Supplementary Materials and Methods

Experimental design

Unpredictable chronic mild stress (UCMS) regimen used in this study has been previously presented (Nollet *et al*, 2011) and is a variant of chronic mild stress procedures described by Willner in rats (Willner, 1997). Briefly, mice were daily subjected to various socio-environmental low intensity stressors according to an unpredictable schedule for a total period of 9 weeks (Figure 1). UCMS-subjected mice were maintained under standard laboratory conditions but were isolated in individual cages (24 cm x 11 cm x 12 cm), while non-stressed controls (non-UCMS mice) were group-housed (*n* = 4 mice/cage) in standard laboratory cages (42 cm x 27 cm x 16 cm) with shelters and tubes. Pharmacological treatment started two weeks after the beginning of the UCMS protocol, and was always maintained until the end of the experiment. Body weight and coat state were assessed weekly as markers of the progression of the UCMS-evoked syndrome. Coat state, which represents an indirect evaluation of grooming behavior, was evaluated by examining the coat on different body parts (head, neck, dorsal area, ventral area, tail, front and hind paws, and genital area). The total score resulted from the sum of scores (0 = well-groomed, 0.5 = moderate degradation, 1 = unkempt); a high score indicates that the coat is in poor condition. Behavioral tests were performed in week 8 (*n* = 14-19 mice/group), at least 18 h after last treatment (all mice were subjected to one test daily, always in the same sequence). To label newborn adult cells in the dentate gyrus (DG), the thymidine analogous 5-bromo-2'-deoxyuridine (BrdU, B-5002, Sigma-Aldrich) was used as it can incorporate into DNA of proliferating cells (Kuhn and Cooper-Kuhn, 2007). BrdU was dissolved in 0.9% NaCl and was administrated to mice intraperitoneally (i.p.) 4 weeks before intracardiac perfusions, at the beginning of the sixth week (4 injections of 75 mg/kg every 2 h, during 2 days). At the end of the UCMS protocol, all mice were subjected to dexamethasone-suppression test 2 h before being perfused. All measures were evaluated by trained experimenters blind to the treatment.

Behavioral testing

Basal locomotor activity. An actimeter was used 2 h (afternoon, i.e. during the dark phase of the cycle), 10 h (night, i.e. during the light phase of the cycle) and 18 h (morning, i.e. at the beginning of the dark phase) after drug administration to assess the long-lasting effects of UCMS and pharmacological treatments on basal locomotor activity of mice in their home cage. Control animals were isolated 24 h before the beginning of the sessions. The cage was placed in the center of the device, which consisted of a 20 x 20 cm square plane with photobeam detectors crossing the plane. The movement of the animal was automatically detected when it crossed the beam, allowing a score to be established. Locomotor activity was measured during 2 h for each time point.

Elevated plus maze. The elevated plus maze (EPM) consists in a plus-cross-shaped maze (27x5 cm) originating from a central platform (5x5 cm) and elevated 38.5 cm above the floor. Two opposite arms were enclosed by walls (15 cm) while two others were opened and brightly lit. Mice were placed in the center area facing one of the closed arms and their movements were tracked by a video camera for 5 min. After each test, the maze was cleaned with 2.0 % ethanol and dried to prevent interference of subsequent tests by olfactory cues. The time spend in open and closed arms, as well as the number of entries to open and closed arms (data not shown), were recorded and analyzed using Ethovision XT 7.1 software (Noldus, Wageningen, The Netherlands). The time spent in the open arms is associated with a reduction of anxiety behavior.

Resident-intruder test. The resident-intruder (R-I) test was performed as previously described (Nollet *et al*, 2011) and consists of the introduction of a novel littermate (C57BL/6 male mice) in the cage in order to measure the aggressiveness of resident mice. Non-UCMS mice were placed in individual cages 24 h before the test, and the UCMS-mice litter was changed 24 h before the test in order to have all animals in the same experimental conditions. The intruder was placed into the home cage of the test animal (resident) in such a way that mice were in opposite corners. The latency of the resident first attack and the number of resident attack(s) (data not shown) were measured over a 6-min period (latency of 360 seconds for non-attacking mice).

Attacking intruders were excluded, without excluding the resident. Depressive-like animals are more agonistic and likely to attack more often and sooner than non-stressed animals (Mineur *et al*, 2003).

Tail suspension test. The procedure of the tail suspension test (TST) followed in this study was derived from the protocol previously described (Steru *et al*, 1985). Mice were suspended by the tail (approximately 1 cm from the tip of the tail) using adhesive tape to a rod 60 cm above the floor. The trials were conducted for a period of 6 min and were video recorded. The behavioral measure was the duration of immobility, interpreted as behavioral despair. Mice were considered immobile only when they hung motionless.

Novelty-suppressed feeding test. The novelty-suppressed feeding (NSF) test used in this study has been formerly described (Surget *et al*, 2008). It consisted of a 33 x 33 x 30 cm box whose floor was covered with 2 cm sawdust. Twelve hours before the test, mice were fasted by removing food from their cages. At the time of testing, a single pellet of food (regular chow) was placed on a white paper positioned in the box center. Mice were placed in the corner, and the latency to chew the pellet was recorded within a 3-min period. This test induced a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the arena. In addition, since drug treatments could have various effects on appetite, the feeding drive of each animal was assessed by measuring the amount of food consumed over 5 min when animals had been returned in their home cage. No difference was observed (data not shown).

Immunohistochemistry

Anesthetized mice (sodium pentobarbital, 40 mg/kg, i.p.) were transcardially perfused through the heart with 80 ml of saline solution followed by 200 ml of 4% paraformaldehyde in 0.1M phosphate buffer (PB) (pH 7.4). Brains were removed, postfixed 2 h in the same fixative, and cryoprotected in a 20% sucrose solution overnight at 4°C before being processed. Coronal sections (35 µm thickness) were cut in a cryostat (Leica CM 3050S) and divided in four series (i.e. one section every

four sections per lot) allowing different immunohistochemical procedures. Neuronal activity within the PVN of the hypothalamus was analyzed in labeling the c-Fos protein. After a series of washes in 50% ethanol and 3% H_2O_2, free-floating sections were incubated at room temperature (RT) in a rabbit anti-Fos antibody (Calbiochem, PC38, 1:5000). Thirty-six hours later, sections were washed in 0.1M PB, incubated 2 h in a biotinylated anti-rabbit IgG (Jackson Immunoresearch, 711-066-152, 1:500) followed by Elite avidin-biotin complex (ABC) kit (Vector Laboratories, PK-6100, 1:100) for 1 hour, and reacted with diamino-benzidine (DAB) (Sigma-Aldrich, D0426) in the presence of cobalt and H_2O_2. Sections were rinsed, mounted on gelatinized glass slides, dehydrated, cleared in Claral (Réactifs RAL) and coverslipped with Eukitt mounting medium (O. Kindler GmbH, Freiburg). In order to assess the effects of UCMS and pharmacological treatments on cell proliferation, the Ki-67 protein marker was used. Immunohistochemistry was performed as above with a rabbit anti-Ki-67 primary antibody (abcam, ab15580, 1:1000) and biotinylated anti-rabbit IgG (1:500) secondary antibody. Sections were reacted with DAB without metal enhancement (Sigma-Aldrich, D4293). Just before being dehydrated, cleared and coverslipped, all sections were counterstained with cresyl violet (Santa Cruz, SC-214775). To assess the effects of the UCMS and the pharmacological treatments on neurogenesis, we labeled immature newborn neurons with doublecortin (DCX) protein immunostaining, and mature new neurons (4-weeks old) by means of immunodetection of both the neuronal-specific nuclear protein NeuN and the marker of cell division BrdU. Free-floating sections were denatured with 2N HCl (30 min at RT) and incubated with primary antibodies for 24 h followed by fluorochrome-labelled secondary antibodies for 2 h at RT and then mounted on slides with Vectashield mounting medium (Vector Laboratories, H-1000). The primary antibodies used were a mouse anti-NeuN (Millipore, MAB377, 1:500), a rat anti-BrdU (Santa Cruz, SC-70441, 1:500), and a goat anti-DCX (Santa Cruz, SC-8066, 1:500). The secondary antibodies were Alexa Fluor dyes (invitrogen) as follow: 647 nm anti-mouse (A-31571, 1:500), 488 nm anti-rat (A-21208, 1:500), and 546 nm anti-goat (A-11056, 1:500). Various negative controls were performed, omitting either the primary or the secondary antibodies.

Image analysis and cell quantification

When staining was obtained with DAB, the number of positive cells was counted using x10 and x20 objective lens with a Leica DM 2000 microscope, whereas with fluorochrome-labeled secondary antibodies, the number of positive cells was counted using a x20 and x40 objective lens with an epifluorescence microscope (Imager.Z2, Zeiss) and AxioVision software (Zeiss). Activity of hypothalamic nuclei related to HPA axis was assessed by analysis of Fos-labeled neurons in the parvocellular nuclei of the PVN whose axons are known to release CRH. The nomenclature and nuclei boundaries used were those defined by Franklin and Paxinos's mouse brain atlas (Franklin and Paxinos, 2008). Fos-positive cells within each nucleus was counted bilaterally in every fourth sections starting from bregma -0.22 to -1.22 mm. Hippocampal cell proliferation and neurogenesis were assessed by analysis of Ki-67-labeled (cell proliferation), DCX-labeled (newborn immature neurons) and BrdU/NeuN-labeled (newborn mature neurons) cells in the granular cell layer (GCL) of the dentate gyrus (DG) (defined as a two-cell soma-wide zone along the base of the GCL) every fourth sections, spanning the entire hippocampus. As dorsal and ventral parts of the hippocampus do not share the same connectivity with afferent and efferent areas (Fanselow and Dong, 2010), and as only the ventral part is known to regulate the HPA axis through polysynaptic neuronal circuit towards the PVN (Herman *et al*, 2005; Mizoguchi *et al*, 2003; Ulrich-Lai and Herman, 2009), cell proliferation and neurogenesis were examined separately in the dorsal (bregma -0.94 to -1.58 mm), intermediate (bregma -1.70 to -3.28 mm) and ventral parts (bregma -3.40 to -3.88 mm) of the hippocampus (Franklin and Paxinos, 2008). The GCL surfaces were determined with Axiovision software from pictures obtained at x10 objective lens at corresponding levels on cresyl violet or NeuN staining to express numbers of immunoreactive cells per square millimeter of respective areas and finally per cubic millimeter by multiplying by the thickness of sections (35 µm). All quantifications were performed by an investigator blind to stress and treatment history.

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