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Michele Bellesi, Fiorenzo Conti. The mGluR2/3 agonist LY379268 blocks the effects of GLT-1 up-regulation on prepulse inhibition of the startle reflex in adult rats. *Neuropsychopharmacology*, 2010, n/a (n/a), pp.n/a-n/a. 10.1038/npp.2009.225 . hal-00501997

**HAL Id: hal-00501997**

**<https://hal.science/hal-00501997>**

Submitted on 13 Jul 2010

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***The mGluR2/3 agonist LY379268 blocks the effects of GLT-1 up-regulation on prepulse inhibition of the startle reflex in adult rats***

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**Running title:** LY379268 blocks CEF-induced PPI impairment

**Number of figures:** 4 | **Table:** 1 | **Number of pages:** (including references): 26

**Number of words:** Abstract: 153 | Introduction: 453 | Material & Methods: 1517

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## ABSTRACT

The main glutamate transporter GLT-1 is responsible for clearing synaptically released glutamate from the extracellular space and contributes to shape glutamatergic transmission. Recently, it has been shown that ceftriaxone (CEF)-induced GLT-1 up-regulation is associated to 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 of hippocampal mGluR2/3-dependent long term depression (LTD). Here, we tested the hypothesis that administration of the mGluR2/3 agonist LY379268 blocks the effect of GLT-1 up-regulation on PPI of the startle. We showed that LY379268 (1 mg/Kg) administration prevented PPI alterations associated to GLT-1 up-regulation, suggesting that CEF-induced PPI impairment was mGluR2/3-dependent. In addition, we demonstrated that CEF-induced GLT-1 up-regulation did not alter the expression of mGluR2/3, and that it occurred at sites of mGluR2/3 expression. These results indicate a novel mechanism by which GLT-1 up-regulation modulates PPI of the startle.

**Key words:** glutamate transporters - GLT-1 – Metabotropic glutamate receptors – Prepulse inhibition

## INTRODUCTION

GLT-1 (EAAT2; SLC1A2), the main glutamate transporter in 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: 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 schizophrenia patients (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, *et al* 2002; Smith, *et al* 2001); 3) the antipsychotic clozapine down-regulates specifically GLT-1 expression and function in *in vivo* and *in vitro* (Melone, *et al* 2001, 2003; Vallejo-Illarramendi, *et al* 2005); 4) the psychotomimetics PCP up-regulates specifically GLT-1 expression and function (Fattorini, *et al* 2008); and 5) pharmacologically induced GLT-1 up-regulation is associated to 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 NMDARs and non-NMDARs (Lewis and Gonzalez-Burgos, 2006; Lewis and Moghaddam, 2006), GLT-1 plays 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 behavioural abnormalities in animal models of schizophrenia, and its analog 404039 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-induced GLT-1 up-regulation 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 up-regulation (Omrani, *et al* 2009).

Here we tested the hypothesis that administration of the selective mGluR2/3 agonist LY379268 blocks the impairment of PPI of the startle reflex associated to GLT-1 up-regulation.

## MATERIAL 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 behavioural testing, rats were briefly handled prior to any procedure (Geyer and Swerdlow, 1998).

### *Drugs and treatments*

Ceftriaxone sodium (CEF, Rocefin, Roche, Milano, Italy) was used to up-regulate 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, i.e., 2 days after CEF withdrawal. Control rats, matched for age, sex and weight, received saline i.p. All injections were administered between 10:00 and 11:00 AM.

### *Prepulse inhibition 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 performed 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 performed 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).

PPI session lasted approximately 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 pseudorandom order); and block 3 (5 pulse only trials). 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:  $PPI = 100 \times [\text{pulse only units} - (\text{prepulse} + \text{pulse units}) / (\text{pulse only units})$  (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 (i.e., at day 10), 4 control rats and 4 CEF-treated rats were anaesthetized with chloral hydrate (300 mg/kg i.p.) and decapitated; the brains were dissected and frontal cortex, hippocampus, and striatum were removed. Homogenization and crude synaptic plasma membranes preparation were carried out as described (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/homogenate). A standard curve with 2, 4, 6, 8 and 10 µg of bovine serum albumine (BSA; A4503, Sigma Chemicals, St. Louis, MO, USA) was drawn for each dosing run. Since housekeeping proteins (e.g.,  $\alpha$ -actin and  $\beta$ -tubulins) are sensitive to experimental treatments (particularly pharmacologic ones) 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 semiquantitative 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 BioRad Chemidoc and Quantity One software using the SuperSignal West Pico (Rockford, IL, USA) chemiluminescent substrate (Bragina, *et al* 2006 for details).

### ***Immunocytochemistry***

After completion of PPI studies (day 10), 4 control rats and 4 CEF-treated rats were anaesthetized with chloral hydrate (300 mg/kg i.p.) and perfused transcardially with a flush (~1 min) of saline followed by 4% paraformaldehyde in phosphate buffer (PB). Brains were removed, post-fixed 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 mGluR-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 (FITC, 1:250; Vector; FI1000/J0114)- or tetramethylrhodamine isothiocyanate (TRITC, 1:250; Molecular Probes, PoortGebouw, The Netherlands, T-2762/6691-1)-conjugated secondary antibodies. Sections were washed, mounted, air-dried and coverslipped using 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-labelled 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-III of the frontal neocortex anterior to the bregma, from stratum lucidum of hippocampal CA1, and from striatum of control (n=4) and CEF-treated (n=4) animals were randomly selected for each fluorescent as 1024 x 1024 pixel images with a planapo x63 objective (numerical aperture 1.4) and pinhole 1.0 Airy unit. To improve signal/noise ratio, 8 frames of each images were averaged; microscopic fields were scanned with a pixel size of 80 nm. Quantitative analysis of puncta staining was performed in randomly selected 8 x 8  $\mu$ m fields from each 1024 x 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 (i.e., between control and treated groups). Following thresholding, images were transformed in binary images using Image J software (v.1.38, NIH, USA.) (Bragina, *et al* 2006) and mean size and number of mGluR2/3 and GLT-1 puncta was calculated. To verify whether GLT-1 up-regulation 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 analysed with two-way ANOVA with CEF and LY379268 as between-subject factors. PPI scores were analyzed with three-way ANOVA with prepulse intensities as within-subject factor and CEF and LY379268 as between-subject factors. Habituation was analyzed with three-way ANOVA with block as within-subject factor and CEF and LY379268 as between-subject factors. In the presence of interaction, pair-wise 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,  $\alpha$  was set at 0.01. Statistical significance for western blotting and immunofluorescence 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  $95.3 \pm 10.5$  for the Saline group;  $83.8 \pm 10.3$  for the Saline + LY group;  $94.2 \pm 14.9$  for the CEF group; and  $80.9 \pm 14.4$  for the CEF + LY group. One-way ANOVA did not reveal any significant difference among the four groups ( $F(3,40)=0.44$ ; NS).

*Table 1 about here*

*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 x LY379268 interaction ( $F(1, 40)=0.15$ ; NS).

*Figure 1 about here*

*Effects on prepulse inhibition.* 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 x 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 x CEF, prepulse intensity x LY379268, or prepulse intensity x CEF x LY379268 interactions were not significant. For defining CEF x LY379268 interaction, we evaluated pair-wise comparisons among the different treatment conditions. Comparison of group Saline and group CEF with two-way ANOVA, with CEF as a 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 x 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 x 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 x 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 within-subject factor, revealed neither significant effect of CEF ( $F[1,40]=0.25$ ; NS) and LY379268 ( $F[1,40]=0.01$ ; NS), nor CEF x LY379268 interaction ( $F[1,40]=0.12$ ; NS), but a significant effect of trial block ( $F[1,40]=57.06$ ;  $P<0.0001$ ). CEF x block, LY379268 x block, CEF x LY379268 x trial block interactions were not significant.

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 to CEF treatment.

#### ***Effects of CEF treatment on mGluR2/3 expression***

To verify whether reduced activation of mGluR2/3 was associated to 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 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 performed immunocytochemical studies in the same forebrain regions, and found that in CEF-treated rats size and number of mGluR2/3 immunoreactive puncta were comparable to controls (Figure 3).

*Figure 2 and 3 about here*

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

***Spatial relationship between GLT-1 up-regulation and mGluR2/3***

CEF treatment up-regulates GLT-1 (Rothstein, *et al* 2005), and CEF-induced GLT-1 up-regulation can be visualized immunocytochemically (Bellesi, *et al* 2009; Omrani, *et al* 2009). Here, we measured mean size of GLT-1 puncta in CEF-treated animals, and showed that it was significantly increased in frontal cortex (by  $49.9 \pm 12.9\%$ ;  $P=0.03$ ), hippocampus (by  $46.2 \pm 10.8\%$ ;  $P=0.03$ ) and striatum (by  $93 \pm 26.1\%$ ;  $P=0.02$ ) compared to controls.

Since 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 up-regulation and mGluR2/3 expression.

*Figure 4 about here*

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 occurs also 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 frontal cortex (by  $61.8 \pm 11.9\%$ ;  $P=0.009$ ), hippocampus ( $41.4 \pm 14.4\%$ ;  $P=0.04$ ) and striatum ( $103.8 \pm 27.2\%$ ;  $P=0.02$ ) (Figure 4).

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

## DISCUSSION

The major result of the present study is that PPI alterations associated to GLT-1 up-regulation 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 fashion 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, where 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 and colleagues (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 (e.g., 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 expression of synaptically-active molecules at non-glutamatergic 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 the present results are concerned, it must be emphasized that, regardless of whether CEF acts on other transmitter systems or not, its effects on PPI are prevented by the GLT-1 antagonist DHK, indicating that GLT-1 up-regulation is the major mediator of the effects of CEF on PPI.

In the present 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 to GLT-1 up-regulation. Although the present findings do not allow

predictions about clinical efficacy of LY379268, it is worth noting that the better assimilated analog LY404039 ameliorates 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 behavioural 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 of glutamatergic transmission induced by NMDA antagonism is more intense than that induced by up-regulation 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 to GLT-1 up-regulation suggests that the reduced activation of mGluR2/3 is a mechanism by which GLT-1 up-regulation 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 to controls. Second, we showed that GLT-1 up-regulation occurred also at the very sites where mGluR2/3 was expressed. To do this, we considered the overlap of fluorescence signals in double-labelled sections as a marker of nearness, a procedure validated by recent electron microscopy studies (Melone, *et al* 2009a). Up-regulation of GLT-1 at sites close to those of mGluR2/3 expression may thus play a crucial role in reducing the amount of glutamate available for mGluR2/3 activation.

GLT-1 up-regulation induced by CEF determines a strong, dihydrokainate 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 play a key role in modulating cognitive flexibility as well as in other complex cognitive functions (Morice, *et al* 2007; Nicholls, *et al* 2008). It is therefore possible that the alterations of LTD observed in animals with GLT-1 up-regulation (Omrani, *et al* 2009) are responsible for the alterations of the PPI described here, and may conceivably contribute to the cognitive impairment observed in schizophrenia patients.

## **DISCLOSURE/CONFLICT OF INTEREST**

The authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

## **ACKNOWLEDGMENTS**

Funded by grants from MIUR and Università Politecnica delle Marche to F.C. We gratefully acknowledge A. Frontini's assistance with confocal microscopy.

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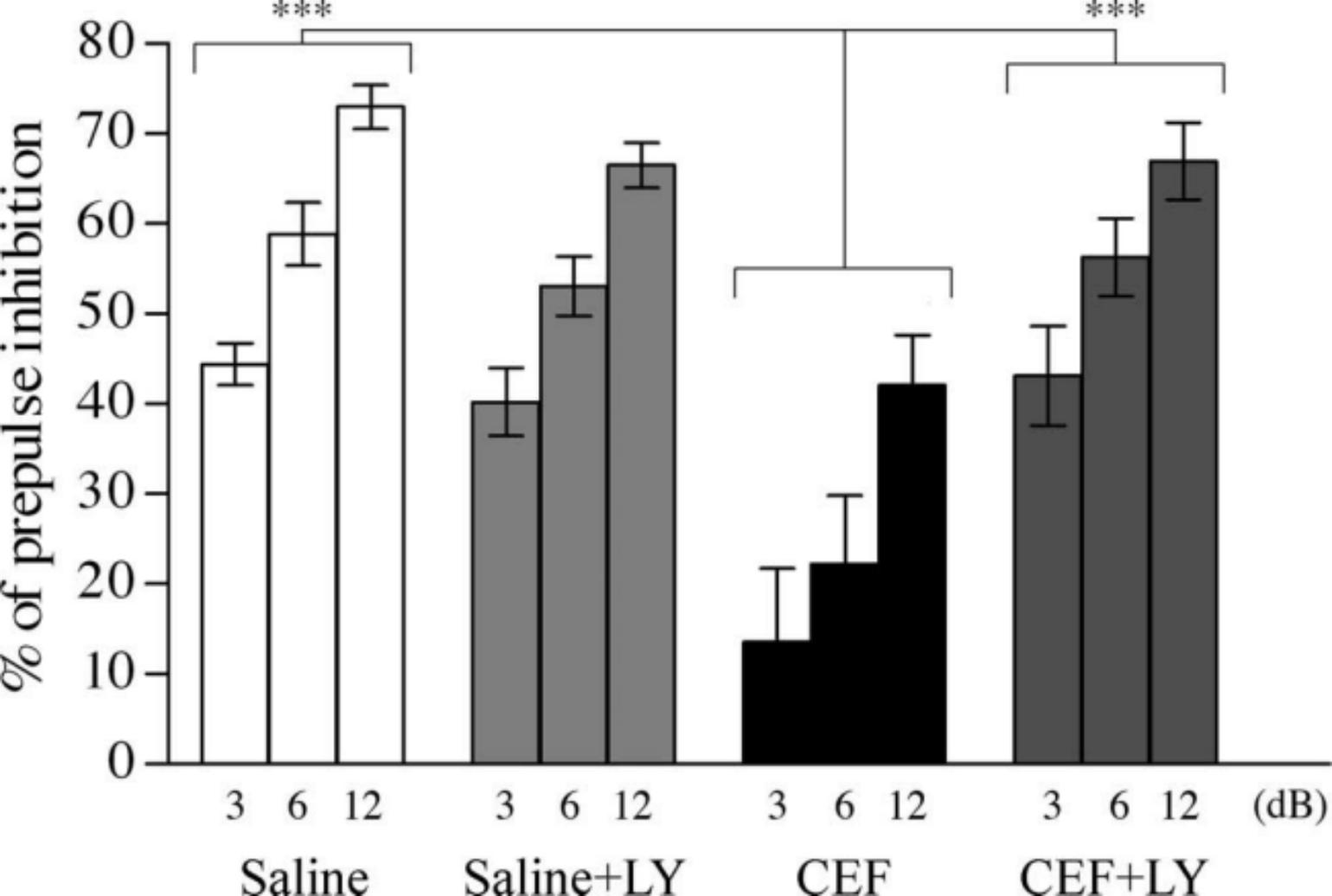
## FIGURE LEGEND

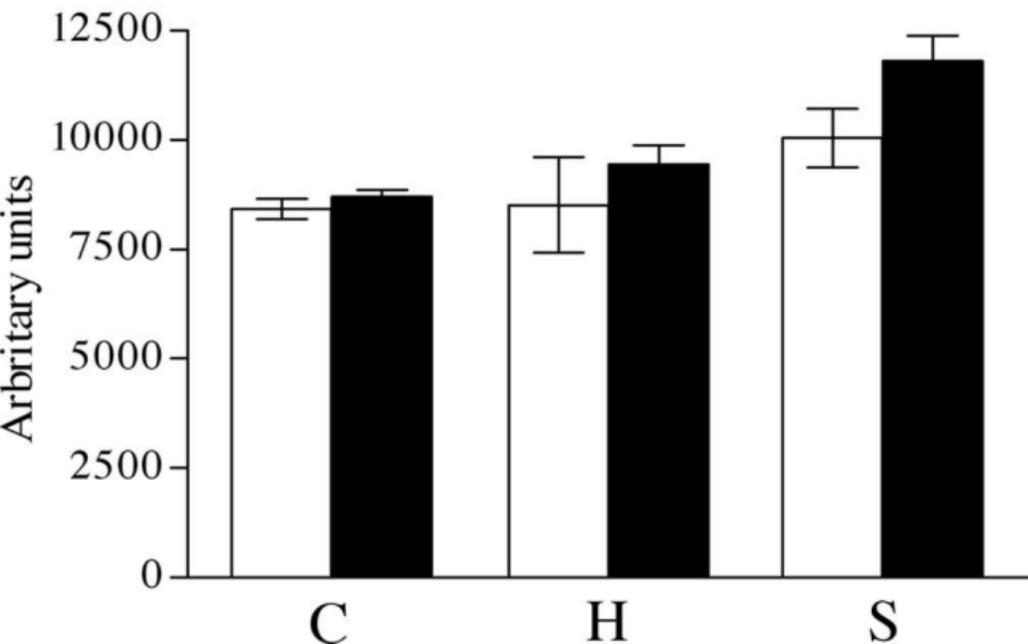
**Figure 1.** Acute administration of the selective mGluR-2/3 agonist LY379268 blocks PPI impairment associated to CEF treatment. Values are %PPI  $\pm$  s.e.m. \*\*\*P<0.001.

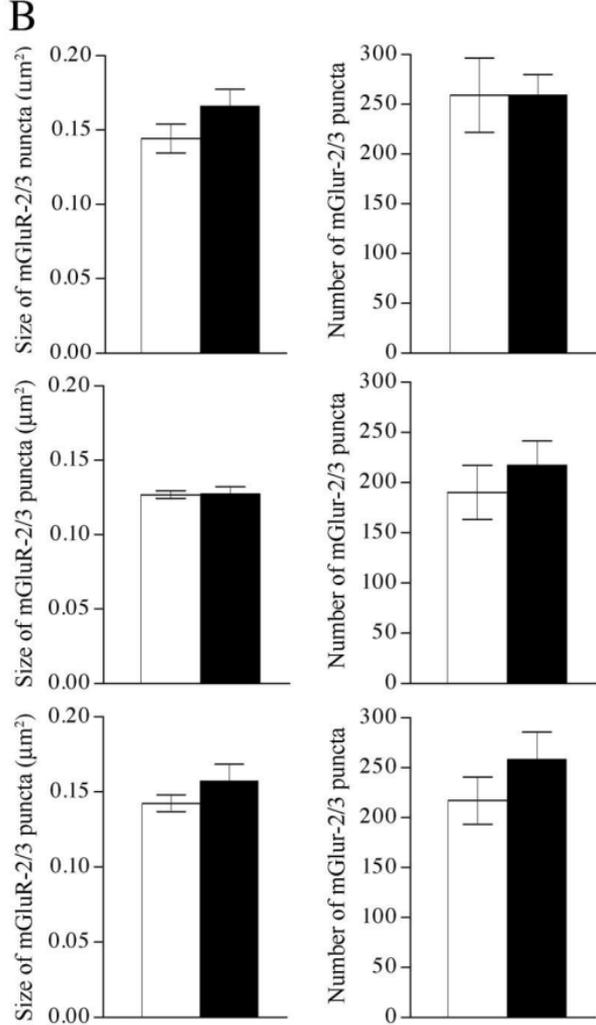
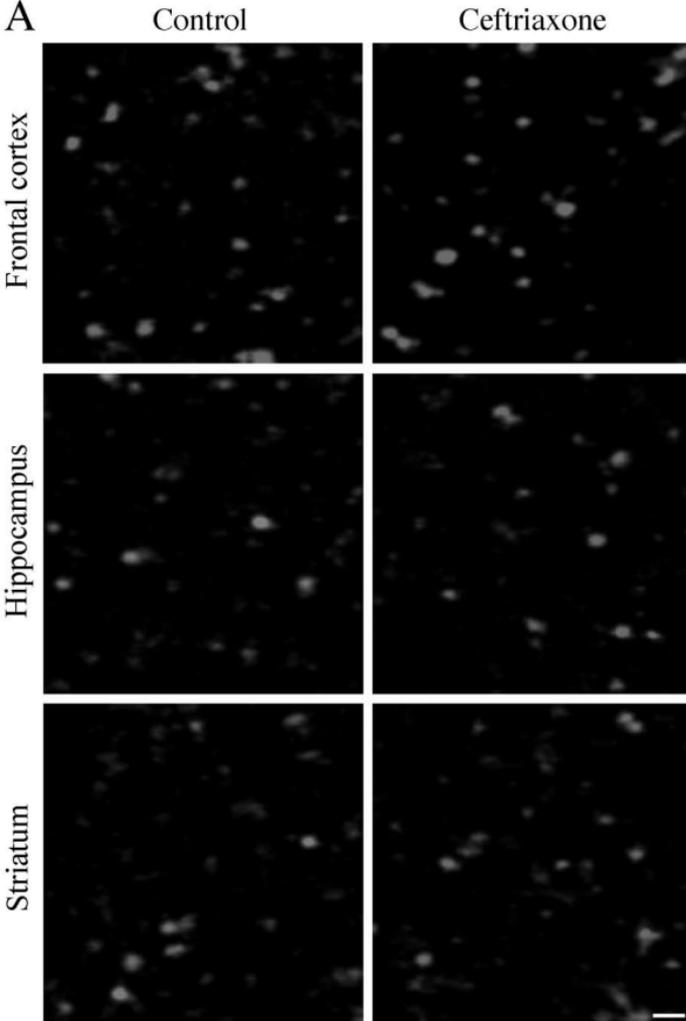
**Figure 2.** CEF treatment is not associated to alterations of mGluR-2/3 expression in forebrain regions regulating PPI. Western blotting studies does not reveal any significant difference between control (*white column*) and CEF-treated (*black column*) rats. C (frontal cortex); H (hippocampus); S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

**Figure 3.** CEF treatment does not affect the distribution of mGluR-2/3 in frontal cortex, hippocampus and striatum. **A**, mGluR2/3 immunoreactive puncta in control and CEF-treated animals. Bar: 0.8  $\mu$ m. **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  $\pm$  s.e.m.

**Figure 4.** GLT-1 up-regulation occurs also in the vicinity of sites of mGluR2/3 expression in 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  $\mu$ m in large frames and 0.4  $\mu$ m 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  $\pm$  s.e.m. \*P<0.05; \*\*P<0.01.





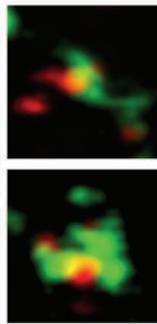
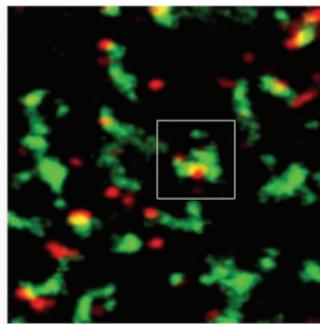
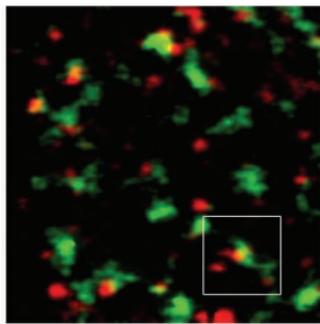


**A**

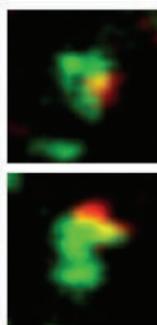
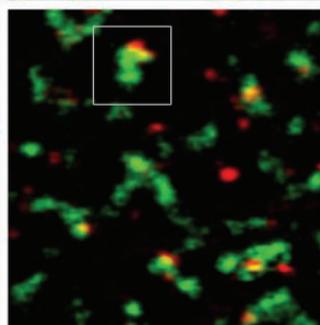
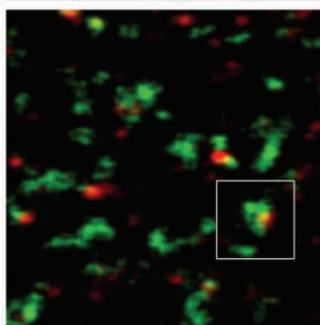
Control

Ceftriaxone

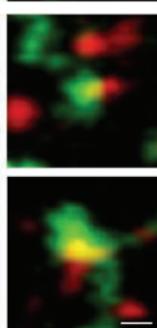
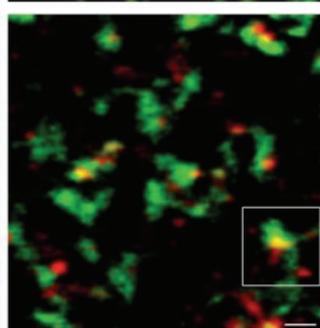
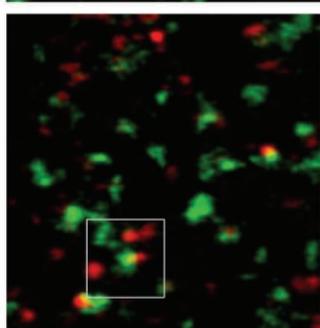
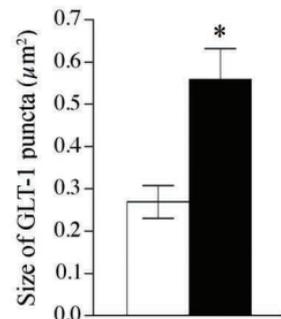
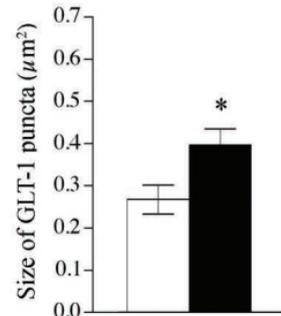
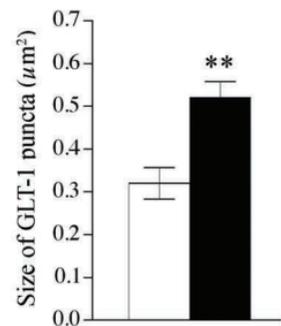
Frontal cortex



Hippocampus



Striatum

**B**

**Table 1.** Startle reflex magnitude and habituation (mean±SEM) of experimental groups.

<b>Group</b>	<b>Startle magnitude</b> second trial block	<b>Habituation</b>	
		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