SUPPLEMENTARY RESULTS

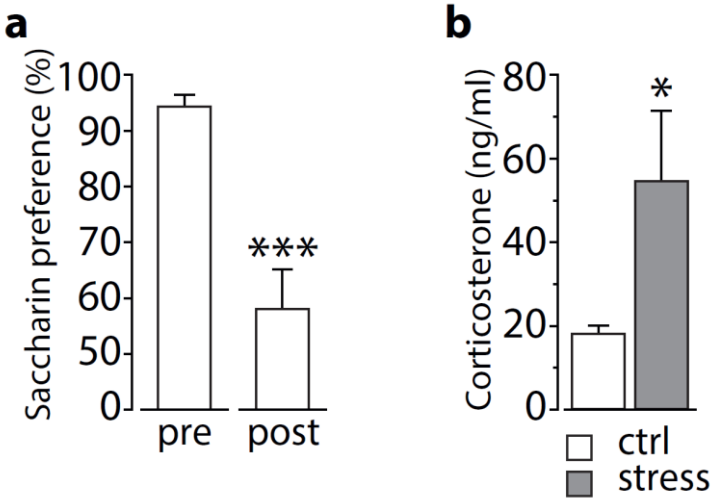

Figure S1. Effects of the exposure to two weeks of stressful condition, before treatment, on anhedonic response and corticosterone levels. (**a**) Saccharin preference before and after the two weeks of stress. Preference for the sweet solution was significantly reduced by stress [$F(1,12) = 17.897$, $p = 0.0012$]. * = $p = 0.0012$. $n = 13$ (**b**) Corticosterone levels in mice kept for two weeks either in the Intellicage in control condition (ctrl) or in the stressful condition (stress). Stress significantly increased corticosterone levels ($U = 5.000$, $n1 = 5$, $n2 = 8$, $p = 0.0281$). Data are mean ± S.E.M.

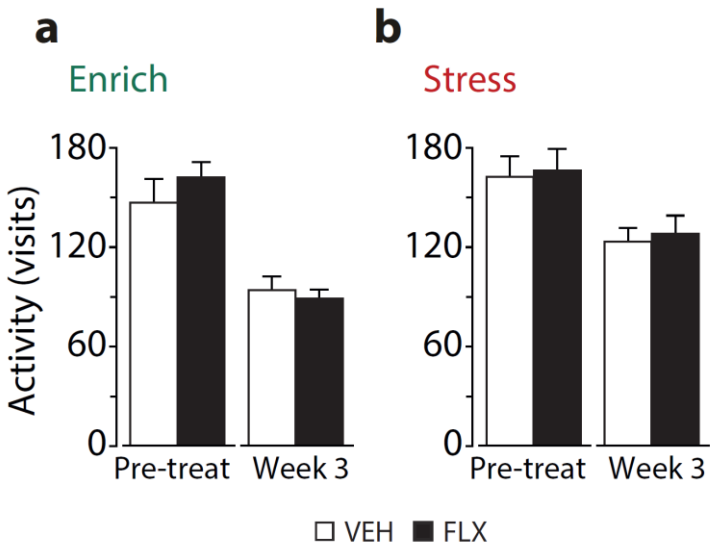

Figure S2. Activity levels in the Intellicage. In both the (**a**) enriched and the (**b**) stressful condition, VEH and FLX mice showed similar activity levels (number of visits). Enrich: VEH, n= 9; FLX, n= 10. Stress, n = 10 in all groups. Data are mean ± S.E.M. * indicate $p < 0.05$ vs respective vehicle group.

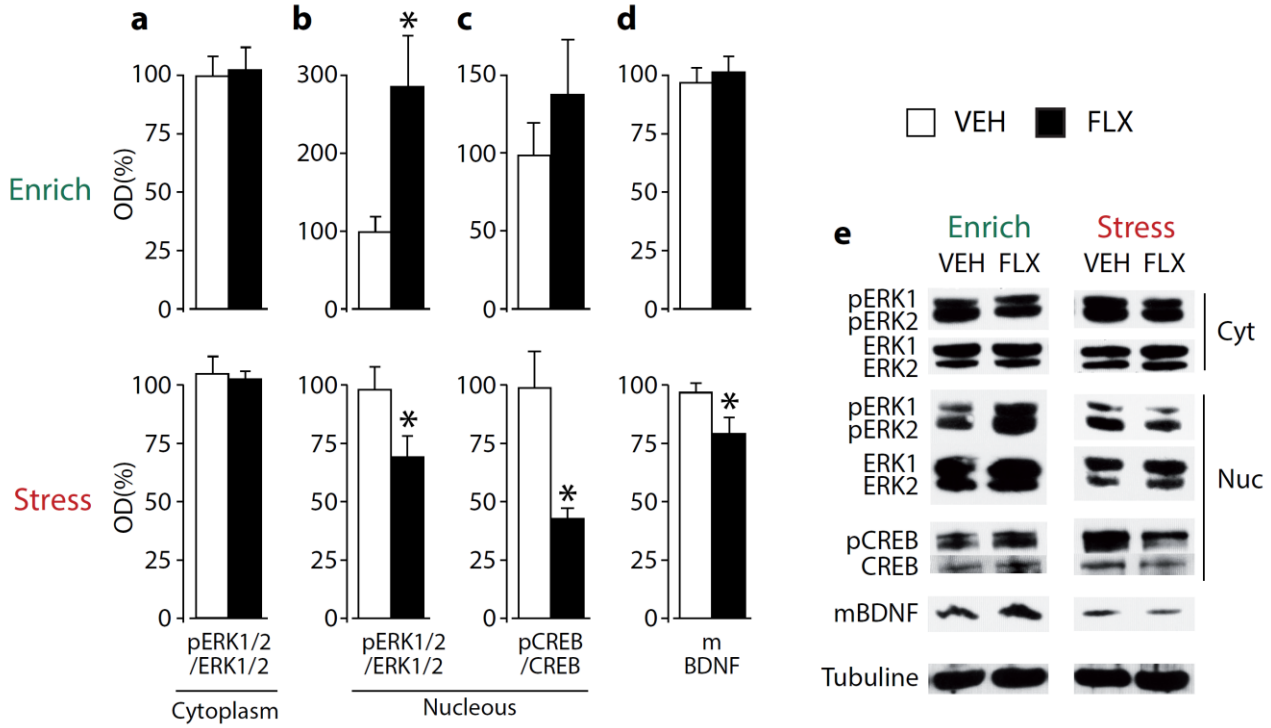

Figure S3. Fluoxetine treatment affects antidepressant signaling pathways according to the quality of the environment in the medial prefrontal cortex. While not affected (**a**) in the cytoplasmic fraction, (**b**) pERK 1/2 – total ERK1/2 ratio was significantly increased in the enriched condition (t= 2.466; p= 0.0431) and significantly reduced in the stressful condition (t= -2.163; p= 0.0483) in the nuclear fraction. In addition, fluoxetine treatment reduced (**c**) CREB activity in the nuclear fraction (t= -3.0761; p=0.02759) and (**d**) the levels of the mature form of BDNF in the total extract (t= -3.076; p= 0.0430). (**e**) Representative Western blots are shown. Total CREB and proBDNF levels were not affected by a chronic treatment with fluoxetine irrespective to the environmental conditions. n= 8 in all groups. Data are mean ± S.E.M. * indicate $p < 0.05$ vs respective vehicle group.

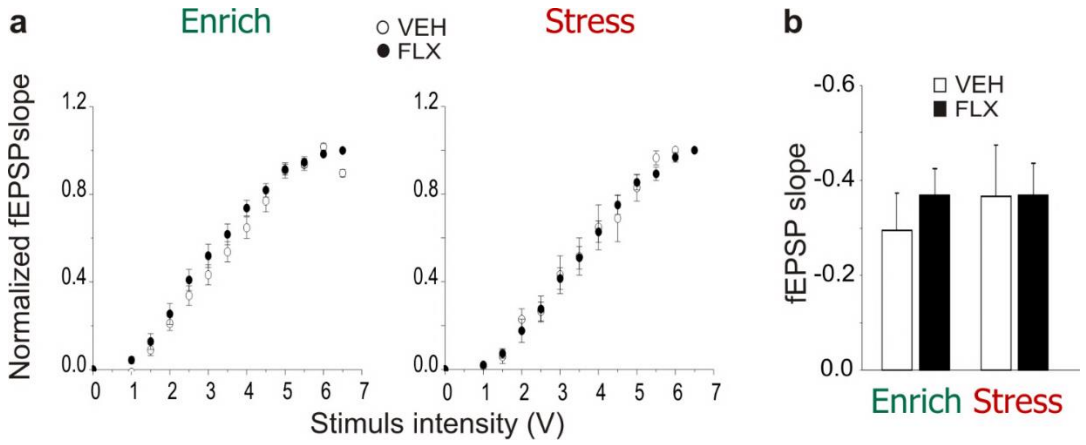

Figure S4. Schaffer collateral-hippocampal CA1 basal responses are similar in FLX and VEH mice both in the enriched and the stressful condition. (**a**) Normalized stimulus intensity response curves (I-O). fEPSP in the CA1 area was increased in slope in an intensity-dependent manner. FLX does not affect I-O both in the enriched and stressful condition (**b**) Population spikes evoked at same fEPSP size. The fEPSP slopes of the first observation of a population spike are similar in all groups. Enrich: VEH, n= 6/2; FLX, n= 11/3. Stress, VEH, n= 5/3; FLX, n= 10/3. Data are mean ± S.E.M.

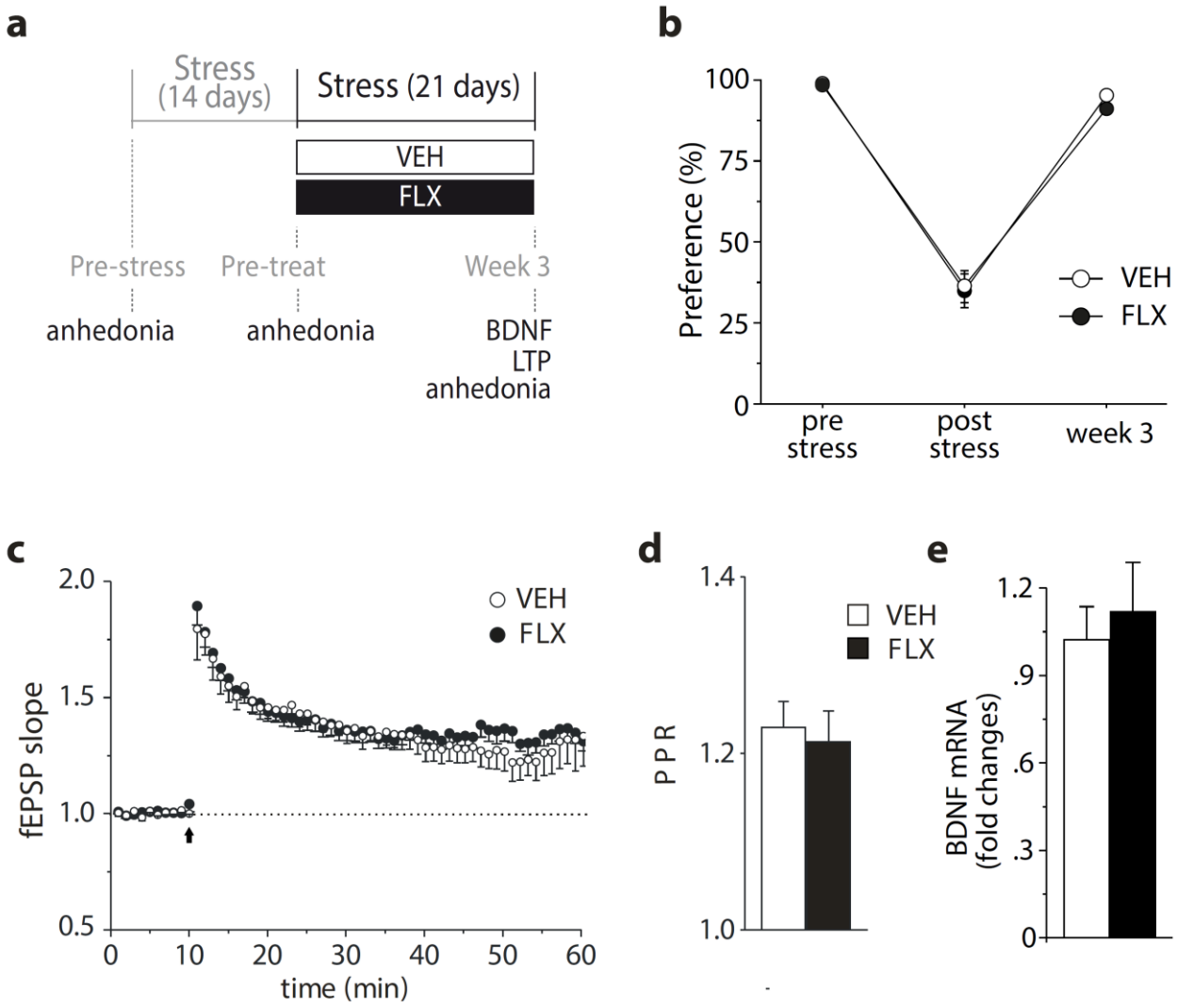

Figure S5. Standard condition. Mice exposed to 2 weeks of stress and afterwards administered with either vehicle or fluoxetine in a standard condition. (**a**) Experimental design of the fluoxetine treatment in the standard condition (**a**) Saccharin preference before and after two weeks of stress and after the administration with either VEH of FLX (week 3). Preference for the sweet solution was similar in the two experimental groups (**b**) CA1 plasticity. In the stress-standard condition, FLX and VEH mice developed a similar LTP (single 100 Hz burst; VEH: n = 9/5; FLX: n = 10/3). Arrows indicate time of application of HFS. (**c**) Fluoxetine did not modify PPR. n=11/3 in all groups. (**d**) Real-Time PCR analysis revealed that BDNF levels were not affected by fluoxetine. n = 6 in all groups. Data are mean ± S.E.M.

MATERIALS AND METHODS

Animals

All experiments were conducted in conformity with European Communities Council Directive 2010/63 and the Italian Decreto L.vo 116/92 and the Veterinary Office of the Canton of Zurich. C57BL/6 male mice 12–15 week old were used and kept under 12-hour light-dark cycle at 22–25°C. Animals were housed in the Intellicage system (TSE-system, NewBehavior AG, Zürich, Switzerland), which is an apparatus for automatic monitoring of mouse behavior. This system is able to score the behavior of each individual living in a social group since each one is identified by a subcutaneous transponder. The Intellicage system consists of a large acrylic cage (20.5 cm high, 58 cm × 40 cm at the top and 55 cm × 37.5 cm at the base, Model 2000 Tecniplast, Buguggiate, VA, Italy), with 4 walls separating each corner from the center so that they form 4 identical chambers to which mice have access by entering a front hole (for a detailed description of the system see[1-3]). The Intellicage system collects data about (i) number and duration of visits in the four corners (activity), (ii) number, duration and side (right or left) of nosepokes and (iii) number, duration and side (right or left) of licks.

Housing condition

For the entire duration of each experiment, animals were housed in the Intellicage, balancing experimental group composition. Five days before being moved to the Intellicage, each animal was injected with a subcutaneous transponder (T-IS 8010 FDX-B; Datamars SA, Switzerland). Food was freely available. The animals have been gradually habituated to the Intellicage environment during a 14-days period. During such period, they were habituated also to the 0.1% of saccharin solution. On the last two days of the habituation period, baseline saccharin preference was measured.

Enriched condition

The Intellicage consists in an enrichment environment because mice are socially housed and exposed to Plexiglas shelters of different colors and shapes (four red transparent Tecniplast plastic nest boxes and four white opaque boxes), and to tissue paper. New paper was provided every 5 days and the plastic shelters were cleaned every week.

Stressful condition

Stress condition consisted in exposing mice each day to a different stressful procedure, randomly chosen among social stress or other stressful procedures provided by the Intellicage. Exposing mice to different stressful procedures was used to prevent habituation to each of these. The stress procedures used are: *Social stress*: moving animals from one Intellicage to the other, creating new social groups and thus forcing mice to re-establish the social hierarchy; *Short open door*: door to access water or saccharin remains open for only 1.5 seconds; *Open door 25%*: door opens only following 25% of nosepokes; *Air puff*: when the mouse enters the corner, it has a 20% chance to receive an air puff. In addition, in the stressful condition, no shelter or tissue paper was provided.

Standard condition

Standard condition consisted in housing mice in standard laboratory condition, i.e. two individuals per cage. Each cage was 33 x 13 x 14 cm Plexiglas box with metal tops and sawdust as bedding. Pellet food and tap water were *ad libitum*.

Behavioral tests

Liking-type anhedonia - Saccharin preference

To assess liking-type anhedonia we measured saccharin preference. In each corner of the Intellicage were present two bottles, one containing tap water and the other containing 0.1 % saccharin solution; both were freely available 24/24 h. Water and saccharin solution were substituted every day. The position of water and saccharin in each corner was counterbalanced across the four corners. Saccharin preference was determined as follows: (saccharin solution consumed/ saccharin solution consumed +water consumed) × 100. Enrich: VEH, n = 23; FLX, n = 22. Stress: VEH, n = 23; FLX, n = 24.

Wanting-type anhedonia - Progressive Ratio

To assess wanting-type anhedonia, i.e. the drive for obtaining a reward, we used the Progressive Ratio reinforcement schedule that utilizes a multiplicative increase in the number of responses (i.e. nosepokes) required to dispense a unit of reinforcer (i.e. saccharin). In particular, while water was always accessible after one nosepoke, saccharin solution was accessible only when a specific number of nosepokes is performed. Such number increases progressively after each series of 8 visits according to the following: 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24. This test was run immediately before and 1 and 3 weeks after the beginning of the fluoxetine treatment. Each test session lasted 24 hrs. During the habituation period and before running the tests, mice were trained 5 times, each one lasting 24 hrs. Enrich: n = 8 in all groups; Stress: VEH, n = 10, FLX, n = 11.

Cognitive Bias

Experimental subject were trained at discriminating between two different light color conditions (blue and yellow) which are randomly presented when the subject enters into the corner. A nosepoke performed in the blue (or yellow) condition leads to a reward (i.e., door opens) while a nosepoke in the yellow (or blue) condition leads to a punishment (air puff). After mice have learnt to discriminate between lights (significant preference for the rewarded stimulus over random choice, i.e. 50%), they are exposed to ambiguous stimuli (simultaneous presentation of blue and yellow light). These stimuli are not reinforced and randomly interspersed with a probability of 0.05 between the reinforced light color stimuli. Nosepoking during the presentation of the ambiguous stimulus was considered as an "optimistic" bias, while refraining was considered as a "pessimistic" bias. Each test session lasted 24 hrs. In order to train mice to discriminate between light color conditions, they were trained 3 times (24 hrs each) during the habituation period. Enrich: VEH, n = 6, FLX, n = 5. Stress: VEH, n = 8, FLX, n = 5.

Treatment

Fluoxetine (Fluoxetine HCl, SantaCruz, USA) was dissolved in water and in saccharin solution and delivered ad libitum in the drinking bottles for 3 weeks. Compared to injection, this administration method allows to avoid the stress due to the manipulation. The solutions were prepared according to the mouse average weight and daily water consumption in order to provide an average daily intake of 30 mg/kg. According to previous studies, such intake allowed to reach an effective fluoxetine serum level around 150 ng/ml[4]. Bottles were wrapped in tin foil to protect the substance from light.

Treatment in the enriched condition

Mice underwent a 14-days stress period consisting in random exposure to different stressful conditions (see above, Stressful condition). Afterwards, mice were exposed for 21 days to the enriched condition while treated with fluoxetine or vehicle.

Treatment in the stressful condition

Mice underwent a 14-days stress period (as above described). Afterwards, they were exposed to a second stress period (21 days) while treated with FLX or vehicle.

Treatment in the standard condition

Mice underwent a 14-days stress period (as above described). Afterwards, they were housed in standard laboratory condition (21 days) while treated with FLX or vehicle.

Histology

Immediately following the behavioural experiments all animals were sacrificed by an overdose of pentobarbital (50mg/kg) and perfused transcardially with cold phosphate buffered saline (PBS) followed by cold 1% paraformaldehyde (PFA) with 15% saturated picric acid. Brains were dissected rapidly and the hippocampi removed. Isolated hippocampi were gently straightened and fixed with 4% PFA in grooves (25 x 4 x 8 mm) carved into PVC blocks. Hippocampi were post fixed in this straightened position for 3 hours, PFA was exchanged every hour. n= 8 in all groups.

Matrix embedding of straightened hippocampi

Randomly selected left or right hippocampi were processed either for immunohistochemistry or for histological cell counting.

For immunohistochemistry, hippocampi were embedded in a gelatine-albumin protein matrix following the protocol developed by Smiley and Bleiwas [5]. In brief, fixed tissue was first cryoprotected by immersion in glycerol. A base layer of protein composed of gelatine-egg-albumin with the cross-linking reagents glutaraldehyde and lysine was prepared in molds (25 x 20 x 14 mm). Hippocampi were positioned in parallel on the slightly hardened base layer (5-6 hippocampi per mold) and gently pushed below the surface. After 10 minutes, the entire mold was filled up with freshly prepared protein matrix. Matrix blocks containing the embedded hippocampi were then cryportocted by immersion into glycerol. Frozen blocks were cut perpendicular to the longitudinal/septotemporal axis of the hippocampi at 40 µm, series of every 10th section were collected and stored in cryoprotection solution until further processing. A reference serie was mounted

immediately in the correct anatomical order and Giemsa-stained (Giemsa stock solution 1.09204.0500, Merck, Darmstadt, Germany) following the protocol of previously described[6].

For cell counts, hippocampi were dehydrated and embedded in HEMA (2-hydroxyethyl methacrylate; Technovit 7100, Heraeus Kulzer GmbH, Wehrheim/Ts, Germany) following the manufacturer's instruction. Again, 5-6 randomly selected hippocampi were embedded in parallel positions in one block. Blocks containing the embedded hippocampi were cut perpendicular to the septotemporal axis at 20 µm. Every 3rd section was collected, mounted and Giemsa-stained as described before.

Immunohistochemistry for Ki67 and DCX

For epitope retrieval, free floating sections were heat treated for 40 min in citrate buffer (Target Retrieval Solution, DAKO; 1:10, pH 6.0). After pre-incubation in 2% normal serum with 0.25% Triton in Tris-buffered saline (TBS) for 60 min at RT, the sections were incubated overnight with primary antibodies against Ki67 (polyclonal Mouse-anti-Ki-67, BD Pharming 1:300) or DCX (polyclonal Goat-anti-Doublecortin, Santa Cruz, 1:250). Incubation in secondary antibody (Goat anti Rabbit 1:300 for Ki67 and Rabbit anti Goat 1:300 for DCX) was followed by incubation with ABC solution (Vectastain). Finally, sections were stained with 3,3' diaminobenzidine (DAB) and mounted. DCX stained sections were counterstained with hematoxylin solution and all sections were dehydrated and cover-slipped.

Cell counts

The total number of DCX positive cells were estimated in every 10th section with the optical fractionator [7-9] using the Stereoinvestigator software (MicroBrightField Inc. Williston, USA) with a 63x oil-immersion lens (ZEISS Plan-Apochromat 63x/1.40 Oil DIC). Cells were counted in a frame of 35 µm x 35 µm with x- and y-step sizes of 75 µm. Total cell numbers (N) were calculated using the formula: $N = \sum Q\text{-} \cdot \frac{1}{asf} \cdot \frac{1}{ssf}$, where ∑Q- is the total number of cells counted, asf is the area sampling fraction and ssf the section sampling fraction. All Ki67 positive cells were counted manually in every 10th section, stained cells in the top focal plane were not considered to prevent over-estimation. Total cell number estimates were calculated by multiplying the cell counts by the section sampling fraction.

Granule cells were estimated in every 12th HEMA embedded section, again using the optical fractionator with a counting frame of 10 µm x 10 µm, a dissector height of 10 µm and step sizes of 110 µm. Total numbers of granule cells was estimated using the same formula as for the DCX+ cells.

Volume measurements

Volumetric measurements were done in all main hippocampal fields. The dentate gyrus was subdivided into molecular layer, dentate gyrus granule cell layer and polymorphic layer (hilus). In the CA1 and CA3 region, the areas of the pyramidal cell layer, basal synaptic field (stratum oriens) and apical synaptic field (CA3: stratum lucidum/radiatum/lacunosum-moleculare; CA1: stratum radiatum/lacunosum-moleculare) were assessed. Volume estimations used the Cavalieri method [10] with Stereoinvestigator software (MicroBrightField

Inc. Williston, USA). Estimation was done in series adjacent to immuno-stained sections (reference serie) in every 10th section using a point-grid size of 60 µm for all areas.

Morphing the hippocampus

The quantitatively assessed data of cell numbers and volumes were collected in the correct anatomical order from septal to temporal. Thanks to the embedding method, we did not lose sections and analysed on average 13.6 (SD=1.5) sections for immunostained cell numbers and volume measurements and 17.5 (SD=2.1) sections for granule cell numbers. For analysis and visualization of the septotemporal distribution of cells and volume, we processed the data as described before [8]. Briefly, counts from individual sections of each animal were morphed into a standardized number of virtual sections, giving all hippocampi the same septotemporal length. The septotemporal length was standardized for all animals by selecting a desired number of virtual sections as a standard length, dividing counts obtained in each section into a number of sub-bins that corresponds to the desired number of sections and, finally, aggregating, across sub-bins, the number of sub-bins that corresponds to the number of sections available in an animal into a virtual section. E.g., three sections with counts of 8, 12 and 6 will be re-binned into 4 virtual sections. Counts are divided into the sub-bins 2, 2, 2, 2, 3, 3, 3, 3, 1.5, 1.5, 1.5, 1.5 and 1.5. Three sub-bins are sequentially aggregated into 4 virtual sections with counts of 6, 8, 7.5 and 4.5. The number of virtual sections selected corresponded to the integer closest to the mean number of sections analysed to minimize data dilution or loss.

RNA extraction, RT-PCR and Real Time PCR

Total RNA was extracted from the hippocampi of animals (n= 8 for each group) chronically treated with fluoxetine of vehicle under enriched or stressful conditions by using GeneElute™ Mammalian Total RNA Moniprep kit (Sigma, St. Louis, MO. USA) with a clean-up step with On-Column DNAse I Digestion Set (Sigma, St. Louis, MO. USA) to remove genomic contamination. Quantity of total RNA was determined using an ND-1000 Spectrophotometer (Nanodrop), and RNA integrity was checked with an Agilent 2100 Bioanalyzer (Agilent Techonologies) to determinate the ratios of 28S–18S ribosomal RNA band intensities (RNA integrity number; RIN). A cutoff of 8 of RIN value was applied in order to ensure a high quality of the samples. One µg of total RNA was reverse transcribed with High Capacity cDNA Reverse Transcription Kit (Life Technologies®, Milan) in 20 µL of reaction mix. Real Time PCR was performed in ABI PRISM 7900 HT using Power SYBR Green mix (Applied Biosystems®, Milan) added of the following primers: total BDNF forward: CCA TAA GGA CGC GGA CTT GTA C and total BDNF reverse: AGA CAT GTT TGC GGC ATC CAG G; p11(S100a10) forward: CTT CAA AAT GCC ATC CCA AA and p11(S100a10) reverse: TAT TTT GTC CAC AGC CAG AGG; GR(Nr3c1) forward: GCG ATA CCA GGA TTC AGA AA and GR(Nr3c1) reverse: GCA AAG CAT AGC AGG TTT CC; MR(Nr3c2) forward: ACA ATT CCA AGC CTG ACA CC and MR(Nr3c2) reverse: TGG TCC TCA AGA GGC AAG TT. The cycling parameters were: 95°C 10 min and 95 °C 15 s, 60° 1 min for 40 cycles. Single PCR products were subjected to a heat dissociation protocol (gradual increase of temperature from 60°C to 95 °C) and agarose gel separation to verify the absence of artifacts, such as primer-dimers or non-specific products. Direct detection of PCR products was monitored by measuring an increase in fluorescence intensity caused by binding of SYBR

GREEN I dye to neo-formed double strand DNA during the amplification phase. Each sample was normalized to the expression of the housekeeping gene GAPDH (NCBI accession number: NM_008084). Cycle threshold (Ct) value was determined by the SDS software 2.2.2 (Applied Biosystems®, Milan) and was utilized to calculate mRNA fold changes using the delta delta ct (ΔΔCt) method (calibrator: average of VEH treated control animals). For an appropriate application of comparative ΔΔCt method, it was demonstrated that amplification efficiency of the target genes and endogenous control gene were approximately equal. n= 8 in all groups.

Protein extraction and Western blotting

For protein extraction hippocampi and medial prefrontal cortices were homogenized by potter (12 stroke at 600 rpm) in lysis buffer containing Hepes 10 mM, EGTA 0.1 mM, sucrose 0.28 M pH 7.4, 1X cOmplete protease Inhibitor Cocktail (Roche, Mannheim, Germany), NaPP 5 mM, NaF 20 mM, Na_3VO_4 1 mM. A fraction of the lysate was collected (total fraction) and the remaining fraction was centrifuged at 1,000 X g for 5 min at 4°C. The supernatants were then transferred in a new tube (cytoplasmic fraction) and the pellets were resuspended in a buffer containing NaCl 120 mM, Hepes 20 mM, EGTA 0.1 mM, Dithiothreitol 0.1 mM, Sodium Pyrophosphate 5 mM, NaF 20 mM, Na3VO4 1 mM, Phenylmethanesulfonyl Fluoride 0.1 mg/mL (nuclear enriched fraction). Protein concentration was determined with a standard protocol using Coomassie® reagent (Sigma-Aldrich, Milan, Italy). Western blots were carried out on 20 µg of total, cytoplasmic or nuclear enriched extracts separated by 10-14% SDS-PAGE and transferred onto PVDF (Millipore) or nitrocellulose membranes. After blocking with 5% non-fat dry milk in TBS-Tween 20 1 hr, membranes were incubate with specific antibodies overnight at 4°C followed by incubation with secondary antibodies (HRP-conjugated anti-rabbit or anti-mouse IgG at the appropriate dilutions) for 1 hr at room temperature (see Table S1). Antibody binding was detected by using Immobilon™ Western Chemiominencent HRP substrate (Millipore). The levels of protein were calculated by measuring the peak densitometric area of the autoradiography analyzed with an image analyzer. The OD for targets signals were normalized according to the OD of β-Tubulin. Phosphorylated and un-phosphorylated proteins were run and detected on the same blot after stripping. Ratios were expressed as percentage of relative control ± SEM. n= 8 in all groups. Each experiment was performed twice and the mean of the OD ratios (target/internal standard) analyzed.

Table S1: antibodies and conditions used in the Western blotting experiments

	Primary antibody	Secondary antibody
ERK 1/2	1:1000 (Cell Signaling, #9102)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
pERK 1/2	1:1000 (Cell Signaling, #9106)	Anti-mouse 1:5000 (Santa Cruz, sc-2005)
CREB	1:1000 (Cell Signaling, #9197)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
pCREB(Ser133)	1:1000 (Cell Signaling, #9191)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
BDNF	1:500 (N-20, Santa Cruz)	Anti-rabbit 1:5000 (Cell Signaling, #7071)

GR	1:1000 (M-20, Santa Cruz)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
GluN1	1:882 (Upstate, #05-432)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
GluN2B	1:5000 (Chemicon, AB1557P)	Anti-rabbit 1:10000 (Cell Signaling, #7071)
GluN2A	1:1000 (Upstate, #06-313)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
GluR1	1:2500 (Millipore, #ABN241)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
GluR2	1:5000 (Millipore, #AB1768)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
PSD95	1:3000 (Millipore, #MABN68)	Anti-mouse 1:5000 (Santa Cruz, sc-2005)
pGluR1(Ser845)	1:500 (Zymed Hsto-line, #368300)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
pGluR1(Ser831)	1:500 (Upstate, #05-823)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
pGluR2(Ser880)	1:5000 (Upstate, #33568)	Anti-rabbit 1:5000 (Cell Signaling, #7071)
β-Tubuline	1:1000 (Santa Cruz, sc-9104)	Anti-rabbit 1:5000 (Cell Signaling, #7071)

Corticosterone levels

Corticosterone levels were measured in all subjects before and after the chronic stress procedure at baseline (i.e., no exposure to acute stress). Blood was collected from the tail 1 hr before lights on. The bleeding procedure consisted in a small and superficial cut in the tail. Blood samples were collected individually in potassium– EDTA coated 10 ml tubes (1.6 mg EDTA/ml blood; Sarstedt, Germany). All samples were kept on ice and later centrifuged at 3000 rpm for 15 min at +4uC. Blood plasma was transferred to Eppendorf tubes for corticosterone determination and stored at 220uC until further analysis. Corticosterone was measured using a commercially available radio-immunoassay (RIA) kit containing 125iodine labeled corticosterone (MP Biomedicals Inc., CA, USA). Vials were counted for 2 min in a gamma-scintillation counter (Packard Minaxi Gamma counter, Series 5000). Sensitivity of the assay was 0.125 mg/dl, inter- and intra-assay variation was less than 10% and 5%, respectively. VEH, n= 6; FLX, n = 7.

Hippocampal slice preparation

Electrophysiological experiments were performed at the end of environmental stimulation (ES) (starting after 21 days of EE to 31 days after). The experiments were performed in agreement with international guidelines on the ethical use of animals from the European Communities Council Directive of 24 November 1986 (86/609 EEC). Hippocampal slices were routinely obtained from 13-14 week-old C57BL/6 mice. Briefly, the animals were decapitated after being anesthetized with halothane. Whole brains were rapidly removed from the skull and immersed for 10 min in ice-cold artificial cerebrospinal fluid (ACSF) solution containing (in mM): NaCl 125, KCl 4.4, CaCl2 2.5, MgSO4 1.5, NaHPO4 1, NaHCO3 26 and glucose 10. The ACSF was continuously oxygenated with 95% O2, 5% CO2 to maintain the proper pH (7.4). Transverse 350 μm thick

slices were cut at 4 C with a vibratome (DSK, Japan) and the appropriate slices were placed in a chamber containing oxygenated ACSF. After their preparation, slices were allowed to recover for 2 h. Individual slices were then transferred to the interface slice-recording chamber (BSC1, Scientific System Design Inc) with a total fluid dead space of approximately 3 ml. Slices were maintained at 30-32° C and constantly superfused at the rate of 1.5 ml/min. Solutions were applied to the slices by a peristaltic pump.

Electrophysiological recordings

At the beginning of each recording, a concentric bipolar stimulating electrode (SNE-100X 50 mm long Elektronik–Harvard Apparatus GmbH) was positioned in the stratum radiatum for stimulation of Schaffer collateral pathway projections to CA1. An ACSF-filled glass micropipette (0.5–1 MΩ) was positioned 200–600 µm from the stimulating electrode for recording orthodromically-evoked fEPSPs. Stimuli consisted of 100 µs constant current square pulses, applied at 0.05 Hz. The intensity of the stimulus was adjusted in each experiment to evoke ~50% of the maximal field potential amplitude without appreciable population spike contamination. Evoked responses were monitored online and stable baseline responses were recorded for at least 10 min. Only the slices that showed stable fEPSP amplitudes were included in the experiments. To analyze the time course of fEPSP slope, the recorded fEPSP was routinely averaged over 1 min (n=3). LTP experiments were performed in ACSF and the averaged fEPSP (45 min post-induction) was normalized to the baseline values (1 min) before LTP induction (HFS, 1 train, 100 Hz, 1-s duration, test strength).

For the paired-pulse ratio (PPR) test, closely spaced consecutive stimuli (50 ms interval) were used, and PPR was calculated as the ratio between the fEPSP amplitude evoked by the second stimulus (A2) over the first (A1; A2/A1). Input–output (I-O) curves of synaptic transmission were measured at the beginning of each experiment and were generated by applying a series of stimuli of increasing intensities to the Schaffer collaterals. fEPSP slopes were normalized to responses produced by the maximal stimulus intensity.

Data acquisition and analysis

Slices were visualized with a Wild M3B (Heerbrugg, Switzerland). fEPSPs were recorded and filtered (1 kHz) with an Axopatch 200 A amplifier (Axon Instruments, CA) and digitized at 10 kHz with an A/D converter (Digidata 1322A, Axon Instruments). Data were stored on a computer using pClamp 9 software (Axon Instruments) and analyzed off-line with Clamp-fit 9 program (Axon Instruments).

Statistical analysis

Two experiments aimed at comparing vehicle vs. fluoxetine-treated mice, independently in the enriched and in the stressful condition, were performed. The direct comparison between the effects of enrichment and stress was beyond the goal of the present study. For this reason and for practical needs, the two experiments were run independently on different mouse cohorts. One-way ANOVA, Student's t-test or, when data were non-normally distributed, non-parametric analyses (Mann-Whitney U test) were used. Only neuroanatomical data were analyzed with a two-way ANOVA considering treatment (VEH vs. FLX) as between-subjects variable and subject as a random factor nested within treatment; longitudinal axis of the hippocampus as repeated

measures within subject. Sample size was chosen to identify differences between experimental groups of about 1.3 times the standard deviation, in a one-way completely randomized ANOVA with a two-sided alpha of 0.05 and a power of 0.80. *Post-hoc* comparisons were performed using the Tukey's HSD test (statistical software Statview II, Abacus Concepts, CA, USA). For electrophysiological and molecular comparisons the Student's t-test was used. Data were controlled for homoscedasticity. Outliers were identified using the Grubbs' test. fEPSP data were analyzed also by third order polynomial regression. Specifically, three curves were independently fitted to (i) enrich- and stress-VEH data, (ii) enrich- and stress-FLX data, (iii) the four groups together. The $\chi 2$ for each fit was estimated. A significant p-value indicated that the fit must be discarded, thus demonstrating that the fitted groups belong to different populations. For subject randomization, three *strata* based on preference for saccharin after stress exposure before treatment were created (low-medium, medium, high-medium) and animals within each stratum were randomly assigned to each experimental group with restricted randomization in order to guarantee balance across treatment groups. Data were collected in a blind fashion. The values were reported as mean ± S.E.M.

REFERENCES

1. Branchi I, Santarelli S, Capoccia S, Poggini S, D'Andrea I, Cirulli F *et al.* Antidepressant treatment outcome depends on the quality of the living environment: a pre-clinical investigation in mice. *PloS one* 2013; **8**(4)**:** e62226.

2. Knapska E, Walasek G, Nikolaev E, Neuhausser-Wespy F, Lipp HP, Kaczmarek L *et al.* Differential involvement of the central amygdala in appetitive versus aversive learning. *Learning & memory* 2006; **13**(2)**:** 192-200.

3. Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA *et al.* The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol Psychiatry* 2009; **14**(8)**:** 764-773, 739.

4. Dulawa SC, Holick KA, Gundersen B, Hen R. Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology* 2004; **29**(7)**:** 1321-1330.

5. Smiley JF, Bleiwas C. Embedding matrix for simultaneous processing of multiple histological samples. *J Neurosci Methods* 2012; **209**(1)**:** 195-198.

6. Iniguez C, Gayoso MJ, Carreres J. A versatile and simple method for staining nervous tissue using Giemsa dye. *Journal of neuroscience methods* 1985; **13**(1)**:** 77-86.

7. Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K *et al.* Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *Apmis* 1988; **96**(5)**:** 379-394.

8. Slomianka L, West MJ. Asymmetry in the hippocampal region specific for one of two closely related species of wild mice. *Brain Res* 1987; **436**(1)**:** 69-75.

9. West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. *The Anatomical record* 1991; **231**(4)**:** 482-497.

10. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K *et al.* Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* 1988; **96:** 379-394.