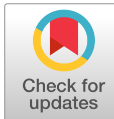

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The positive allosteric modulator at mGlu2 receptors, LY487379, reverses the effects of chronic stress-induced behavioral maladaptation and synaptic dysfunction in the adulthood

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Chronic stress induces maladaptive neural responses in several brain areas including hippocampus (McEwen et al., 2016). It has been demonstrated that chronic stress exposure induced a down-regulation of the putative presynaptic type 2 metabotropic glutamate (mGlu2) receptors (Nasca et al., 2015), which would reduce the negative feedback role exerted by these receptors. The reduced availability of these receptors would enhance glutamate overflow in the hippocampus, supporting the hypothesis that hippocampal glutamatergic neurotransmission plays a key etiopathological determinant in stress-induced neuropsychiatric disorders. Since modulation of glutamatergic neurotransmission has been shown to represent an interesting pharmacological tool to treat

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psychiatric disorders (Peterlik et al., 2016; Matrisciano et al., 2016), in the present study we have investigated the effects of the mGlu2 receptor positive allosteric modulator (PAM) LY487379. The rational bases of our study were: i) chronic restraint stress (CRS) application in C57/BALB6 mouse induced a loss of resilience at the behavioral, biochemical and electrophysiological level (Nasca et al., 2015); ii) a superimposed familiar stressor (restraint) but not unfamiliar (i.e., forced swim stress) completely reversed the effects of CRS (Nasca et al., 2015). Using the CRS model, in the present study we have investigated the effects of LY487379, an mGlu2 PAM, as well as a superimposed familiar stressor (acute restraint stress – ARS), on the immobility time at the tail suspension test and electrophysiological profile of glutamatergic transmission in the dentate gyrus (DG).

All procedures were carried out in accordance with the Italian guidelines of animal care (D.Lgs 4/3/2014 n. 26). Male C57/BALB6 (7-wk-old, 25–30 g; Charles River) mice were housed four per cage under controlled conditions (12-h light/dark cycle, 22 °C, food and water ad libitum). The restraint device contained four 0.4-cm air holes and allowed mice to stretch their legs without impairing circulation to the limbs but not to move within the tube. For the chronic stress procedure, mice were placed in the restrainers for 2 h per day for 21 consecutive days (Nasca et al., 2015). Naive age-matched not-stressed animals were used as controls. After 21 d of CRS, on day 22, additional groups of CRS-exposed mice were: (i) exposed to restraint stress (CRS+ARS); (ii) administered with the selective allosteric potentiator of mGlu2 receptor LY487379 [2,2,2-Trifluoro-*N*-[4-(2-methoxyphenoxy)phenyl]-*N*-(3-pyridinylmethyl) ethanesulfonamide] (CRS+PAM) (Fig. 1A). Tail suspension test (TST) was performed as previously described (Nasca et al., 2015). Briefly, 24 h after the last CRS session or 2 h after the ARS exposure, mice were suspended 50 cm above the floor by adhesive tape (approximately 1 cm from the tip of the tail). Immobility was defined as the absence of movement and the total duration of immobility during a 6-min test were recorded. An additional experimental CRS group of mice was subjected to TST 20 min after subcutaneous (s.c.) administration of LY487379 (Tocris) (30 mg/kg, dissolved in peanut oil) (CRS+PAM). The dose of

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LY487379 was chosen on the demonstration that this dosage significantly reduced both amphetamine- and phencyclidine-induced hyperlocomotor activity without affecting basal locomotor activity (Galici et al., 2005). For electrophysiological studies, mice were deeply anesthetized and killed by decapitation 24 h after the last CRS session or 2 h after the ARS exposure (CRS and CRS+ARS, respectively) (Fig.2A). On the other hand, the effect of LY487379 on hippocampal electrophysiological parameters was investigated by adding the PAM to the bath solution. Parasagittal hippocampal slices were obtained as previously described (Nasca et al., 2015). Individual slices were placed in a recording chamber, on the stage of an upright microscope (Nikon, Japan) and submerged in a continuously flowing (3 ml/min) solution at 28 °C (±0.2 °C). Individual neurons were visualized through a 40× water-immersion objective (Nikon, Japan) connected to infrared video microscopy (Hamamatsu, Japan). Borosilicate glass electrodes (3–7 MΩ), pulled with a PP 83 Narishige puller, were filled with a solution containing the following (in mM): CsMeSO3 (115), CsCl (10), CaCl2 (0.45), EGTA (1), Hepes (10), QX-314 (5) Na-GTP (0.3), Mg-ATP (4.0), pH adjusted to 7.3 with CsOH. Whole-cell voltage clamp (at -70 mV holding potential) and current clamp recordings were carried out with a MultiClamp 700B amplifier (Axon Instruments, Foster City, USA), filtered at 1kHz and digitized (10kHz). The spontaneous excitatory postsynaptic currents (sEPSC) were recorded from granule neurons of ventral DG. For paired-pulse ratio (PPR) experiments, paired-pulse stimuli (50 ms inter-pulse interval) were elicited with stimulating electrode placed in the medial perforant pathway. All experiments were performed in presence of GABA antagonist picrotoxin (100 µM). The concentration of LY487379 (30 µM) was selected in agreement with previous electrophysiological studies demonstrating its efficacy in modulating mGlu2 receptors (Schaffhauser et al., 2003; Odagaki et al., 2013).

Twenty-one day CRS resulted in an increased behavioral immobility time at the TST; however, 2 h after a known ARS, immobility time at the TST was decreased in CRS mice. LY487379, which is by itself devoid of any effect in the TST, also reduced the immobility time of CRS-exposed

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mice to levels not different from the control group (Fig. 1B). sEPSC recorded from dentate granule cells from CRS mice and controls revealed an increased frequency in CRS neurons compared to control neurons, whereas no significant difference was found in the amplitude of the same events although a trend toward reduction was observed (Fig.2A). Interestingly, the sEPSC frequency of the CRS group was reversed by ARS exposure (CRS+ARS, Fig. 2A), whereas no difference was observed in the amplitude of the same events (Fig.2A). To investigate the role of mGlu2 in the altered glutamatergic transmission observed, LY487379 (30 μM, 10 min) was added to the superfusion medium of hippocampal slices obtained from CRS animals. Remarkably, LY487379 was able to reverse the increased frequency of sEPSCs in the CRS group (Fig.2A). Next, we studied the probability of neurotransmitter release using a paired pulse ratio (PPR) protocol. We observed a reduction of PPR in CRS group (Fig.2B) compared with control neurons, which is known to be associated with increased probability release (Fioravante et al., 2011). Acute restraint stress application, as well as application of LY487379 in the superfusion medium, restored PPR to control level (Fig. 2B). Notably, bath application of LY487379 in control neurons did not produce *per se* any change both in sEPSCs and PPR (Fig.2A-B).

Although we did not investigate the biochemical mechanism by which a chronic restraint stress application induces hippocampal synaptic dysfunction and behavioral maladaptation, the present study shows that administration of the positive allosteric modulator at mGlu2 receptors mimics the effect of a single familiar stress.

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Figure 1: Effects of ARS and LY487379 on CRS-induced behavior

A) Schematic representation showing the time course of the stress challenge procedure and behavioral/electrophysiological analyses in the different experimental groups analyzed. B) Histograms (mean ± SEM) of Tail Suspension Test, * $p < 0.05$, ** $p<0.01$, (minimum 5 mice for group, F=6,780, $p<0.001$; ANOVA followed by Tukey-Kramer Multiple Comparisons Test).

Figure 2: Chronic stress increases the probability release of glutamate in the dentate gyrus

A) Histograms (mean ± SEM) show the sEPSCs amplitude (left) and frequency (right) recorded from neurons of different groups. On top of each plot representative traces are shown. B) Histograms (mean ± SEM) show the paired-pulse ratio (PPR; right) recorded from neurons of different groups. Data are presented as mean ± SEM, * $p < 0.05$, ** $p<0.01$, *** $p<0.001$, (minimum 7 neurons for group, F=14,018, $p<0.001$; ANOVA followed by Tukey-Kramer Multiple Comparisons Test).

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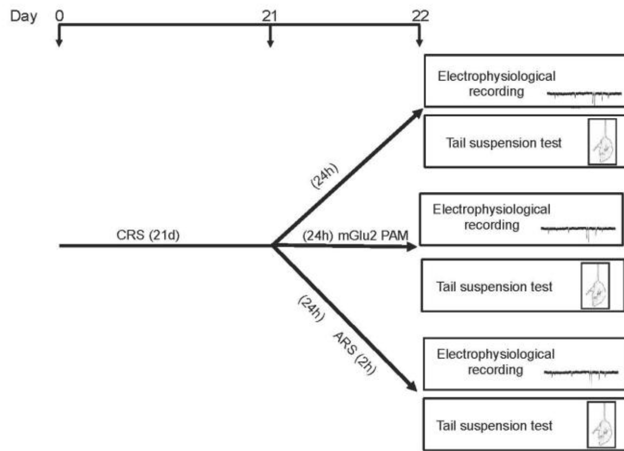

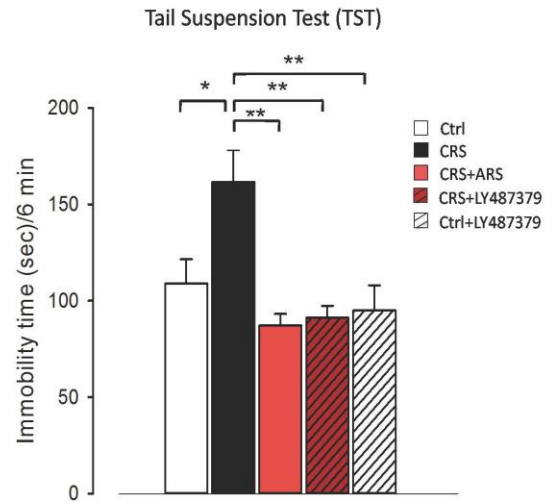

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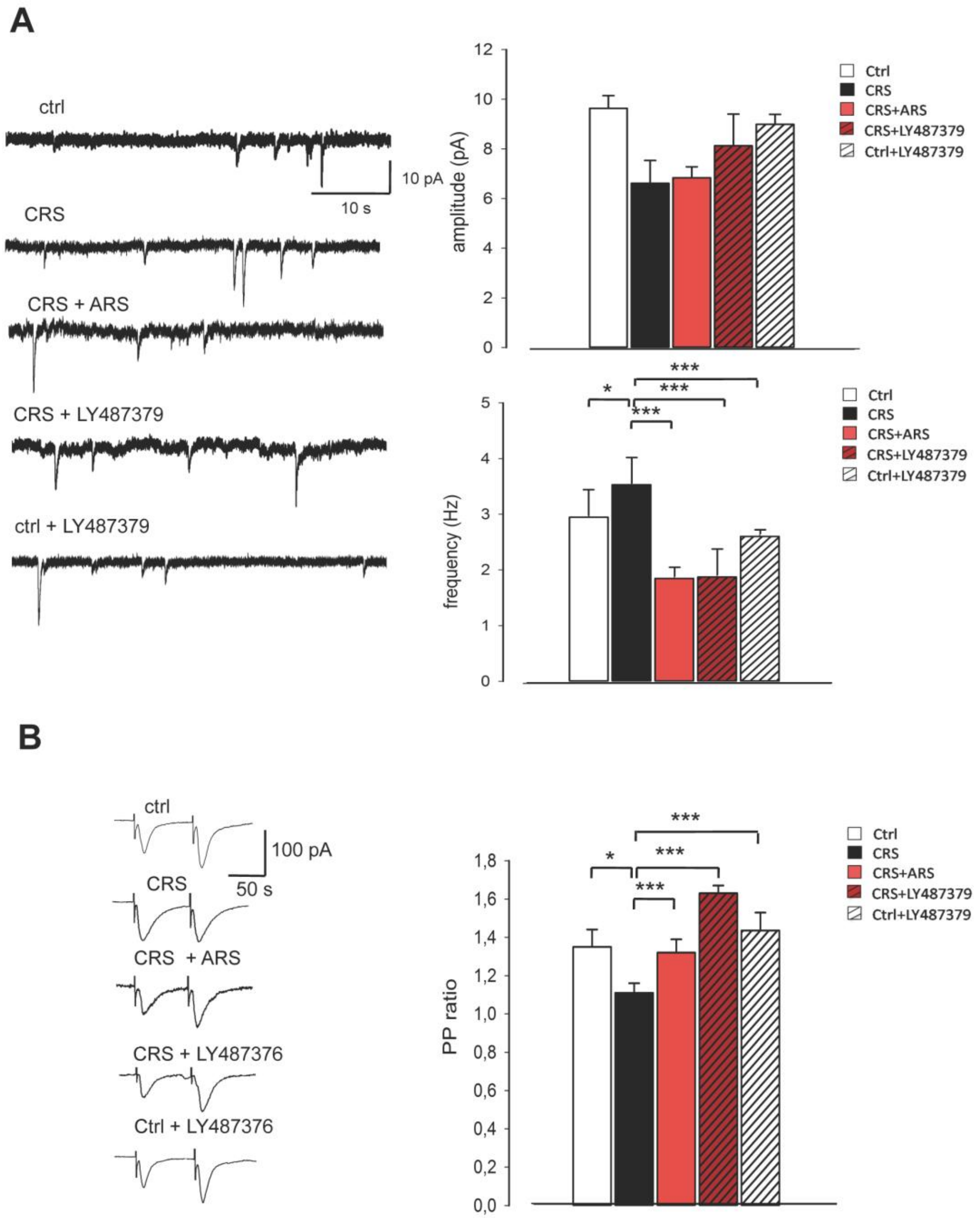

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