sprague–Dawley rat brains in cooled artificial cerebro-spinal fluid containing (in mM) NaCl 119, KCl 2.5, $MgCl_2$ 1.3, $CaCl_2$ 2.5, NaH_2PO_4 1.0, $NaHCO_3$ 26.2 and glucose 11 and bubbled with 95% O_2 and 5% CO_2. Whole-cell voltage-clamp recording techniques were used (32–34 °C, 2 mL/min, submerged slices) to measure holding currents and synaptic responses of dopaminergic neurons of VTA that were identified by the presence of a large I_h current (Johnson & North, 1992) immediately after obtaining a whole-cell configuration. As I_h is present in ≈90% of dopamine neurons (Cameron *et al*., 1997; Neuhoff *et al*., 2002) we are aware that its presence is not an unequivocally identification criterion. However, in a previous study (Cruz *et al*., 2004) we have confirmed the tight correlation of I_h with tyroxine hydroxylase mRNA using single cell RT-PCR for and found it sufficient to obtain highly significant difference between experimental and control cells. The holding potential was −50 mV and the access resistance was monitored by a hyperpolarizing step to −80 mV with each sweep every 10 s. Experiments were terminated if the access resistance varied more than 20%. Synaptic currents were evoked by stimuli (0.1 ms) at 0.05 Hz through bipolar stainless steel electrodes positioned rostral to the VTA. The internal solution contained (in mM): CsCl 130, NaCl 4, $MgCl_2$ 2, EGTA 1.1, HEPES 5, Na_2ATP 2, Na_2-creatine-phosphate 5, Na_3GTP 0.6 and spermine 0.1. DHPG and mGluR-LTD could reliably be evoked independent of the duration of whole-cell configuration prior to induction, indicating that this form of LTD does not wash out (see Fig. 2B for an example of induction after 20 min of recording). Currents were amplified (Axopatch 1D), filtered at 1 kHz and digitized at 5 kHz (National Instruments Board PCI-MIO-16E4, Igor, Wave Metrics, Lake Oswego, OR). The liquid junction potential was small (−3 mV) and therefore traces were not corrected. In experiments where the stimulus artefact contaminated the initial phase of the EPSC, it was mathematically subtracted to increase the accuracy of the *I–V* plots. Without that correction the mean reversal potential at baseline was 7 ± 5 mV, with the correction it was 2.4 ± 0.2 mV. All experiments were carried out in the presence of D-APV (50 μM) and picrotoxin (100 μM) and EPSCs were fully sensitive to CNQX (10 μM). mGluR-antagonists were preincubated for 30 min. Compiled data are expressed as means ± SEM. For statistical comparisons the non parametric Mann–Whitney, Wilcoxon matched or the paired Student's *t*-tests were used and the level of significance was taken at $P < 0.05$.

Drugs

D-2-Amino-5-phosphonovalericacid (D-APV); 3,5 dihydrophenylglycine (DHPG); 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF109203X); Tyr-D-Ala-Gly-Nme-Phe-Gly-ol (DAMGO), 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1H-pyrazole-3-carboxamide (AM251); 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and 7-hydroxyimino-cyclopropa(b)chromen-1a-carboxylate ethyl ester (CPCCOEt) were bought from Tocris; 6,Cyano-7-nitroquinoxaline-2,3-dione (CNQX), spermine; Joro spider toxin (JST); K3-1,2-bis(aminophenoxy)ethane-*N,N,N,N*-tetra-acetic acid (BAPTA) and Picrotoxin (PTX) were obtained from Sigma (Buchs, Switzerland).

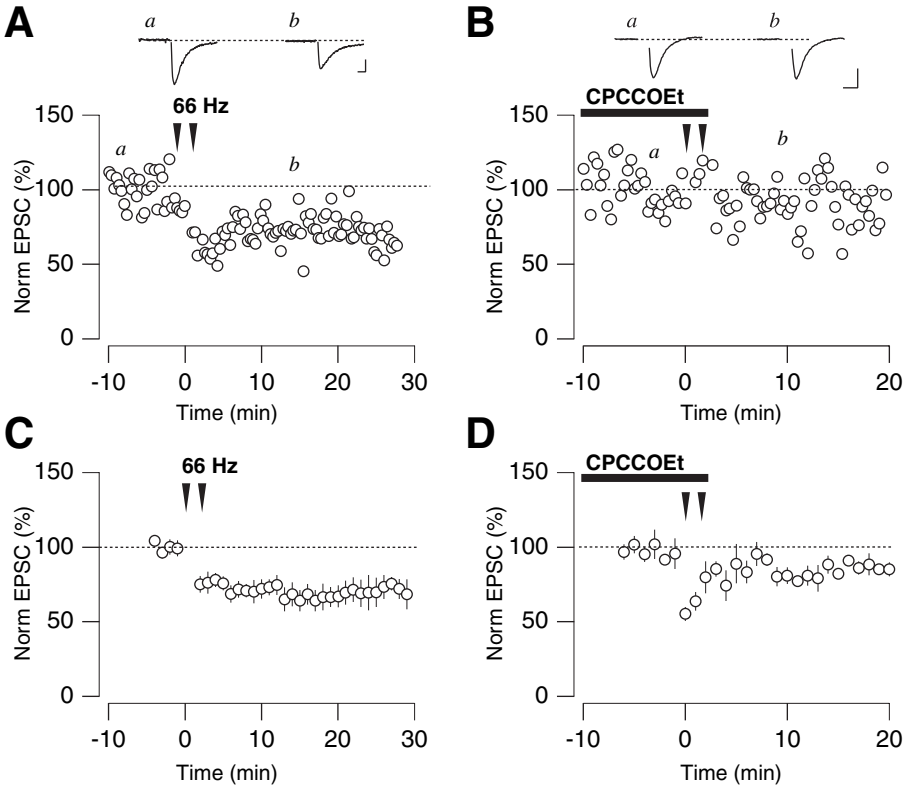

FIG. 1. LTD induced by synaptic activation of mGluRs in the VTA. (A) Brief trains of high frequency stimuli (five stimuli at 66 Hz) induced a long-lasting depression. (B) mGluR-LTD is inhibited by the mGluR1 antagonist CPCCOEt (100 μM). (C and D) Amplitude vs. time plots for all experiments ($n = 10/6$). Scale bars, 50 pA/5 ms.

Results

Co-existence of two distinct forms of LTD in DA neurons of the VTA

To investigate the role of mGluR activation on AMPAR mediated excitatory postsynaptic currents (EPSCs) we applied brief bursts of extracellular stimulation rostral to the VTA, likely activating preferentially afferents from the prefrontal cortex. Such a protocol is sufficient to activate mGluR on DA neurons. For example, brief trains (five stimuli) at 66 Hz give rise to a K(Ca) mediated slow IPSC (Fiorillo & Williams, 1998), longer trains at similar frequencies (> 10 stimuli) evoke a combined IPSC-EPSC that also reflects the activation of transient receptor potential (Trp) channels (Gee *et al*., 2003; Kim *et al*., 2003; Tozzi *et al*., 2003; Bengtson *et al*., 2004). In the presence of the NMDAR blocker, D-APV, we monitored AMPAR-EPSCs elicited every 10 s by single shock stimulation and observed that two

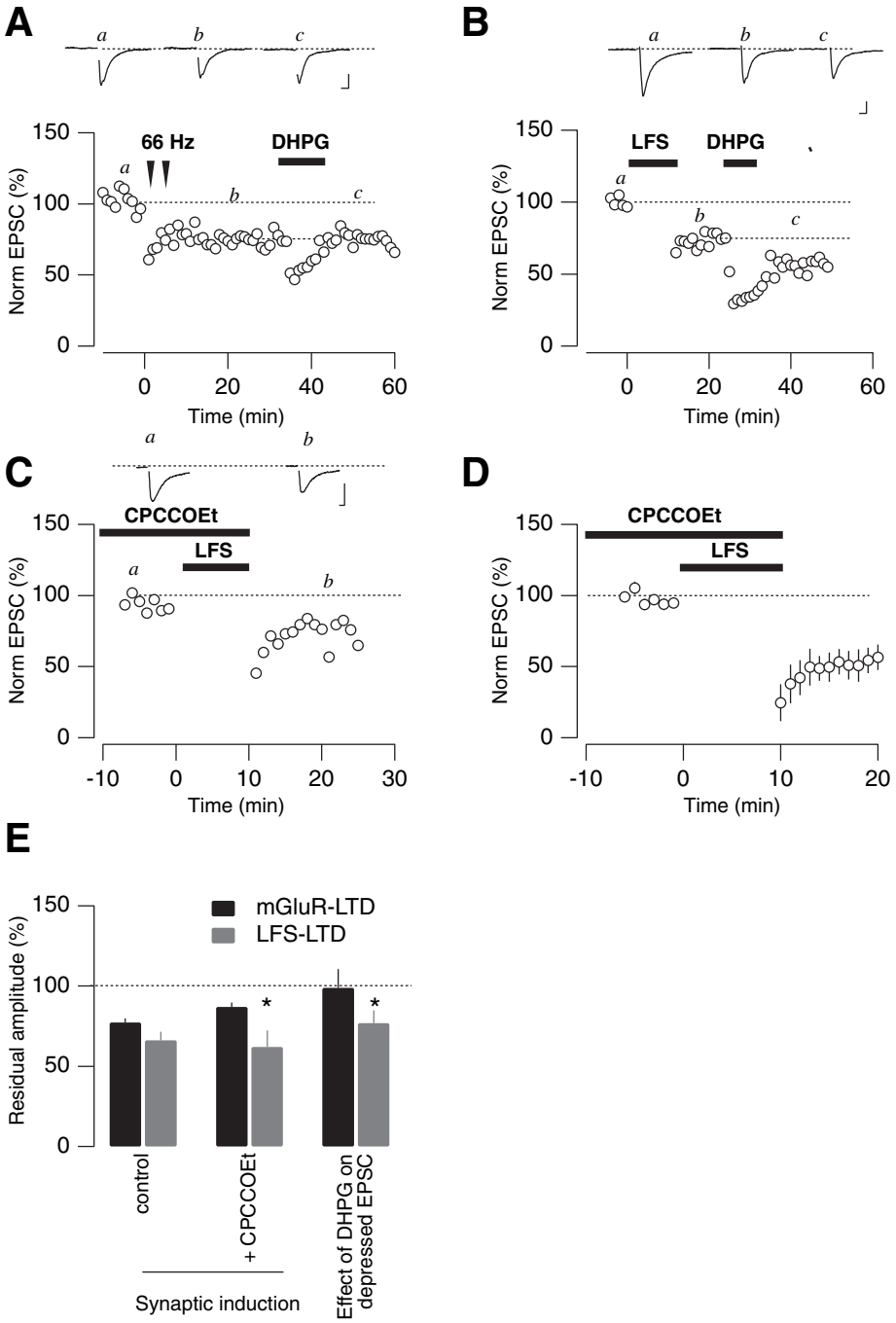

FIG. 2. Two distinct forms of LTD coexist in the VTA. (A) Synaptically induced mGluR-LTD occluded the effects of subsequent DHPG (20 μM) application. (B) A train of 600 stimuli at 1 Hz (LFS) induced a long-lasting depression that did not occlude subsequent application of DHPG. (C and D) LFS-LTD was not blocked by mGluR1 antagonist CPCCOEt as shown in a single example (C) and the group data (D, $n = 6$). (E) Bar graph indicating that LFS-LTD was of similar magnitude as mGluR-LTD but that only the latter was sensitive to CPCCOEt. In addition, normalized to the synaptically induced depression, subsequent effects of DHPG lead to a further depression ($n = 6$ for all cases, $^*P < 0.05$) only with LFS-LTD. For all panels, insets show traces at corresponding time points. Scale bars, 50 pA/5 ms.

brief trains of five stimuli at 66 Hz were sufficient to induce a substantial long-lasting depression (32.8 ± 1.2%, $n = 10$, $P < 0.01$ for a hypothetical mean of 0), which was inhibited by the selective mGluR1 antagonist, CPCCOEt (100 μM, 13.1 ± 2.7%, $n = 6$, $P < 0.01$ for the comparison of the two conditions, Fig. 1). Conversely, in line with previous studies (Jones *et al*., 2000; Thomas *et al*., 2000), low frequency stimulation (LFS, 1 Hz for 10 min) elicited a form of LTD independent of mGluR activation (33.8 ± 5.4%, $n = 6$, Fig. 2B–D). In fact, LFS-LTD was reliably induced in the presence of CPCCOEt (38 ± 10.3, $n = 6$, Fig. 2C and D) and the application of the mGluR I agonist, 3,5 dihydrophenylglycine (DHPG), after the induction of LFS-LTD caused a further depression (23.15 ± 8%, $n = 6$, $P < 0.05$ for a hypothetical mean of 0, Fig. 2B). Conversely, after LTD by short trains at 66 Hz, the effects of DHPG were occluded (1.1 ± 11.6, $n = 6$, not significantly different form a hypothetical mean of 0, Fig. 2A). In conclusion we have demonstrated the existence of a form of LTD of excitatory afferents on DA neurons of the VTA that depends on the activation of mGluRs (mGluR-LTD). Figure 2E summarizes the group data confirming the distinct induction mechanisms for the two forms of LTD that coexist at these synapses.

LTD induced by the group I mGluR agonist DHPG

To elucidate the mechanisms involved in the expression of mGluR-LTD, we took advantage of the above-established pharmacological induction protocol. DHPG, bath-applied for 5 min, induced a depression of the AMPAR EPSCs measured at −50 mV that had two phases. The first phase was of rapid onset, very strong (56 ± 3% $n = 12$) but transient, while the second phase lasted beyond agonist application and was consequently called DHPG-LTD (Fig. 3A and B). As with mGluR-LTD, the induction of DHPG-LTD was inhibited by CPCCOEt (100 μM, 12.1 ± 7.4%, $n = 6$ vs. 39.1 ± 7.6%, $n = 12$ in control condition, $P < 0.05$), but not by MPEP (10 μM, 36.5 ± 2.3%, $n = 8$, $P > 0.05$), suggesting mGluR1 rather than mGluR5 as the relevant receptor (Fig. 3C and D). Both phases of the synaptic depression depended on intracellular calcium; the initial depression was partially blocked and subsequent DHPG-LTD was abolished in the presence of BAPTA in the pipette (10 mM, 3.9 ± 12.4%, $n = 5$, Fig. 3E). Similar to LTD in other brain regions (Oliet *et al*., 1997; Chung *et al*., 2003), a protein kinase C (PKC) inhibitor (here GF109203X applied 1 μM) also blocked DHPG-LTD in the VTA (1.7 ± 11.6%, $n = 6$, Fig. 3F).

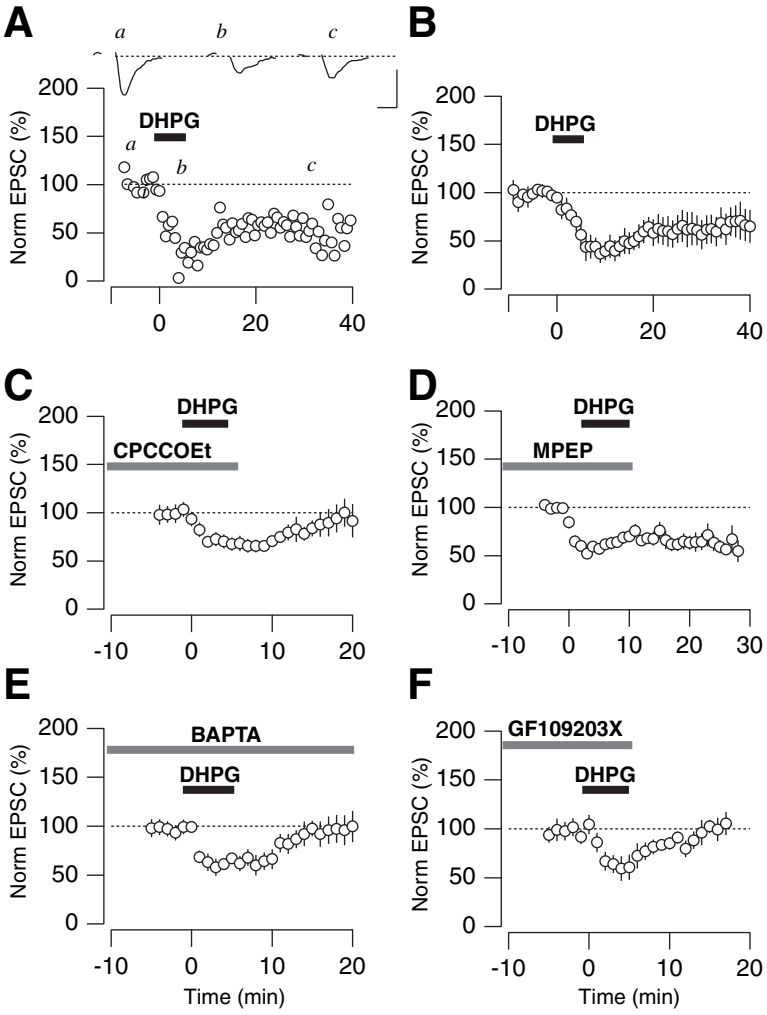

FIG. 3. The mGluRI agonist DHPG induces a long-term synaptic depression (DHPG-LTD). (A) Representative example of DHPG-LTD induced by applying DHPG in presence of D-APV (50 μM). The inset shows a single EPSC from a typical experiment before, during and after the DHPG application (Scale bars, 50 pA/5 ms). (B) On average DHPG depressed EPSCs to 60.9 ± 7.6% of control ($n = 12$). (C and D) The mGluR1 selective antagonist CPCCOEt blocked DHPG-LTD (C, $n = 6$) while MPEP (10 μM) did not block the depression (D, $n = 8$). (E) DHPG-LTD was blocked by BAPTA in the patch pipette (10 mM, $n = 5$). (F) DHPG-LTD was not induced in the presence of the PKC blocker GF109203X (1 μM, $n = 6$).

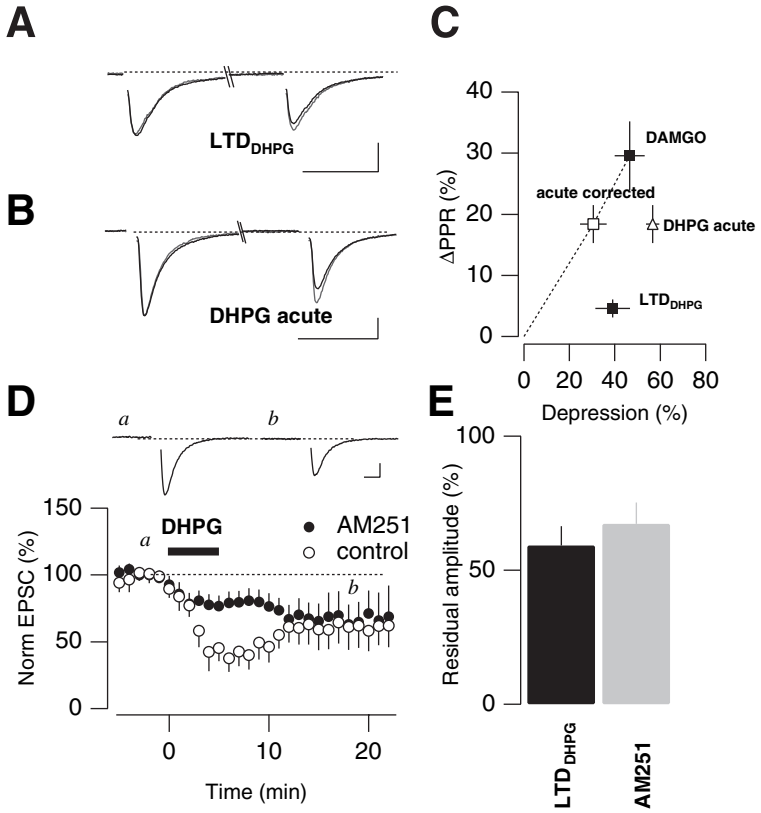

FIG. 4. Paired pulse ratio (PPR) changes with DHPG-LTD and independence of CB1R activation. (A) Scaled synaptic responses to a paired pulse at 70 ms interval before (black trace) and 20 min after application of DHPG (grey trace). Notice the absence of increase in the amplitude of the scaled second response. (B) For comparison PPR significantly increased during the initial transient depression. Scale bars, 50 pA/20 ms. (C) Change in PPR ratio as a function of depression. As the transient depression is the sum of developing LTD and the decrease of transmitter release, the magnitude of the depression was mathematically corrected (acute corrected) subtracting the depression at 5 min in the presence of AM251 (21.6 ± 5.4%, Fig. 4D). As a control the μ-opioid agonist DAMGO caused an even stronger increase of PPR. To estimate the trajectory relating PPR and depression, the 'acute corrected' and the DAMGO data points as well as the origin (point 0,0) were connected (dotted line). Note that this line is far from the small change observed with DHPG-LTD. (D) In the presence of the CB1 antagonist AM251 (10 μM) the fast transient depression was abolished while DHPG-LTD was still present. The insets show a single EPSC from a typical experiment before and after the DHPG application in presence of AM251 ($n = 10$). (E) Bar graph indicating residual EPSC-amplitude after DHPG application with and without AM251 ($n = 10$ or 12).

Change in paired pulse ratio (PPR) with mGluR-LTD

PPR, a measure of the presynaptic release probability did change only slightly with DHPG-LTD (4.6 ± 1.4%, $n = 6$, $P < 0.05$, Fig. 4A and C). In contrast, PPR during the transient depression strongly increased suggesting underlying presynaptic inhibition of the EPSCs (18.4 ± 3.1%, $n = 5$, $P < 0.01$, Fig. 4B). As a control, the μ-opioid receptor selective agonist DAMGO also caused a significant increase in the PPR (29.6 ± 5.6%) for a depression of 46.6 ± 6.6% ($n = 6$). These results suggest that the transient depression is mediated by a change in release probability. Conversely, DHPG-LTD is more likely expressed by a postsynaptic mechanism.

As endocannabinoids are known to regulate afferent transmitter release in response to mGluR activation (Varma *et al*., 2001), we applied DHPG in the presence of the CB1R antagonist AM251 to elucidate the origin of the initial transient depression. AM251 (2 μM) blocked the initial depression (21.6 ± 5.4%, $n = 10$, vs. 56 ± 3%, $n = 12$ $P < 0.01$) but had no significant effect on the magnitude of DHPG-LTD (32.7 ± 8%, $n = 10$, vs. 39.1 ± 7.6%, $n = 12$, $P > 0.05$, Fig. 4D and E), similar to observations in the hippocampus (Rouach & Nicoll, 2003). We conclude that the activation of postsynaptic mGluRs may initially trigger the release of endocannabinoids that transiently inhibit EPSCs, and also induces a long-lasting depression that may be expressed by a postsynaptic mechanism. In fact, the transient initial depression reflects the sum of the presynaptic inhibition and the slowly developing DHPG-LTD. The above experiment allowed us to mathematically estimate the magnitude of each component and to draw the PPR change – depression trajectory (Fig. 4C). Although there is a small increase of PPR with DHPG-LTD, this value was very different from the estimated trajectory and a presynaptic mechanism therefore cannot account for this form of LTD.

Rectifying synaptic AMPAR responses in DA neurons of the VTA

The pharmacologically isolated AMPAR component (recordings in the presence of 50 μM D-APV and full sensitivity to 10 μM CNQX) of the EPSC reversed at 2.4 ± 0.2 mV ($n = 12$). Interestingly, in the constant presence of a defined concentration of intracellular polyamines (see Materials and methods), we found that EPSCs at negative potentials were significantly larger compared to responses at the symmetrical positive potentials. This is a property referred to as inward rectification and indicative of AMPAR lacking the edited GluR2 subunit (Fig. 5A and B). The rectification index (RI) measured by dividing the EPSC amplitude measured at +40 mV by the amplitude measured at −70 mV was on average $RI_{+40/-70mV} = 0.31 \pm 0.02$ ($n = 19$), which differed significantly

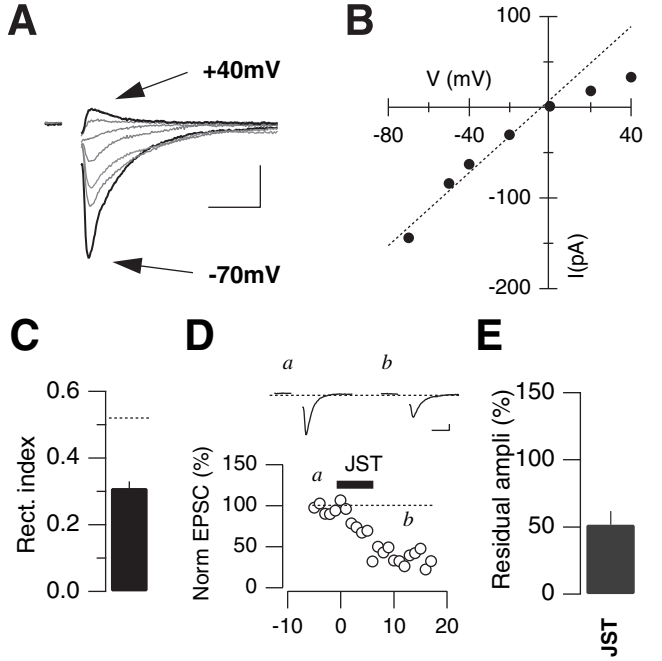

FIG. 5. Synaptic currents in dopamine cells of VTA exhibit properties of GluR2-lacking AMPARs. (A) Examples of evoked AMPA-mediated synaptic responses at membrane potentials of −70 mV to +40 mV (average of consecutive 20 sweeps; scale bar, 50 pA/5 ms). (B). Example of the rectifying current-voltage (*I–V*) relationship of AMPAR mediated EPSCs in an individual dopamine cell. The dotted line represents a linear fit of the EPSC amplitudes recorded between −70 mV and 0 mV. (C) Averaged rectification index (I_{+40mV}/I_{-70mV}) of AMPAR-mediated responses. The dashed line indicates the rectification index corresponding to a linear *I–V* curve that reverses at 2.5 mV (no rectification). (D) Evoked EPSCs were inhibited by Joro spider toxin (JST, 500 nM) within minutes. Insets show representative averaged traces before and after the pharmacological intervention (Scale bar, 50 pA/5 ms). (E) Bar graph illustrating averaged residual amplitude after 10 min of JST application ($n = 6$).

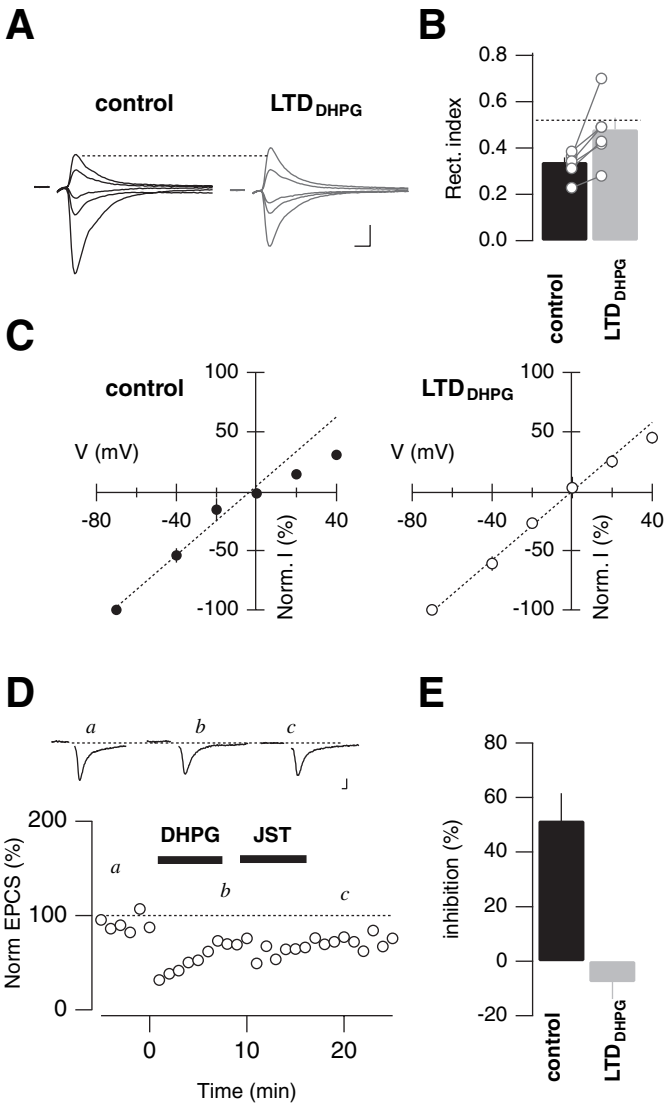

FIG. 6. Loss of EPSCs rectification after DHPG application. (A) Representative example of EPSCs recorded at −70, −40, −20, +20 and +40 mV before and after the induction of DHPG-LTD. (B) Change in rectification index ($EPSC_{+40mV}/EPSC_{-70mV}$) after DHPG-LTD induction for six experiments. On average rectification index was 0.34 ± 0.02 before and 0.48 ± 0.05 after DHPG-LTD induction. (C) *I–V* plots of EPSCs before and after the induction of DHPG-LTD (all EPSCs are normalized to the amplitude measured at −70 mV, $n = 9$). Dotted lines represent linear fits between −70 and −20 mV. (D) Example of a recording indicating that JST was ineffective after induction of mGluR-LTD. (E) EPSCs inhibition induced by JST in control condition (same data as Fig. 5D) and after mGluR-LTD induction.

from the value calculated for a linear *I–V* relationship for a reversal potential of 2.4 mV ($RI_{+40/-70mV} = 0.52$, $P < 0.01$, Fig. 5C). The presence of GluR2-lacking synaptic AMPARs was pharmacologically confirmed by the sensitivity of the EPSCs to Joro spider toxin (JST 500 nM), a selective blocker of AMPARs devoid of GluR2 (depression of 51.5 ± 10.1%, $n = 6$, Fig. 5D and E). Taken together, we conclude that AMPAR on DA neurons of the VTA contain a substantial proportion of GluR2-lacking AMPA receptors. This is in contrast to previous studies (Thomas *et al*., 2000; Faleiro *et al*., 2004), where bath-applied *N*-(4 hydroxyphenyl-propanoyl-)-spermine did not have a significant effect on EPSCs in VTA slices of mice, and EPSCs in rats had similar absolute amplitudes at +40 and −40 mV. These differences may be due to a loss of sensitivity to external polyamines below −80 mV (Tikhonov *et al*., 2000; Magazanik *et al*., 2003), the absence of spermine in the pipette or reflect differences between species, age or basal state of sensitization (see also Discussion).

Loss of rectification with DHPG-LTD and mGluR-LTD

To test the possibility that a redistribution of AMPARs could mediate DHPG-LTD, we measured the amplitude at potentials ranging from −70 mV to +40 mV immediately before and 20 min after the pharmacological induction of the DHPG-LTD. We found that with DHPG-LTD the inward rectification was lost and *I–V* curves became linear (Fig. 6A). For each individual cell ($n = 6$), RI became larger with DHPG-LTD, which on average led to an increase of $RI_{+40/-70mV} = 0.34 \pm 0.02$–$0.48 \pm 0.05$, $P < 0.05$ for paired comparison (Fig. 6B). The latter value did not significantly differ from the value calculated for a linear *I–V* relationship reversing at 2.4 ± 0.2 mV ($RI_{+40/-70mV} = 0.52$, Fig. 6B). *I–V* curved derived from all cells ($n = 9$) indicate a loss of rectification with DHPG-LTD (Fig. 6C). In addition, depressed EPSCs became insensitive to JST. The EPSC previously depressed by mGluR-LTD was unaffected by JST (−7.7 ± 6.2%, $n = 6$, not significantly different from a hypothesized change of 0%, Fig. 6D and E).

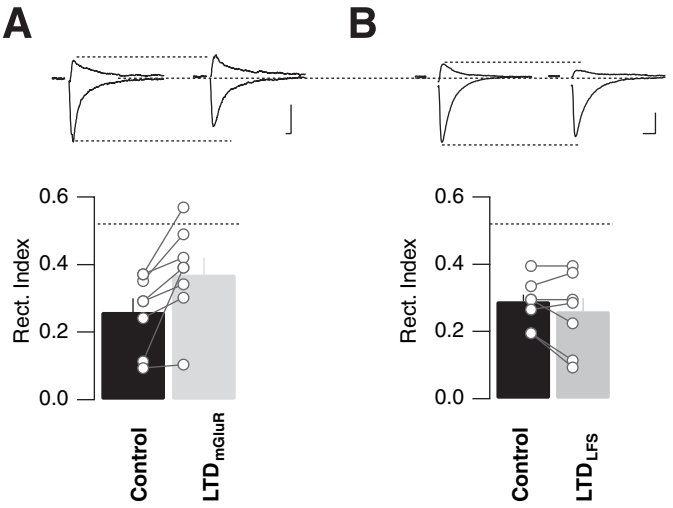

FIG. 7. Change in rectification with mGluR-LTD but not LFS-LTD. (A) Brief trains of high frequency stimuli (five stimuli at 66 Hz) induced mGluR-LTD that was associated with a change of the rectification index ($EPSC_{+40mV}/EPSC_{-70mV}$). On average rectification index was 0.27 ± 0.04 before and 0.39 ± 0.05 after LTD ($n = 8$). Insets show representative sweeps at −70 mV and +40 mV before and after the induction of mGluR-LTD. (B) LFS-LTD in contrast left the rectification index unchanged ($n = 7$). Insets show representative traces before and after LFS-LTD induction. Scale bars, 50 pA/5 ms (for all panels).

Similar increases of the RI were also observed with synaptic induction of LTD. While mGluR-LTD consistently caused an increase of RI (0.27 ± 0.04–0.39 ± 0.05, $P < 0.01$ $n = 8$, Fig. 7A), LFS-LTD did not change this parameter (0.29 ± 0.02 vs. 0.26 ± 0.04, $P > 0.05$, Fig. 7B). A direct comparison between DHPG-LTD and LFS-LTD (Fig. 6B vs. Fig. 7B) as well as mGluR-LTD and LFS-LTD (Fig. 7A vs. Fig. 7B) reveal significant differences in both cases ($P < 0.05$).

Taken together, these results suggest that after mGluR-LTD induction EPSCs were mediated by AMPARs that contained edited GluR2.

Discussion

We show that pharmacological or synaptic activation of group I mGluRs triggers an indistinguishable LTD. We reveal that excitatory synapses in DA neurons of the VTA contain AMPARs that lack the GluR2 subunit and we provide strong evidence that this mGluR-LTD is associated with an exchange of native receptors for GluR2 containing AMPARs.

Under baseline conditions we found that the EPSCs are strongly rectifying, which is the electrophysiological signature of AMPARs that do not contain edited GluR2 subunits. In fact, editing in the pore-lining region of GluR2 is required to abolish the polyamine block (Bowie & Mayer, 1995; Kamboj *et al*., 1995; Koh *et al*., 1995) and to make AMPAR calcium impermeable (Geiger *et al*., 1995). Consequently, transgenic mice expressing increasing fractions of nonedited GluR2-containing receptors (Feldmeyer *et al*., 1999) and knock out mice devoid of GluR2 (Jia *et al*., 1996; Mainen *et al*., 1998) also show rectifying synaptic responses. In wild-type animals, GluR2-lacking AMPARs have previously been found exclusively in GABAergic neurons, including stellate cells of the cerebellum, superficial lamina neurons of the dorsal horn (Engelman *et al*., 1999) and interneurons of the amygdala (Mahanty & Sah, 1998) and hippocampus (Laezza *et al*., 1999). Our data now indicate that such receptors are also present in functional synapses of dopamine neurons.

Synapses that contain such AMPARs can still express various forms of synaptic plasticity, including LTD (Meng *et al*., 2003). For example, when AMPARs lacking GluR2 were strongly stimulated, the EPSCs underwent a potentiation that resembled LTP, but was independent of NMDAR (Jia *et al*., 1996; Mainen *et al*., 1998; Meng *et al*., 2003). Conversely, in synapses that normally express GluR2, LTD depends on a mobile pool of constitutively recycling AMPARs (Lüscher *et al*., 1999; Lüthi *et al*., 1999). Here we show that a synapse that normally contains AMPARs lacking GluR2 can still undergo LTD using two distinct mechanisms. In response to LFS and voltage-gated calcium channel activation synaptic strength decreases in a protein kinase A-dependent manner (Gutlerner *et al*., 2002). In addition, we find that brief bursts activating mGluRs remove native AMPARs for GluR2 containing receptors. The depression at −50 mV may be due to the smaller single channel conductance of GluR2 containing AMPARs (Swanson *et al*., 1997) and/or involve a decrease of the actual number of receptors. This scenario is similar to the events observed in stellate granular cells of the cerebellum, where calcium entry through AMPARs is sufficient to trigger the receptor switch (Liu & Cull-Candy, 2000).

The activation of group I mGluRs has several additional effects in DA neurons of the VTA. The selective agonist DHPG triggers an inward current presumably mediated by Trp channels (Gee *et al*., 2003; Kim *et al*., 2003; Tozzi *et al*., 2003; Bengtson *et al*., 2004), increases the intracellular calcium (Morikawa *et al*., 2000; Morikawa *et al*., 2003) and leads to the release of endocannabinoids (Fig. 4) like in several other brain regions (Varma *et al*., 2001; Wilson & Nicoll, 2002). In the VTA, CB1 receptors are indeed expressed on presynaptic terminals of excitatory afferents and depolarization-induced suppression of excitation (DSE) has been observed (Melis *et al*., 2004). Therefore endocannabinoids could in principle mediate mGluR-LTD. However we can discard this possibility based on our observation that a selective CB1R antagonist only blocked the transient depression, while the magnitude of mGluR-LTD remained unchanged. This is in contrast to a presynaptic form of mGluR-LTD that has been described on excitatory synapses in the striatum (Gerdeman *et al*., 2002), the nucleus accumbens (Robbe *et al*., 2002) and on inhibitory synapses of the hippocampus (Chevaleyre & Castillo, 2003).

Appropriate synaptic stimulation can also activate Trp channels as well as K(Ca) channels leading to a slow EPSC or IPSC, respectively (Fiorillo & Williams, 1998; Bengtson *et al*., 2004). Our experiments using the Ca^{2+} chelator BAPTA suggest that mGluR-LTD depends on an initial Ca^{2+} increase, but occurs in parallel with some other effectors, as Trp currents for example were enhanced in the presence of BAPTA (not shown). We have found that PKC is required for mGluR-LTD in the VTA, which suggest that an interaction with the scaffolding protein PICK1 may be involved (Xia *et al*., 1999). This is similar to mGluR-LTD in the hippocampus (Oliet *et al*., 1997) that is also PKC dependent and may be mediated by AMPAR internalization (Xiao *et al*., 2001). Moreover, overexpression of PICK in hippocampal neurons led to an increase of AMPAR EPSC rectification while interfering with the PDZ domain of PICK had the opposite effect (Terashima *et al*., 2004).

The mGluR1-dependent LTD in the VTA described here could play an important role in opposing the LTP that may be to be associated with behavioural sensitization. The induction of sensitization takes place in the VTA and is expressed by neuroadaptive changes in the nucleus accumbens. This is based on the fact that NMDA (Karler *et al*., 1989) and dopamine receptor 1 (Kalivas & Stewart, 1991) antagonists directly applied into the VTA block sensitization. The underlying mechanism may involve the synaptic insertion of new AMPARs, particularly homomeric receptors containing the GluR1 subunit (Carlezon & Nestler, 2002). This hypothesis is strongly

supported by the observation that viral overexpression of the GluR1 subunit in dopamine neurons of VTA alone is sufficient to cause sensitization in drug-naïve animals (Carlezon *et al*., 1997). Reports on increases in GluR1 remain controversial (Fitzgerald *et al*., 1996; Lu *et al*., 2002), maybe because they rely on a redistribution of existing receptor pools to the synapse and may therefore not involve changes in mRNA and total protein levels (Self, 2002).

The observation that our drug naïve animals show rectification may indicate that there is a dynamic equilibrium between sensitization and full desensitization that could also be influenced by environmental factors, such as stress (Saal *et al*., 2003). Alternatively, the presence of GluR2-lacking AMPARs may be developmentally regulated (Kelly *et al*., 2004), with rectification still present in the rats used here (P15–P21) compared to the mice used by Thomas *et al*. (2000) (P28–P60). In any case the emphasis here is on the observation that mGluR-LTD may represent a mean to shift the equilibrium towards GluR2-containing AMPARs and therefore reverse sensitization.

It is important to realize that the mGluR-LTD described here not only changes the efficacy of excitatory afferents to depolarize DA neurons but also fundamentally changes the quality of the transmission. As changes in the fraction of calcium-permeable AMPARs regulate Ca entry, this may be a way to control the cascades of molecular events that contribute to sensitization. Our study demonstrates the existence of GluR2 lacking AMPARs and provides experimental evidence for a corollary of the hypothesis that sensitization may be expressed by the insertion of AMPARs made predominantly of homomeric GluR1 (Carlezon & Nestler, 2002). In this model the subunit composition of synaptic AMPARs on DA neurons would go from mainly GluR1-containing receptors in the sensitized state to heteromeric GluR1/GluR2-containing AMPARs in the fully de-sensitized state. At baseline, both receptor populations would be present. mGluR-LTD would be a mechanism that would shift the balance towards the heteromeric state and therefore oppose sensitization.

The link that we provide here between mGluR1 activation in the VTA and LTD further supports its implication in sensitization as a decrease of mGluR1 function leads to inherent behavioural sensitization without any prior drug exposure (Kalivas *et al*., 2003).

Pharmacological interventions at this receptor, for example through positive modulation, may be a way to determine synaptic efficacy and calcium permeability on the sliding scale between sensitized and naïve neurons in order to reverse drug sensitization in addicts.

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Abbreviations

AM251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1H-pyrazole-3-carboxamide; BAPTA, 1,2-bis(aminophenoxy)ethane-*N,N,N,N*-tetra-acetic acid; CB1, cannabinoid receptor 1; CPCCOEt, 7-hydroxyimino-cyclopropa(b)chromen-1a-carboxylate ethyl ester; DA, dopamine; DAMGO, Tyr-D-Ala-Gly-Nme-Phe-Gly-ol; D-APV, D-2-amino-5-phosphonovaleric acid; DHPG, 3,5 dihydrophenylglycine; EPSC, excitatory postsynaptic current; GF109203X, 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide; JST, Joro spider toxin; LTD/LTP, long-term depression/potentiation; LFS, low frequency stimulation; mGluRs, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; NMDAR, *N*-methyl-D-aspartate receptor; PKC, protein kinase C; PPR, paired pulse ratio; RI, rectification index; Trp, transient receptor potential; VTA, ventral tegmental area.

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