**Supplementary Information**

**Materials and methods**

All experimental procedures were carried out according to the National Institutes of Health, The Rockefeller University Institutional Animal Care and Use Committee guidelines and to the European (86/609/EEC) and Italian (D.Lgs 116/92) guidelines of animal care. All efforts were made to minimize animal suffering.

For the purpose of exploring the possible mechanistic link of stressors to an epigenetic control of the glutamatergic synapse (fig.5 in the text), a second series of studies was carried out focusing on the effects of ARS as group since the overall effect is sufficiently strong to be significant even without sorting into what we designed as LS and HS mice. The scatter plots for the time spent in the light chamber at the light-dark test for vehicle-, spironolactone- and Ru486-treated mice show the ranges of values within each group (fig. SI 1).

**Coat-state rating scale**

The coat-state evaluation was based on a modified version of the fur scale (45). The coat-state assessment was carried out by two blind observers before the start of each behavioral test session during the entire time course of the CUS. At each time point, the red eye conjunctiva and the brightness and smoothness of the coat in the back and abdomen was recorded assigning to each animal a score from 1 to 4 based on the presence of red conjunctiva of eyes and the percentage of the coat brightness and coat stains. The resulting scores of each animal were averaged over the four weeks of CUS. The coat-state scores were assigned as follow (Table SI 1):

- The coat is completely shiny (100%) and smooth with no patches in both the back and the abdomen. Mice have clear conjunctiva. Score=1
- Mice show a largely shiny coat (>80%) and a few spiky patches (<10%) in either the back or the abdomen. Mice have clear conjunctiva. Score=2
- Mice show a slightly shiny coat (<50%) and some fur loss (<25%) in either the back or the abdomen. Mice have clear conjunctiva. Score=3

- Mice show an opaque coat with a low brightness (<10%) and spread fur loss (<40%) in either the back or the abdomen. Mice show red eye conjunctiva. Score=4

**Sucrose preference test**

The sucrose preference test has been performed has previously described (26). The choice of using the 24hrs model is based on our previous experience since we noticed in the past that, after a chronic stress paradigm, the anhedonia-like behavior is well evident after 12 and 24 hrs with a trend toward a decrease after 48hrs followed by again a marked increase after 72hrs.

**Gene expression analysis by qPCR**

Tissue samples were weighed before homogenization and about 60–90mg of hippocampal tissue or 60mg of prefrontal cortex tissue was lysed. Total RNA was extracted using Qiazol reagent and RNeasy mini Kit (Qiagen, US), according to the manufacturer's instructions. RNA quality was checked using the 2100 Bioanalyzer (Agilent Technologies, US) and quantified spectrophotometrically. The acceptance criteria for RNA quality was a 260/280 ratio ≥ 1.80 and ≤ 2.20. 2µg of total RNA was then used for cDNA synthesis according to the manufacturer's instructions of the high capacity cDNA reverse transcription kit (Life Technologies, US). The reverse transcribed reaction was carried out according the follow condition steps: 25°C/10 min, 37°C/120 min and 85°C/5 min. qPCR was performed according to the protocols of the manufacturer (Life Technologies, US), using TaqMan Universal PCR Master Mix and gene-specific primers, synthetized by Applied Biosystem Company (Life Technologies, US). GAPDH was used as a reference control. The following PCR conditions were used: an initial incubation of 50°C for 2 min and a denaturation step of 95°C for 10 min, followed by 40 cycles of 95°C/15 s and 60°C/1 min. All reactions were performed in triplicate. The threshold cycle (CT), which correlates inversely with the levels of target mRNA, was measured as the number of cycles at which the reporter fluorescence emission exceeds the preset threshold level. The amplified transcripts were quantified using the comparative CT method (46) with the formula for relative fold change $=2^{\Delta\Delta CT}$.

**Western Blot analysis**

Hippocampal and prefrontal cortex tissues were dissected and stored at −80 °C. On the day of the experiment, tissues were homogenized at 4 °C on 0.1% SDS-lysis buffer containing protease inhibitors (1 mM PMSF, 1 μg/mL aprotinin, and 1 μg/mL of leupeptin) and phosphatase inhibitors (1mMNaF, 1 mM Na3VO4, and 1 mM glycerol-2-phosphate) with a motordriven Teflon-glass homogenizer (300 × g). Homogenates were centrifuged at 17,000 × g at 4 °C for 20 min, and the supernatant was used for protein determinations. Samples containing 30 μg proteins were resuspended in SDS-bromophenol blue reducing buffer containing 40 mM DTT. Proteins were separated by 8% (wt/vol) SDS-polyacrylamide gel and then electroblotted on nitrocellulose membranes (Whatman). Filters were blocked overnight at 4 °C in TTBS buffer (100 mM Tris·HCl, 0.9% NaCl, 0.1% Tween 20, pH 7.4) containing 5% (wt/vol) non-fat dry milk, and then incubated overnight at 4 °C with the following primary antibodies: mouse anti-mGlu2 receptor (1 μg/mL; Abcam, US), and mouse anti-β-actin (1:5,000; Sigma Aldrich, US). Blots were washed three times with TTBS buffer and then incubated for 1 h with secondary antibodies (1:5,000; peroxidase-coupled anti-rabbit or anti-mouse; EMD Millipore, US). Immunostaining was revealed by enhanced chemiluminescence (Amersham Biosciences).

Data provided by the manufacturer shows that mGlu2 antibody has a high specificity in recognizing the metabotrophic glutamate receptor 2, but not metabotrophic glutamate receptor 3. The antibody was tested by blocking with their respective immunizing peptide to confirm its specificity. Immunoreactivity was also confirmed using positive control lysate in western blots.

**Chromatin immunoprecipitation**

The ChIP assay has been performed as previously described (16) with some modifications. Briefly, genomic DNA was extracted from half hippocampus of stressed mice and controls. Tissue was incubated with 500 μL of PBS 1× containing 1% formaldehyde at 37 °C for 10 min, supplemented with a mixture of protease inhibitors. After being washed three times with cold PBS 1×, tissue was homogenized in 100 μL of PBS 1x and then, to obtain consistent chromatin fragmentation, the lysates were sonicated in 200 μL of 1x SDS shearing buffer (supplied by the Covaris kit, Life technologies)

in the Covaris sonicator. The ChIP procedure was carried out by following the protocol provided by the ChIP assay kit (Ez-Magna ChIP, Upstate Chemicals, Millipore, US). An aliquot (10%) of the sonicated lysate was used for the quantification of the total amount of DNA in sample extracts before immunoprecipitation. The remaining solution was incubated overnight at 4°C with anti–acetyl-K27-histone H3 antibodies (0.05 μg/mL; Abcam, US). The immunoprecipitated DNA was released from the antibody complex by proteinase-K digestion. After phenol-chloroform extraction and ethanol precipitation, the DNA pellet was resuspended in 20 μL of diethyl pyrocarbonate (DEPC) water. The extract was used for detection and quantification of the mGlu2 and mGlu3 receptor gene promoter and for the house-keeping b-actin gene. Chromatin from formaldehyde-crosslinked tisses was also electrophoresed through a 2% agarose gel that shows chromatin fragments with a length between 200bp and 1000bp.

**References**

45. Mineur YS, Belzung C, Crusio WE. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. *Behav. Brain Resear*. 2006; **175**, 43–50.

46. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). Method. *Methods* 2001; **25,** 402-8.

26. Nasca C, Orlando R, Marchiafava M, Boldrini P, Battaglia G, Scaccianoce S et al. Exposure to predator odor and resulting anxiety enhances the expression of the α2 δ subunit of voltage-sensitive calcium channels in the amygdala. J Neurochem. 2013; 125, 649-56.

16. Nasca C, Xenos D, Barone Y, Caruso A, Scaccianoce S, Matrisciano F et al. L-acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. Proc Natl Acad Sci U S A 2013; 110, 4804-9.

## Figure Legends

### Figure SI 1

Scatter plots show the range of individual variation in the time spent in the light chamber at the light-dark test for vehicle-, spironolactone- and RU486-treated mice with (ARS) and without (Ctrl) acute restraint stress. In spite of the range of individual variation within each group, the overall effect of stress and the ability of spironolactone, but not RU486, to block the stress effect are sufficiently strong to be significant (see legend to Fig 5e).

### Table SI 1

Schema of the evaluation criteria for the assignment of the score at the coat-state rating scale

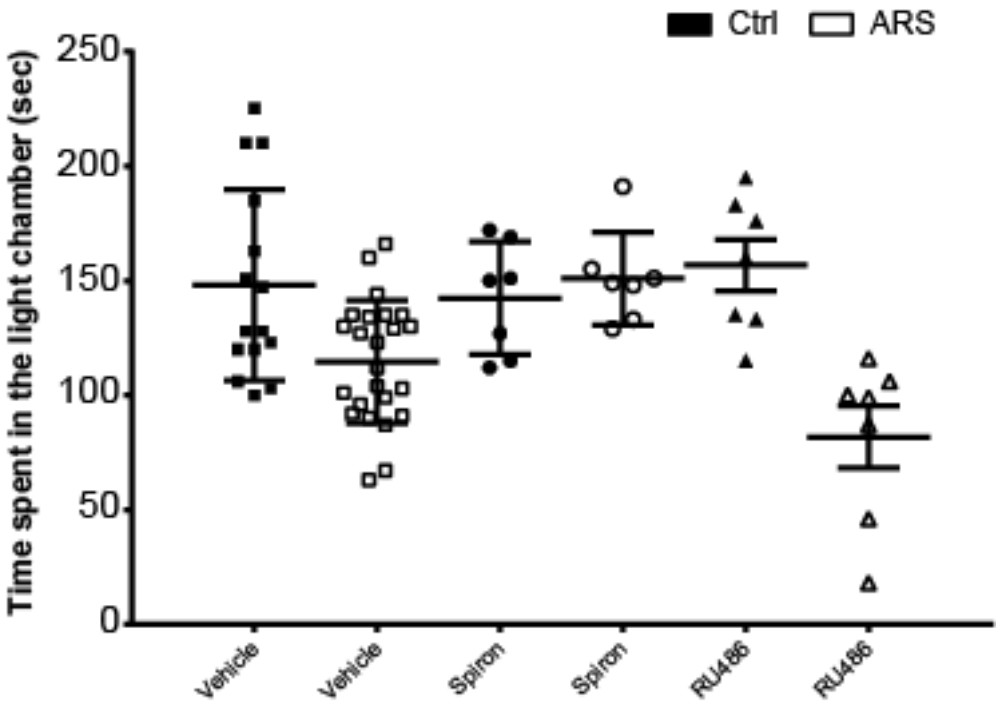
Ctrl
ARS
Time spent in the light chamber (sec)
250
200
150
100
50
0
Vehicle
Vehicle
Spiron
Spiron
RU486
RU486

**Figure SI 1**

| Body area | Measure | Scores | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| Coat in the back | Brightness (%) | Shiny (100%) | Mostly shiny (>80%) | Slightly shiny (<50%) | Opaque (<10%) |
| | Smoothness (% patches) | No patches | Spiky patches (<10%) | Slight fur loss (<25%) | Large fur loss (<40%) |
| Coat in the abdomen | Brightness (%) | Shiny (100%) | Mostly shiny (>80%) | Slightly shiny (<50%) | Opaque (<10%) |
| | Smoothness (% patches) | No patches | Spiky patches (<10%) | Slight fur loss (<25%) | Large fur loss (<40%) |
| Eyes | Red conjunctiva | No | No | No | Yes |

**Table SI 1**