Neuropsychopharmacology (2010) 35, 1253–1260
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www.neuropsychopharmacology.org

The mGluR2/3 Agonist LY379268 Blocks the Effects of GLT-1 Upregulation on Prepulse Inhibition of the Startle Reflex in Adult Rats

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The main glutamate transporter GLT-1 is responsible for clearing synaptically released glutamate from the extracellular space and contributes to the shaping of glutamatergic transmission. Recently, it has been shown that ceftriaxone (CEF)-induced GLT-1 upregulation is associated with an impairment of the prepulse inhibition (PPI) of the startle reflex, a simple form of information processing that is reduced in schizophrenia, and determines a strong reduction in hippocampal metabotropic glutamate receptor (mGluR)2/3-dependent long-term depression. In this study, we tested the hypothesis that administration of the mGluR2/3 agonist LY379268 blocks the effect of GLT-1 upregulation on PPI of the startle. We showed that administration of LY379268 (1 mg/kg) prevented PPI alterations associated with GLT-1 upregulation, suggesting that CEF-induced PPI impairment was mGluR2/3 dependent. In addition, we showed that CEF-induced GLT-1 upregulaton did not alter the expression of mGluR2/3, and also that it occurred at sites of mGluR2/3 expression. These results indicate a novel mechanism by which GLT-1 upregulation modulates PPI of the startle.
Neuropsychopharmacology (2010) **35,** 1253–1260; doi:10.1038/npp.2009.225; published online 13 January 2010

Keywords: glutamate transporters; GLT-1; metabotropic glutamate receptors; prepulse inhibition

INTRODUCTION

GLT-1 (EAAT2; SLC1A2), the main glutamate transporter in the mammalian forebrain, is responsible for the largest proportion of total glutamate transport (Rothstein *et al*, 1996; Tanaka *et al*, 1997; Conti and Weinberg, 1999; Danbolt, 2001). Owing to its perisynaptic localization (Rusakov and Lehre, 2002; Melone *et al*, 2009a), GLT-1 regulates glutamate spillover, thereby modulating the activation of glutamate receptors (Scanziani *et al*, 1997; Min *et al*, 1998; Mitchell and Silver, 2000; Tzingounis and Wadiche, 2007).

For its fundamental role in regulating Glu levels, GLT-1 is involved in the pathophysiology of several neuropsychiatric diseases (Beart and O'Shea, 2007; Lauriat and McInnes, 2007; Sheldon and Robinson, 2007). Some evidence suggests a role for GLT-1 in the pathophysiology of schizophrenia as follows: (1) a susceptibility locus for schizophrenia is probably located within or near the *GLT-1* gene (Deng *et al*, 2004), and this gene is reportedly dysregulated in patients with schizophrenia (Shao and Vawter, 2008); (2) GLT-1 immunoreactivity is increased in the thalamus, striatum, and prefrontal cortex of schizophrenia patients (Matute *et al*, 2005; McCullumsmith and Meador-Woodruff, 2002; Smith *et al*, 2001); (3) the antipsychotic clozapine specifically downregulates GLT-1 expression and function both *in vivo* and *in vitro* (Melone *et al*, 2001, 2003; Vallejo-Illarramendi *et al*, 2005); (4) the psychotomimetics PCP specifically upregulates GLT-1 expression and function (Fattorini *et al*, 2008); and (5) pharmacologically induced GLT-1 upregulation is associated with an impairment of the prepulse inhibition (PPI) of the startle reflex, a neurophysiological parameter altered in schizophrenia patients and in animal models of schizophrenia, in a dihydrokainate (DHK)-reversible manner, and worsens PCP-induced PPI alterations (Bellesi *et al*, 2009; Melone *et al*, 2009b). These data support the hypothesis that, in concert with both NMDARs and non-NMDARs (Lewis and Gonzalez-Burgos, 2006; Lewis and Moghaddam, 2006), GLT-1 has a role in the dysfunction of glutamatergic transmission that contributes to the disturbance of information processing occurring in schizophrenia (Venables, 1960; McGhie and Chapman, 1961; Andreasen, 1997, 2000; Tononi and Edelman, 2000).

Targeting metabotropic glutamate receptors (mGluRs) is a practical strategy for regulating abnormal glutamatergic transmission (Moghaddam, 2004). The selective mGluR2/3 agonist LY379268 attenuates behavioral abnormalities in

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Received 22 October 2009; revised 2 December 2009; accepted 7 December 2009

animal models of schizophrenia, and its analog LY404039 is effective in a schizophrenia phase 2 clinical trial (Cartmell *et al*, 1999, 2000; Clark *et al*, 2002; Imre *et al*, 2006; Imre, 2007; Patil *et al*, 2007; Harrison, 2008). Recently, we have shown that ceftriaxone (CEF)-induced GLT-1 upregulation impairs hippocampal long-term depression (LTD) in a DHK-reversible manner by limiting activation of mGluR2/3, thus indicating that a reduced function of mGluR2/3 mediates the effects of GLT-1 upregulation (Omrani *et al*, 2009).

In this study, we tested the hypothesis that administration of the selective mGluR2/3 agonist LY379268 blocks the impairment of PPI of the startle reflex associated with GLT-1 upregulation.

MATERIALS AND METHODS

Animals

Adult Sprague–Dawley male rats (initial weight 280–300 g; Charles River, Milano, Italy) were used. Their care and handling were approved by the Ethical Committee for Animal Research of Università Politecnica delle Marche. Experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC), and all efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were kept under a 12-h dark–light cycle and permitted food and water *ad libitum*. Rats were caged in pairs and handled within 24 h of arrival and then over at least 1 week before testing; to minimize stress during behavioral testing, rats were briefly handled before any procedure (Geyer and Swerdlow, 1998).

Drugs and Treatments

CEF sodium (CEF, Rocefin, Roche, Milano, Italy) was used to upregulate GLT-1 (Rothstein *et al*, 2005; Bellesi *et al*, 2009; Omrani *et al*, 2009). CEF was dissolved in saline (0.9% NaCl) and administered intraperitoneally (i.p.) at a dose of 200 mg/kg for 8 consecutive days (Bellesi *et al*, 2009). LY379268 (2453, Tocris Bioscience, Bristol, UK) was dissolved in saline and administered i.p. in a single dose of 1 mg/kg at day 10, ie, 2 days after CEF withdrawal. Control rats, matched for age, sex, and weight, received saline i.p. All injections were administered between 1000 and 1100 hours.

PPI of the Startle Reflex

Experimental design. Four experimental groups ($n = 11$ for each group) were studied: the first group received saline for 8 days, followed 2 days after by a single injection of saline (Saline group); the second group received saline for 8 days, followed 2 days after by a single injection of LY379268 (saline + LY group); the third group received CEF for 8 days, and an injection of saline 2 days after CEF withdrawal (CEF group); and the fourth group was administered CEF for 8 days, followed by a single injection of LY379268 2 days after CEF withdrawal (CEF + LY group). All acoustic startle experiments were conducted in an acoustic startle chamber (SR-LAB; San Diego Instruments, San Diego, CA, USA). Two days before treatment inception, a brief startle session was performed to obtain experimental and control groups matched for startle reactivity (matching session; Geyer and Swerdlow, 1998; Martinez *et al*, 1999). In all experimental groups, PPI test was conducted at day 10 (2 days after CEF withdrawal) to allow complete clearance of the antibiotic (Rebuelto *et al*, 2003), 30 min after the injection of saline or LY379268.

Testing procedures. For matching startle sessions, rats were placed in a startle chamber and exposed to 5 min of 70-dB background noise, followed by 11 pulse-only trials (40-ms noise burst at 120 dB); intertrial interval was 15 s (Geyer and Swerdlow, 1998).

The PPI session lasted $\sim$14 min, and consisted of 5 min of acclimation (70-dB background), followed by 4 trial types: pulse-only trials (40-ms noise burst at 120 dB) and 3 prepulse trials (20-ms noise bursts 3, 6, or 12 dB above background, followed 100 ms later by a 40-ms noise burst at 120 dB). Sessions included 3 blocks: block 1 (6 pulse-only trials), block 2 (10 pulse-only trials and 5 prepulse trials for each prepulse tone presented in a pseudorandom order), and block 3 (5 pulse-only trials). The intertrial interval was 15 s.

Data collection and analysis. Experimental groups were matched for startle reactivity, which was calculated by averaging pulse-alone startle amplitude (2–11 trials) of each animal during matching session (Geyer and Swerdlow, 1998).

In all animals, startle reflex magnitude was measured by averaging the startle amplitude of pulse-only trials of block 2 (10 trials). The degree of PPI (in %) was calculated according to the formula: PPI = 100 × (pulse-only units − (prepulse + pulse units))/(pulse-only units) (Geyer and Swerdlow, 1998), where pulse-only units were startle magnitudes recorded from block 2. Habituation was assessed as the decrement in response magnitude across blocks (block 1, trials 2–6 and block 3, trials 32–36).

Western Blotting

After completion of PPI studies (ie, at day 10), 4 control rats and 4 CEF-treated rats were anesthetized with chloral hydrate (300 mg/kg i.p.) and decapitated; the brains were dissected and the frontal cortex, hippocampus, and striatum were removed. Homogenization and crude synaptic plasma membrane preparation were carried out as described previously (Danbolt *et al*, 1990). Bio-Rad Protein Assay (Bio-Rad Laboratories, GmbH, München, Germany) and a Beckman DU 530 spectrophotometer (Beckman Coulter, Fullerton, CA, USA) were used to determine the total amount of protein in each homogenate (3–4 measurements per homogenate). A standard curve with 2, 4, 6, 8, and 10 μg of bovine serum albumin (A4503, Sigma Chemicals, St Louis, MO, USA) was drawn for each dosing run. As housekeeping proteins (such as α-actin and β-tubulins) are sensitive to experimental treatments (particularly pharmacologic treatments) and to diverse physiological conditions and have therefore some limitations as internal standard (Ferguson *et al*, 2005), 3–6 measurements were made for each brain region of each animal; to minimize procedural variables, homogenates from treated and control animals

were loaded onto the same gel (Bragina *et al*, 2006). For a semi-quantitative analysis, we drew standard curves of increasing concentration of total protein from controls ($n = 4$) to define a linear range for immunoblot densitometric analysis (Bragina *et al*, 2006). Nitrocellulose filters were probed with mGluR2/3 antibodies (AB1553; Millipore, Billerica, MA, USA; 1 : 200; 20 μg of total protein; Testa *et al*, 1998). After exposure to secondary antibodies (Vector, Burlingame, CA, USA), bands were visualized by Bio-Rad Chemidoc and Quantity One software using the SuperSignal West Pico (Rockford, IL, USA) chemiluminescent substrate (see Bragina *et al*, 2006 for details).

Immunocytochemistry

After completion of PPI studies (day 10), 4 control rats and 4 CEF-treated rats were anesthetized with chloral hydrate (300 mg/kg i.p.) and perfused transcardially with a flush ($\sim$1 min) of saline, followed by 4% paraformaldehyde in phosphate buffer (PB). The brains were removed, postfixed in the same fixative for 7 days (Chen *et al*, 2004), and cut on a Vibratome in 50-μm parasagittal sections, which were collected in PB in serial groups of 5 and then used for immunocytochemistry (Minelli *et al*, 2001; Chen *et al*, 2004; Melone *et al*, 2005). Sections were incubated in a solution containing a mixture of GLT-1 (1 : 1000; Millipore; AB1783; Bragina *et al*, 2006) and mGlurR-2/3 (1 : 50; Millipore; AB1553; Testa *et al*, 1998) primary antibodies. Sections were then exposed to a solution containing a mixture of affinity-purified fluorescein isothiocyanate (1 : 250; Vector; FI1000/J0114)- or tetramethylrhodamine isothiocyanate (1 : 250; Molecular Probes, PoortGebouw, The Netherlands, T-2762/6691-1)-conjugated secondary antibodies. Sections were washed, mounted, air-dried, and coverslipped using the Vectashield mounting medium (H-1000; Vector), and finally examined with a Leica TCS SL confocal laser scanning microscope equipped with an argon (488 nm) and helium/neon (543 nm) laser. Control experiments with single-labeled sections and sections incubated with two primary antibodies and one secondary antibody or with one primary and two secondary antibodies revealed neither bleed-through nor cross-reactivity. For all immunocytochemical studies, sections from controls and CEF-treated animals were processed in parallel to minimize procedural variables.

Data collection and analysis. Fields from layers II to III of the frontal neocortex anterior to the bregma, from the stratum lucidum of hippocampal CA1, and from the striatum of control ($n = 4$) and CEF-treated ($n = 4$) animals were randomly selected for each fluorescent as 1024×1024 pixel images with a planapo $\times 63$ objective (numerical aperture 1.4) and pinhole 1.0 Airy unit. To improve the signal–noise ratio, 8 frames of each image were averaged; microscopic fields were scanned with a pixel size of 80 nm. Quantitative analysis of puncta staining was performed in randomly selected $8 \times 8\,\mu m^2$ fields from each 1024×1024 pixel image (10 fields for each region for each animal). Image processing was performed on Photoshop CS2 (Adobe Systems, San Jose, CA, USA); optimal resolution of punctate staining was obtained by setting threshold values as described elsewhere (Bellesi *et al*, 2009). Threshold values did not differ across conditions (ie, between control and treated groups). After thresholding, images were transformed in binary images using the Image J software (v.1.38, NIH, USA.) (Bragina *et al*, 2006) and the mean size and number of mGluR2/3 and GLT-1 puncta was calculated. To verify whether GLT-1 upregulation occurred at sites where mGluR2/3 was expressed, the mean size of GLT-1 puncta contacting mGluR2/3 puncta (a contact was defined as the presence of fluorescence signals overlap) was estimated.

Statistical Analysis

Startle reactivity was analyzed using one-way ANOVA. Startle reflex magnitudes were analyzed using two-way ANOVA with CEF and LY379268 as between-subject factors. PPI scores were analyzed using three-way ANOVA with prepulse intensities as a within-subject factor and CEF and LY379268 as between-subject factors. Habituation was analyzed with three-way ANOVA with block as a within-subject factor and CEF and LY379268 as between-subject factors. In the presence of interaction, pairwise comparisons were conducted with two-way ANOVA with CEF or LY379268 as a between-subject factor and prepulse intensity or block as a within-subject factor. For multiple comparisons, α was set at 0.01. Statistical significance for western blotting and immofluorescence studies was evaluated by two-sided *t*-test ($\alpha = 0.05$). Statistical analysis was performed using SPSS (v.13.0).

RESULTS

Effects of LY379268 on PPI Impairment Induced by CEF Treatment

Mean values of startle reactivity in all experimental groups were 95.3 ± 10.5 for the Saline group, 83.8 ± 10.3 for the saline + LY group, 94.2 ± 14.9 for the CEF group, and 80.9 ± 14.4 for the CEF + LY group. One-way ANOVA did not reveal any significant difference among the four groups ($F((3, 40) = 0.44$; NS)).

Effects on startle magnitude. CEF treatment and a single LY379268 injection had no significant effect on startle magnitude (Table 1). Two-way ANOVA of startle magnitude with CEF and LY379268 as between-subject factors revealed neither significant effects of CEF ($F(1, 40) = 0.02$; NS) and LY379268 ($F(1, 40) = 0.12$; NS) nor CEF $\times$ LY379268 interaction ($F(1, 40) = 0.15$; NS).

Effects on PPI. LY379268 blocked PPI impairment induced by CEF administration (Figure 1). Three-way ANOVA of PPI with CEF and LY379268 as between-subject factors and prepulse intensity as a within-subject factor showed a significant effect of CEF ($F(1, 40) = 14.96$; $P < 0.0001$) and LY379268 ($F(1, 40) = 8.07$; $P = 0.007$), a significant CEF $\times$ LY379268 interaction ($F(1, 40) = 17.52$; $P < 0.0001$), and a significant effect of prepulse intensity ($F(2, 80) = 95.3$; $P < 0.0001$). Prepulse intensity $\times$ CEF, prepulse intensity $\times$ LY379268, or prepulse intensity $\times$ CEF $\times$ LY379268 interactions were not significant. For defining CEF $\times$ LY379268 interaction, we evaluated pairwise comparisons among the different treatment conditions. Comparison of group Saline and group CEF with two-way ANOVA, with CEF as a

Table 1 Startle Reflex Magnitude and Habituation (Mean ± SEM) of Experimental Groups

Group	Startle magnitude second trial block	Habituation	
		First trial block	Last trial block
Saline group ($n=11$)	102.3 ± 17.7	134.5 ± 21.8	68.2 ± 15.1
Saline+LY group ($n=11$)	92.6 ± 25.8	145.2 ± 27.7	73.5 ± 12.9
CEF group ($n=11$)	98.9 ± 18.7	139.1 ± 26.9	84.9 ± 22.5
CEF+LY group ($n=11$)	95.5 ± 18.4	152.3 ± 38.7	64.5 ± 14.2

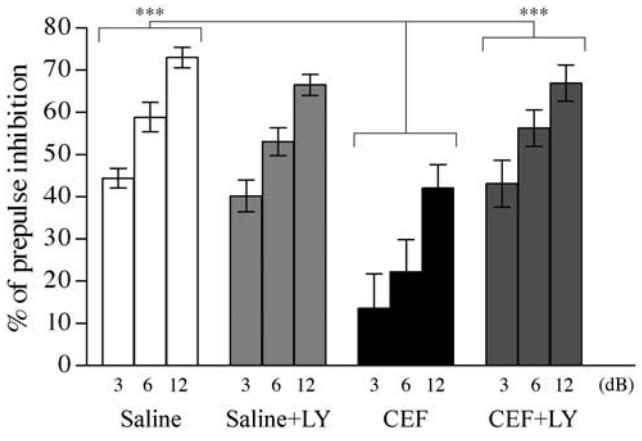

Figure 1 Acute administration of the selective mGlur-2/3 agonist LY379268 blocks PPI impairment associated with CEF treatment. Values are %PPI ± SEM ***$P<0.001$.

between-subject factor and prepulse intensity as a within subject factor, showed a significant effect of prepulse intensity ($F(2,40)=41.83$; $P<0.0001$) and of CEF ($F(1,20)=25.71$; $P<0.0001$), but not of CEF × prepulse intensity interaction ($F(2,40)=0.57$; NS). Comparison of group Saline and group Saline/LY with two-way ANOVA, with LY379268 as a between-subject factor and prepulse intensity as a within-subject factor, showed a significant effect of prepulse intensity ($F(2,40)=80.47$; $P<0.0001$) but not of LY379268 ($F(1,20)=2.58$; NS) or of LY379268 × prepulse intensity interaction ($F(2,40)=0.15$; NS). Comparison of group CEF and group CEF/LY with two-way ANOVA, with LY379268 as between-subject factor and prepulse intensity as within-subject factor, showed a significant effect of prepulse intensity ($F(2,40)=33.11$; $P<0.0001$) and of LY379268 ($F(1,20)=14.96$; $P=0.001$) but not of LY379268 × prepulse intensity interaction ($F(2,40)=1.03$; NS).

Effects on habituation. CEF treatment and a single LY379268 injection had no effects on startle habituation to repeated acoustic pulses (Table 1). Three-way ANOVA of startle magnitudes, with CEF and LY379268 as between-subject factors and trial block as a within-subject factor, revealed neither a significant effect of CEF ($F(1,40)=0.25$; NS) and LY379268 ($F(1,40)=0.01$; NS), nor a CEF × LY379268 interaction ($F(1,40)=0.12$; NS), but a significant effect of trial block ($F(1,40)=57.06$; $P<0.0001$). CEF × block, LY379268 × block, CEF × LY379268 × trial block interactions were not significant.

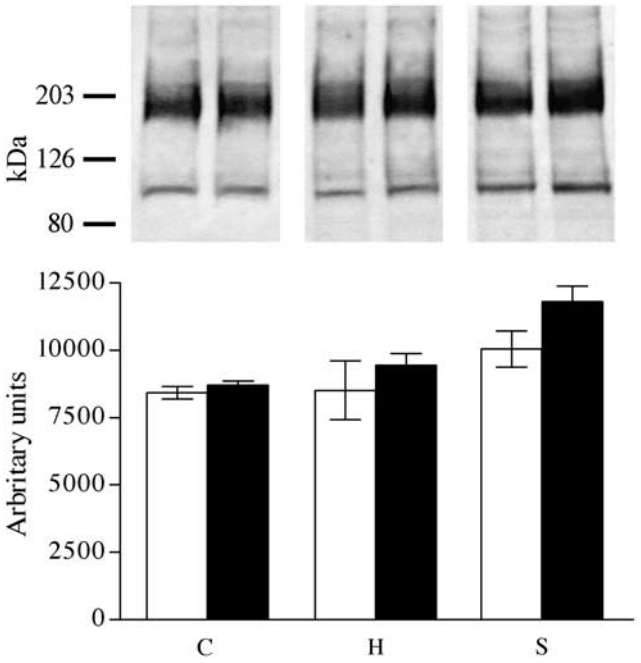

Figure 2 CEF treatment is not associated with alterations of mGluR-2/3 expression in forebrain regions regulating PPI. Western blotting studies do not reveal any significant difference between control (white column) and CEF-treated (black column) rats. C, frontal cortex; H, hippocampus; S, striatum.

These studies showed that PPI alterations induced by CEF treatment were blocked by the selective mGluR2/3 agonist LY379268, indicating that reducing mGluR2/3 activation contributes to PPI impairment associated with CEF treatment.

Effects of CEF Treatment on mGluR2/3 Expression

To verify whether reduced activation of mGluR2/3 was associated with an alteration of its expression, we studied mGluR2/3 protein levels in forebrain regions regulating PPI (Swerdlow *et al*, 2001). In rats treated with CEF for 8 days, western blotting studies showed that mGluR2/3 expression was not significantly different from controls in the frontal cortex, hippocampus, and striatum, thus indicating that CEF treatment does not change the amount of mGluR2/3 expressed (Figure 2). In addition, to rule out the possibility that CEF altered the distribution of mGluR2/3, we carried out immunocytochemical studies in the same forebrain regions, and found that in CEF-treated rats, size and number of mGluR2/3 immunoreactive puncta were comparable with controls (Figure 3).

These results showed that PPI impairment induced by CEF treatment was not associated with an altered expression of mGluR2/3.

Spatial Relationship Between GLT-1 Upregulation and mGluR2/3

CEF treatment upregulates GLT-1 (Rothstein *et al*, 2005), and CEF-induced GLT-1 upregulation can be visualized immunocytochemically (Bellesi *et al*, 2009; Omrani *et al*,

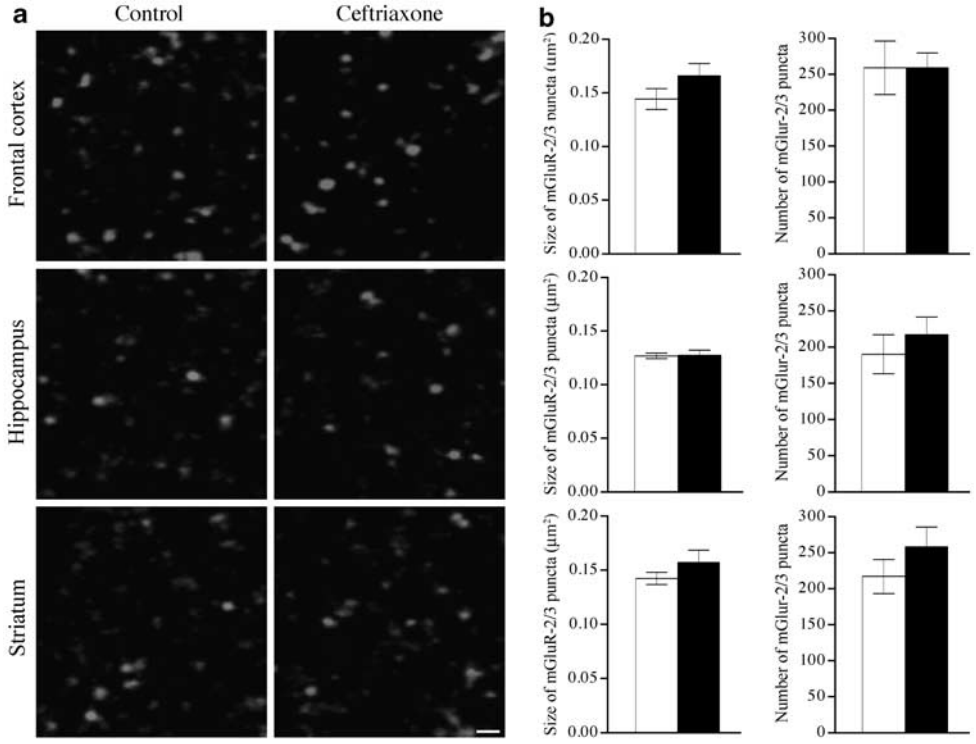

Figure 3 CEF treatment does not affect the distribution of mGlur-2/3 in the frontal cortex, hippocampus, and striatum. (a) mGluR2/3 immunoreactive puncta in control and CEF-treated animals. Bar: 0.8 μm. (b) Quantitative analysis showed that mean size and number of mGluR2/3 puncta are comparable in control (white column) and CEF-treated (black column) rats. Values are mean ± SEM.

2009). In this study, we measured the mean size of GLT-1 puncta in CEF-treated animals, and showed that it was significantly increased in the frontal cortex (by 49.9 ± 12.9%; $P = 0.03$), hippocampus (by 46.2 ± 10.8%; $P = 0.03$), and striatum (by 93 ± 26.1%; $P = 0.02$) compared with controls.

As both GLT-1 (Melone *et al*, 2009a) and mGluR2/3 (Ferraguti and Shigemoto, 2006) are localized perisynaptically, we sought to define the anatomical relationship between GLT-1 upregulation and mGluR2/3 expression.

In both controls and CEF-treated animals, confocal images showed a consistent degree of overlap between the two fluorescent signals, indicating a close spatial relationship between mGluR2/3 and GLT-1. To verify whether the increased expression of GLT-1 also occurs at the very sites expressing mGluR2/3, we estimated the mean size of GLT-1 puncta showing clear overlap with mGluR2/3 puncta (Figure 4), and showed that it was significantly increased in the frontal cortex (by 61.8 ± 11.9%; $P = 0.009$), hippocampus (by 41.4 ± 14.4%; $P = 0.04$), and striatum (by 103.8 ± 27.2%; $P = 0.02$) (Figure 4).

These results indicated that GLT-1 upregulation occurred in the vicinity of mGluR2/3 receptors, thus suggesting that GLT-1 upregulation has a role in determining the reduced activation of mGluR2/3 observed in PPI experiments.

DISCUSSION

The major result of this study is that PPI alterations associated with GLT-1 upregulation are blocked by the selective mGluR2/3 agonist LY379268.

PPI of the startle reflex is a model of sensorimotor gating that is tested in a similar manner in humans and rats; for this reason, it is widely used to study the neurobiology of schizophrenia and the effects of medications (Geyer and Swerdlow, 1998; Dawson *et al*, 2000; Swerdlow *et al*, 2000; Geyer *et al*, 2001, 2002). Most importantly, although impaired startle PPI is shared by several neuropsychiatric diseases, it is consistently observed in schizophrenia, in which it is believed to be an index of the deficient sensorimotor gating underpinning sensory flooding and cognitive fragmentation (Braff and Geyer, 1990; Braff *et al*, 1992, 2001, 2007; Swerdlow *et al*, 1994, 2006; Minassian *et al*, 2007).

Rothstein *et al* (2005) showed that CEF robustly stimulates GLT-1 expression, but not that of the other glutamate transporters, through increased *GLT-1* gene transcription. This finding has been replicated in different laboratories (eg, Chu *et al*, 2007; Ouyang *et al*, 2007; Lee *et al*, 2008), including ours (Bellesi *et al*, 2009; Omrani *et al*, 2009). We also showed that CEF increases GLT-1a expression in forebrain regions that regulate PPI of the startle (Bellesi *et al*, 2009). Whether CEF alters the expression of synaptically active molecules at nonglutamatergic synapses is currently unknown, although to date no evidence for an effect of CEF on other neurotransmitter system(s) has been published. As far as these results are concerned, it must be emphasized that, regardless of whether CEF acts on other transmitter systems, its effects on PPI are prevented by the GLT-1 antagonist DHK, indicating that GLT-1 upregulation is the major mediator of the effects of CEF on PPI.

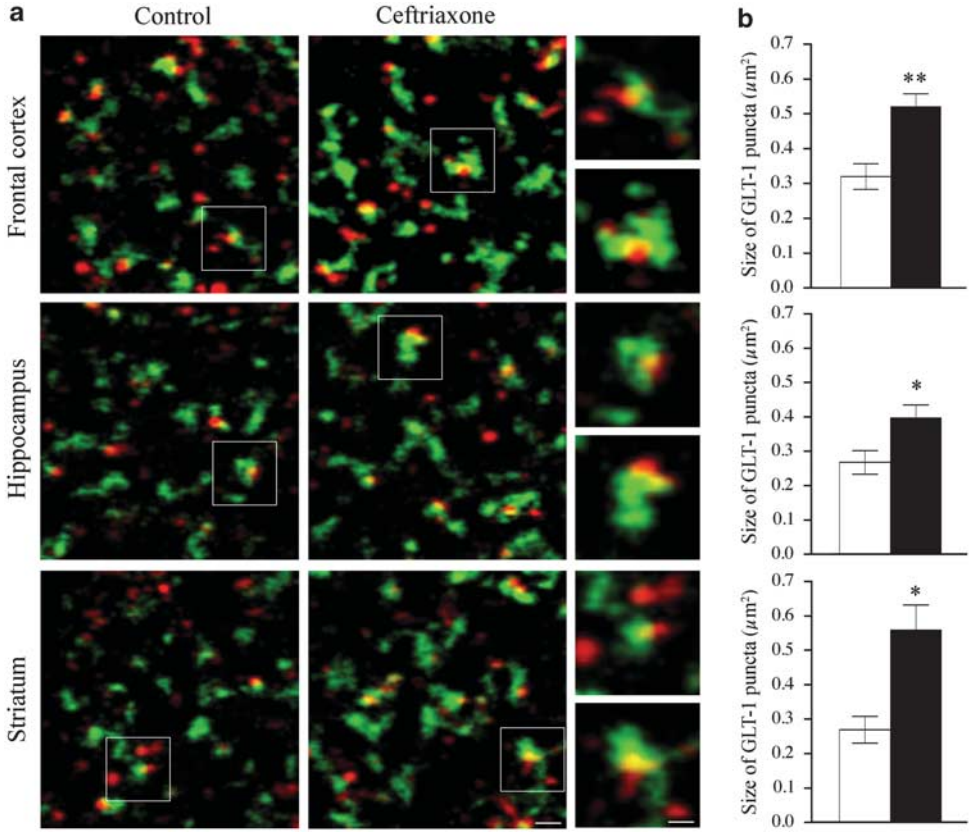

Figure 4 GLT-1 upregulation occurs also in the vicinity of sites of mGluR2/3 expression in the frontal cortex, hippocampus, and striatum. (a) Double-labeling studies showing GLT-1 puncta (green) contacting mGluR2/3 puncta (red) in control and in CEF-treated rats. Framed regions are reproduced enlarged in the right column to illustrate that GLT-1 puncta are larger in CEF-treated animals. Bar: 0.8 μm in large frames and 0.4 μm in small frames. (b) Quantitative evaluation of the size of GLT-1 puncta contacting mGluR2/3 positive puncta in control (white columns) and CEF-treated (black columns) rats. Values are mean ± SEM. $^{*}P<0.05$; $^{**}P<0.01$.

In this paper, we showed that LY379268, a compound effective in animal models of schizophrenia (Cartmell *et al*, 1999, 2000; Clark *et al*, 2002; Imre *et al*, 2006; Imre, 2007), blocked PPI impairment associated with GLT-1 upregulation. Although the present findings do not allow predictions about clinical efficacy of LY379268, it is worth noting that the better assimilated analog LY404039 ameliorates both positive and negative symptoms in schizophrenia patients (Patil *et al*, 2007; Harrison, 2008). Some studies reported that LY379268 failed to block PPI alterations (but not other behavioral abnormalities) induced by administration of NMDA antagonists (Cartmell *et al*, 1999, 2000; Clark *et al*, 2002; Imre *et al*, 2006). The different results may be explained by the different paradigms used to impair PPI; indeed, it is conceivable that the reduction in glutamatergic transmission induced by NMDA antagonism is more intense than that induced by the upregulation of GLT-1, and that the modulating effect of LY379268 on glutamatergic transmission is not sufficient to restore PPI induced by NMDA antagonists.

The ability of LY379268 to prevent PPI abnormalities associated with GLT-1 upregulation suggests that the reduced activation of mGluR2/3 is a mechanism by which GLT-1 upregulation impairs PPI. mGluR2/3 activation depends on the spread of glutamate from release sites to the perisynaptic zone, where these receptors are predominantly located (Scanziani *et al*, 1997; Min *et al*, 1998; Mitchell and Silver, 2000; Ferraguti and Shigemoto, 2006); and GLT-1 controls extracellular levels of glutamate and it is mainly localized perisynaptically (Melone *et al*, 2009a). Thus, by modulating glutamate spillover, GLT-1 influences activation of mGluR2/3 (Rusakov and Kullmann, 1998; Barbour, 2001; Diamond, 2002; Tzingounis and Wadiche, 2007; Zheng *et al*, 2008; Omrani *et al*, 2009). Our western blotting and immunocytochemical studies are consistent with this view. First, we demonstrated that both expression and distribution of mGluR2/3 in forebrain regions regulating PPI were unchanged in CEF-treated rats compared with controls. Second, we showed that GLT-1 upregulation also occurred at the very sites where mGluR2/3 was expressed. To do this, we considered the overlap of fluorescence signals in double-labeled sections as a marker of nearness, a procedure validated by recent electron microscopy studies (Melone *et al*, 2009a). Upregulation of GLT-1 at sites close to those of mGluR2/3 expression may thus have a crucial role in reducing the amount of glutamate available for mGluR2/3 activation.

GLT-1 upregulation induced by CEF determines a strong, DHK-sensitive, impairment of mGluR-dependent LTD (Omrani *et al*, 2009). The possibility that a widespread impairment of LTD can induce cognitive alterations, including PPI abnormalities, has been investigated in animal models of schizophrenia. Mice lacking the dopamine transporter display PPI deficits (Ralph *et al*, 2001; Barr *et al*, 2004; Yamashita *et al*, 2006), and exhibit an impairment

of hippocampal LTD (Morice *et al*, 2007). On these bases, it has been suggested that LTD could have a key role in modulating cognitive flexibility as well as in other complex cognitive functions (Morice *et al*, 2007; Nicholls *et al*, 2008). Therefore, it is possible that alterations of LTD observed in animals with GLT-1 upregulation (Omrani *et al*, 2009) are responsible for the alterations of the PPI described in this study, and may conceivably contribute to the cognitive impairment observed in schizophrenia patients.

ACKNOWLEDGEMENTS

This study was funded by grants from MIUR and Università Politecnica delle Marche to FC. We gratefully acknowledge A Frontini's assistance with confocal microscopy.

DISCLOSURE

The authors declare no conflict of interest.

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