Effect of agomelatine on memory deficits and hippocampal gene expression induced by chronic social defeat stress in mice

Vincent Martin1, Najib Allaïli2, Marine Euvrard1, Tevrasamy Marday4, Armance Riffaud1, Bernard Franc5, Elisabeth Mocaër3, Cecilia Gabriel3, Philippe Fossati4, Stéphane Lehericy2 & Laurence Lanfumey1

Chronic stress is known to induce not only anxiety and depressive-like phenotypes in mice but also cognitive impairments, for which the action of classical antidepressant compounds remains unsatisfactory. In this context, we investigated the effects of chronic social defeat stress (CSDS) on anxiety-, social- and cognitive-related behaviors, as well as hippocampal Bdnf, synaptic plasticity markers (PSD-95, Synaptophysin, Spinophilin, Synapsin I and MAP-2), and epigenetic modifying enzymes (MYST2, HDAC2, HDAC6, MLL3, KDM5B, DNMT3B, GADD45B) gene expression in C57BL/6J mice. CSDS for 10 days provoked long-lasting anxious-like phenotype in the open field and episodic memory deficits in the novel object recognition test. While total Bdnf mRNA level was unchanged, Bdnf exon IV, MAP-2, HDAC2, HDAC6 and MLL3 gene expression was significantly decreased in the CSDS mouse hippocampus. In CSDS mice treated 3 weeks with 50 mg/kg/d agomelatine, an antidepressant with melatonergic receptor agonist and 5-HT2C receptor antagonist properties, the anxious-like phenotype was not reversed, but the treatment successfully prevented the cognitive impairments and hippocampal gene expression modifications. Altogether, these data evidenced that, in mice, agomelatine was effective in alleviating stress-induced altered cognitive functions, possibly through a mechanism involving BDNF signaling, synaptic plasticity and epigenetic remodeling.

Chronic social stress, which in humans often originates from social relationships encountered throughout the entire life14-16, has been proposed to be a major cause for depression1. Stress can lead to multiple psychological and neurobiological disorders such as cognitive deficits often associated with depression1. In many cases, in addition to emotional-related symptoms, depressed patients also suffer from cognitive problems including memory loss, attentional impairment, executive dysfunction and poor decision making. However, and despite the different families of antidepressant compounds available to treat depressive symptoms, cognitive deficits are still not adequately alleviated by regular antidepressant therapies15-16.

Social stress can easily be transposed to rodents, as their society life organization is based on a clearly identified social hierarchy1. To this aim, the chronic social defeat stress (CSDS) paradigm has been developed and validated to determine the behavioral and molecular effects of social stress15-16. This stress is based on repeated subordination to unfamiliar dominant in its own territory. Following repeated exposures to aggressive resident CD-1 mice, intruder C57BL/6J mice display various behavioral deficits such as social avoidance, anxiety-like phenotype, despair-like behavior and a reduction in sweet preference11-13. In addition, CSDS-exposed animals also exhibit cognitive impairments14-16, related to those observed in patients subjected to social stress, that suggest a marked dysregulation of the hippocampus, a brain structure known to play a crucial role in memory processes17, and to be directly altered by social stress18.

1Centre de Psychiatrie et Neurosciences, INSERM UMR 894, Université Paris Descartes, Paris, France. 2Centre de Neuroimagerie de Recherche – CENIR– Inserm UMR1127 - CNRS 7225, Institut Cerveau Moelle - ICM, Sorbonne Universités, UPMC UMR S 1127, Paris, France. 3Institut de Recherches Internationales Servier, IRIS, Suresnes, France. 4Social and Affective Neuroscience - SAN Laboratory - Inserm U 1127 - CNRS UMR 7225- Institut du Cerveau et de la Moelle- ICM - Sorbonne Universités, UPMC UMR S 1127, Paris, France. 5Correspondence and requests for materials should be addressed to L.L. (email: laurence.lanfumey@inserm.fr)
The effect of antidepressant drugs on memory alterations has been assessed on several chronic stress models, but their efficacy is still a matter of debate19–22. Among the different antidepressant compounds, agomelatine is an agonist at melatonergic MT1 and MT2 receptors and a 5-HT2C receptor antagonist displaying antidepressant and anxiolytic properties in several validated animal models. Agomelatine is also able to modulate neuroplasticity in both basal conditions and in models of depression (see refs 23, 24 for review).

Moreover, agomelatine at antidepressant doses has been shown to display beneficial effects on memory in naïve animals after acute25 and chronic treatment26 in the rat NOR test. Another study also reported that agomelatine was able to reverse the memory impairments provoked by chronic mild stress in mice as assessed in the NOR test and the Morris water maze27. In this line, agomelatine was also shown to block memory impairment induced by a stress in rats trained in a spatial memory task28 and to improve social memory in prenatally stressed rats29. Recently, we demonstrated that chronic agomelatine could prevent hippocampal epigenetic modifications that occurred in mice following chronic ultra-mild stress protocol, suggesting that agomelatine could achieve its mechanism of action through epigenetic remodeling30. In order to further explore the consequences of CSDS on anxiety, social behavior and memory functions, as well as hippocampal gene expression, we performed a series of molecular and behavioral experiments and showed that the consequences of such a social stress, in particular those related to cognition, could be reversed by chronic agomelatine treatment in mice.

**Materials and Methods**

**Animals and treatments.** Adult male C57BL/6j mice (7 week-old; Charles River Laboratories, l’Abresle, France) were housed 4–6 per cage under standard laboratory conditions (22 ± 1 °C, 60% humidity, 12-h light-dark cycle with lights on at 07:00, food and water *ad libitum*) for one week before starting experiments. All procedures concerning animal care and treatment were carried out in accordance with the protocols approved by the ethical committee # C2EA-05 Charles Darwin for the use of experimental animals and were licensed by the Directorate General for Research and Innovation (French Ministère de l’Enseignement Supérieur et de la Recherche), under protocol authorization # 00966.02.

Agomelatine (50 mg/kg/day, Servier Laboratoires, Suresnes, France) or its vehicle HEC (hydroxyethylcellulose 1%, Servier Laboratoires) were administered intraperitoneally (i.p.) on a daily basis for 21 days (days 11–31), 2 hours before the dark phase.

**Chronic social defeat stress (CSDS).** Chronic social defeat stress was performed as previously described9,10. Male CD-1 retired breeder mice were screened for aggressive behavioral response measured by the latency to initial aggression that should be less than 60 s in at least two consecutive 180 s-screening sessions31. 24 h before the first defeat, mice were singly housed in one side of a standard cage separated by a clear perforated Plexiglas divider, which allowed sensory but not physical contact. An experimental C57BL/6j intruder mouse was exposed to a CD-1 aggressor for 10 min, during which the intruder mouse was attacked and displayed subordinate posturing. To avoid physical injury, mice were briefly isolated in case of over-aggressive behavior from the CD-1. After a 10-min defeat session, the experimental mouse was placed for the rest of the day on the opposite side of the divider. This procedure was repeated for 10 consecutive days (D1–D10), using a different aggressor CD-1 mouse every day, between 10:00 and 11:00 am. Control mice were housed by pair in a similar cage, separated by a clear Plexiglas divider, and never exposed to CD-1 mice. Immediately after the last defeat session, stressed and control mice were singly housed in a new standard cage.

In order to assess the effect of chronic agomelatine treatment on stress-induced behavioral deficits, susceptible mice were divided into two homogenous groups: “stressed-AGO” that included stressed mice treated for 3 weeks with agomelatine (50 mg/kg/day, i.p.) and “stressed-HEC” that included stressed mice treated with vehicle (HEC) in the same conditions. Control mice treated for 3 weeks with HEC were called “control-HEC”. Mice were weighted once a week in order to monitor body weight gain. Neither stress nor treatments significantly modified body weight (See Table S1), although there is a trend for a higher increase in weight after social stress compared to controls which actually fits with previous data on the leptin resistance-induced by social stress32.

**Behavioral testing.** On D11, defeated and control animals were tested in the open field and social avoidance tests, in order to select the susceptible, which displayed anxiety- and social avoidance phenotypes, and the resilient mice9,10, and agomelatine or HEC chronic treatments were initiated at the same day. Similar behavioral tests were replicated on D29 to assess the effect of treatments. In addition, on D30 and D31, a novel object recognition test was carried out to evaluate the effect of stress and treatments on episodic memory (see Fig. 1).

**Open field test (OF).** 24 h after the last defeat session (D11), mouse anxiety levels and locomotor activity were tested in the open field, consisted of four open boxes (50 × 50 cm) separated by Plexiglas walls and equipped with an infrared floor for automatic exploratory behavior detection (View Point, Lyon, France). A virtual zone (20 × 20 cm) was delimited in the center of the open field. Mice were placed in the boxes, and left free to explore for 1 h under low light conditions (5 lux). The number of entries, the time spent at the center and the total distance covered in the entire open field were measured using a video tracking system (Viewpoint).

**Social avoidance test.** Social avoidance was assessed three hours after the open field test, as previously described9,10. The test consisted in two consecutive 150 s-sessions, under low light conditions (5 lux). Mice were subjected to the test in the same open field boxes they were previously habituated during the open field test. In the first session, experimental mice were allowed to explore the open field containing an empty circular wire cage (18 × 9 cm) located at one end of the field. In the second session, conditions were identical except that the circular cage contained a CD-1 aggressive mouse (defined as “target”). A virtual interaction zone (area projecting 8 cm around wire cage) was delimited, and the time spent in this zone was scored during both sessions using a video tracking system (Viewpoint). Social interaction behavior was estimated as interaction ratio: (time spent in the
interaction zone in the presence of target/time spent in the interaction zone in the absence of target) * 100. Mice were classified either as susceptible with ratio below 100 or as resilient with ratio above 100 on a social avoidance test performed at D11, as described in the literature. Within the CSDS-subjected group, only susceptible mice were included for the subsequent experiments. No such sorting was performed within the control group.

Table 1. Primer sequences used for mRNA expression analyses by RT-qPCR with SYBR Green technology.

| Genes       | 5’ primers                          | 3’ primers                          |
|-------------|-------------------------------------|-------------------------------------|
| BDNF        | AAAACATGATGGCAGGGGATAGACATGTTTGCGGCATCC | TAGACATGTTTGCGGCATCC |
| BDNF IV     | CTCTGCGAGCTGACAATTGAACTGCAATGCGGCCA | GAAGTGTACAGTGTCCGCTGTCCTT |
| MLYT2       | GTATCCGGATGCCAATGCTGTTTCTCTG     | ATGCCACCATACCAACAGG |
| HDAC2       | AGGAGGAGGCCGAGGAACAGG           | CAGAGGCCAGCCAGGAGGAGG |
| MLL3        | TCCGGATAGTTGCCGCCCTCTAAAGGAG    | AGAATGGGAGATGAGGGG |
| KDM5B       | GTCTGAGGGTCCATCCTTCTCTG      | ATGCCACATCACACAGGG |
| DNMT3B      | GTATGAGGAGGCCGAGAAGG           | CAGAGGCCAGCCAGGAGG |
| GADD45B     | GTTCGCTGCGAACAGAATGCA         | TGGCTTTTCCAGGAAACTCT |
| PSD-95      | TCTGCTGCGAGGACGTAAGAAGACG     | AAGCCATCGGGAACCTCCTG |
| Synaptophysin| TCCTTGTACAGTGTCCGCTGTCCTT   | TCCCTTTTCCAGGAAACTCT |
| Spinophilin | AAGGGGAGGGGAGGAGGAGG          | GCCCATCTGAGGAAACATCT |
| Synapsin 1  | CAGGCACTGGGCAAATACTCT         | TCCGAAGAAGGGCACTGTC |
| MAP-2       | TCAGGAGACAGGAGAGGAGGA      | GTGTGGAGGAGGAGGAGG |
| 3-actin     | CCACTTGATCCAGGAGCCTT         | CCGAAGAAGGGAGGAGG |

Figure 1. Study design. Mice were submitted to CSDS for 10 days and then treated 21 days with either agomelatine or its vehicle HEC i.p. Anxiety- and depressive-like phenotypes were assessed at day 11 and day 29 using the social avoidance and open field test, and memory was tested using the novel object recognition test at days 30 and 31. Finally, animals were sacrificed at day 32 for molecular analysis. CSDS, chronic social defeat stress; HEC, hydroxyethylcellulose; NOR, novel object recognition test; OF, open field; SA, social avoidance test.

Figure 2. Effect of chronic social defeat stress and chronic agomelatine (50 mg/kg/day i.p.) on social interaction in the social avoidance test. (A) The day after the last defeat session (Day 11), mice subjected to CSDS can be split into two groups: the susceptible mice, which display a significant reduction in the interaction ratio compared to control mice, and the resilient mice, which do not behave differently from non-stressed group and display a significant higher interaction ratio than the susceptible mice. (B) On Day 29, no significant difference was found between control-, stressed mice treated with HEC and stressed mice treated with agomelatine. Each bar is the mean ± S.E.M. of n = 19 (control mice), 11 (stressed HEC mice) and 11 (stressed AGO mice). AGO, agomelatine; HEC, hydroxyethylcellulose.
Novel object recognition test (NOR). The test was performed as previously described with slight modifications. The apparatus consisted of a black open arena (30 × 30 cm), with three types of objects (colored plastic cylinders, white plastic boxes and transparent glass bottles). Room light was kept at 5 lux. Mice were trained to the apparatus for 15 min in the morning of D30, and the test was carried out in the afternoons of D30 and D31.

During the acquisition phase, two identical objects were placed in the arena, on the right and left corners, at 7 cm-distance from the walls. Mice were introduced in the arena and left free to explore for 5 min, and returned to their home cage. After a 1h-intertrial interval (ITI1), one of the objects was replaced by a different one, and the mice were reintroduced in the arena for another 5 min-session (recognition phase 1, D30). The procedure was replicated 24 h (ITI24) after the first recognition phase, with a different new object (recognition phase 2, D31). In both testing phases, the time spent by the mice exploring the objects (touching with their nose or forelimbs, or sniffing at a distance less than 1 cm) was manually scored from recorded video using XNote StopWatch software. The recognition index was calculated as follow: (time spent exploring the novel object - time spent exploring the familiar object)/(time exploring both novel and identical objects). Mice that explored both objects less than 5 s during recognition phase 1 or 2 were excluded.

Quantification of RNA levels by quantitative RT-PCR. On D32, at the end of treatment, animals were sacrificed by cervical dislocation and the hippocampus was quickly dissected, frozen in liquid nitrogen and kept at −80°C for molecular analysis. Total mRNA was extracted using TRI Reagent Solution (Life Technologies, Saint Aubin, France) following manufacturer's instructions. Reverse transcription was performed with High Capacity cDNA Reverse Transcription kit (Applied Biosystem, Courtaboeuf, France) with the following cycling protocol: 25°C for 10 min, 37°C for 2 h and 85°C for 5 s. cDNA samples were stored at −20°C. Amplification reactions were performed with KAPA SYBR FAST qPCR Master Mix (Clinisciences, Nanterre, France) following manufacturer's instructions using the 7300 Real Time PCR System (Applied Biosystem, Courtaboeuf, France). The following cycling protocol was applied: 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The list of primers used is indicated in Table 1. β-actin was used as housekeeping gene for normalizing gene expression results. The 2^ΔΔCT (Delta Delta Comparative Threshold) method was used to quantify the fold change in mRNA expression in the different experimental groups.

Statistical analysis. Data (displayed as mean ± S.E.M.) were analyzed using Prism 5 Software (GraphPad, San Diego, USA). To compare “control-HEC”, “stressed-HEC” and “stressed-AGO” groups, a one-way analysis of variance (ANOVA) was used, followed by Newman Keuls multiple comparisons test. When variances were
Results

Effect of CSDS and chronic agomelatine treatment on depression-related behavior in the social avoidance test. The interaction ratio was used to discriminate susceptible and resilient mice to chronic stress among a batch of mice all subjected to CSDS. As shown in Fig. 2A and S1, social avoidance test led to the segregation of susceptible mice (n = 24), and resilient mice (n = 17).

Social avoidance test was then replicated on D29, after a 3-week treatment with either agomelatine or HEC in CSDS-exposed mice, or HEC in control mice (Fig. 2B and S1). Although a Kruskal-Wallis test failed to reveal any overall difference between the three groups of mice (p = 0.16), it can be noted that stress still tended to reduce interaction ratio, but there was no effect of the agomelatine treatment.

Effect of CSDS and chronic agomelatine treatment on anxiety-related behavior in the open field. In order to assess the effect of CSDS on anxiety-like behavior, mice were subjected to the open field test on D11 (Fig. 3A). Susceptible mice displayed a significant hypolocomotion in the entire open field (p < 0.0001, Student's t-test with Welch's correction), and a decreased number of entries (p < 0.0001) and time spent at the center of the open field (p = 0.0007) compared to control mice.

The open field test was repeated on D29, to evaluate the effect of treatments on stress-induced deficits (Fig. 3B). After a 3 week chronic vehicle treatment, one way ANOVA followed by Newman-Keuls multiple comparisons test showed that stressed-HEC displayed a significantly decreased in the recognition index compared to control-HEC mice [F(2,41) = 3.209, p < 0.05] (Fig. 4B). In contrast, a one-way ANOVA showed that stressed-AGO mice did not significantly differ from control-HEC [F(2,30) = 0.8906, p > 0.05] and that the recognition ratio was significantly increased in comparison to that of stressed-HEC mice [F(2,30) = 3.679, p < 0.05].
reduced in CSDS mice, compared to non-stressed mice \([F(2,43) = 3.538, p < 0.05]\).

ANOVA followed by Newman-Keuls multiple comparisons test showed that was decreased by CSDS in HEC-treated mice \([F(2,37) = 2.528, p = 0.0923]\).

Figure 5. Long-lasting effect of chronic social defeat stress and chronic agomelatine on total Bdnf and Bdnf exons IV and VI mRNA expression in the hippocampus. The effects of CSDS and agomelatine treatment on hippocampal Bdnf gene expression were determined after 3 weeks of agomelatine (50 mg/kg/day, i.p.) treatment following 10 days of CSDS. CSDS and chronic agomelatine treatment did not induce any change in total Bdnf and Bdnf exon VI mRNA expression. However, Bdnf exon IV gene expression was significantly reduced in stressed-HEC mice, but the level of Bdnf exon IV mRNA was not different between control-HEC and stressed-AGO mice. *p < 0.05, one-way ANOVA followed by Newman-Keuls multiple comparisons test. Each result is expressed as the mean ± S.E.M. of \(n = 21\) (control mice), 12 (stressed HEC mice) and 12 (stressed AGO mice).

| Gene         | Control HEC | Stressed HEC | Stressed AGO | Control HEC vs Stressed HEC | Stressed HEC vs Stressed AGO |
|--------------|-------------|--------------|--------------|----------------------------|------------------------------|
| PSD-95       | 1.003 ± 0.018 | 0.9500 ± 0.022 | 1.019 ± 0.025 | n.s                         | n.s                          |
| Synaptophysin| 1.011 ± 0.038 | 0.8867 ± 0.048 | 0.9367 ± 0.035 | n.s                         | n.s                          |
| Spinophilin  | 1.007 ± 0.029 | 1.020 ± 0.044 | 1.023 ± 0.031 | n.s                         | n.s                          |
| Synapsin I   | 1.003 ± 0.019 | 0.9750 ± 0.029 | 1.008 ± 0.026 | n.s                         | n.s                          |
| MAP-2        | 1.003 ± 0.019 | 0.9236 ± 0.022 | 1.017 ± 0.037 | *                           | #                            |

Table 2. Long-lasting effect of chronic social defeat stress and chronic agomelatine on synaptic plasticity markers mRNA expression in the hippocampus. The effects of CSDS and agomelatine treatment on hippocampal gene expression of synaptic plasticity markers were tested after 3 weeks of agomelatine (50 mg/kg/day, i.p.) treatment following 10 days of CSDS. Neither CSDS nor chronic agomelatine modified PSD-95, Synaptophysin, Spinophilin and Synapsin I gene expression. However, mRNA expression of MAP-2 was decreased in stressed mice treated with vehicle compared with controls and stressed mice treated with agomelatine. *p < 0.05 “Control HEC” vs “Stressed HEC”, **p < 0.05 “Stressed HEC” vs “Stressed AGO”, one-way ANOVA followed by Newman-Keuls multiple comparisons test. Each result is expressed as the mean ± S.E.M. of \(n = 18\) (control mice), 11 (stressed HEC mice) and 11 (stressed AGO mice).

Effect of CSDS and chronic agomelatine treatment on total Bdnf and Bdnf exons IV and VI mRNA expression. mRNA expression of total Bdnf and Bdnf exons IV and VI was quantified in the hippocampus of control-HEC, stressed-HEC and stressed-AGO mice. As shown in Fig. 5, while a one-way ANOVA showed that CSDS and agomelatine treatment failed to induce any expression modification in total Bdnf \([F(2,44) = 0.4390, p = 0.6474]\) and Bdnf exon VI \([F(2,43) = 0.9738, p = 0.3858]\) gene expression, the same analyze followed by Newman-Keuls multiple comparisons test showed that Bdnf exon IV mRNA level was significantly reduced in stressed mice, compared to non-stressed mice \([F(2,43) = 4.871, p < 0.01]\). Interestingly, stressed-AGO mouse Bdnf exon IV mRNA level did not differ from that of control-HEC \([F(2,43) = 1.767, p > 0.05]\).

Effect of CSDS and chronic agomelatine treatment on synaptic plasticity markers mRNA expression. A one-way ANOVA showed no difference between the three groups for PSD-95, Synaptophysin, Spinophilin and Synapsin I gene expression \([F(2,39) = 2.534, p = 0.0923]; F(2,39) = 2.528, p = 0.0928]; F(2,39) = 0.06687, \(p = 0.9354\) and F(2,39) = 0.487, \(p = 0.6181\), respectively] (Table 2). In contrast, a one-way ANOVA followed by Newman-Keuls multiple comparisons test showed that MAP-2 hippocampal mRNA level was decreased by CSDS in HEC-treated mice \([F(2,37) = 3.538, p < 0.05]\), and that this effect was prevented by chronic agomelatine administration \([F(2,37) = 3.555, p < 0.05]\).

Effect of CSDS and chronic agomelatine treatment on epigenetic modifying enzymes mRNA expression. A one-way ANOVA showed that gene expression of histone acetyltransferase MYST2 was not modified in any of the groups \([F(2,39) = 0.3391, p = 0.7145]\) (Table 3). In contrast, CSDS decreased the expression of histone deacetylases HDAC2 \([F(2,37) = 4.534, p < 0.05\), one-way ANOVA followed by Newman-Keuls multiple comparisons test] and HDAC6 mRNA \(p < 0.05\), Kruskal-Wallis test followed by Dunn’s multiple comparisons test). Interestingly, this effect was prevented by chronic agomelatine treatment \([HDAC2: F(2,37) = 3.249, p < 0.05\) and HDAC6: \(p < 0.05\), respectively]. At histone methylation level, a one-way ANOVA followed by Newman-Keuls multiple comparisons test indicated that CSDS reduced the expression of the histone methyltransferase...
natural propensity of mice to interact with unfamiliar mice34. Here, the social avoidance test, performed 24 h after the end of the last defeat session, revealed that 59% of total mice subjected to the stress protocol were stressed mice chronically treated with the vehicle showed reduced gene expression, and this effect was reversed by chronic agomelatine treatment. *p < 0.05 “Control HEC” vs “Stressed HEC”, #p < 0.05 “Stressed HEC” vs “Stressed AGO”, one way ANOVA followed by Newman-Keuls multiple comparisons test (HDAC2 and MLL3) or Kruskal-Wallis test followed by Dunn's multiple comparisons test (HDAC6). Each result is expressed as the mean ± S.E.M. of n = 19 (control mice), 10 (stressed HEC mice) and 10 (stressed AGO mice). AGO, agomelatine; HEC, hydroxyethylcellulose.

Table 3. Long-lasting effect of chronic social defeat stress and chronic agomelatine on histone acetylation/methylation and DNA methylation modifying enzymes mRNA expression in the hippocampus. The effects of CSDS and agomelatine treatment on hippocampal gene expression of enzymes known to modify histone acetylation and methylation as well as DNA methylation were tested after 3 weeks of agomelatine (50 mg/kg/day, i.p.) treatment following 10 days of CSDS. No significant differences were found between the three groups for MYST2, KDM5B, DNMT3B and GADD45B mRNA expression. However, concerning HDAC2, HDAC6 and MLL3, stressed mice treated with the vehicle showed reduced gene expression, and this effect was reversed by chronic agomelatine treatment. *p < 0.05 “Control HEC” vs “Stressed HEC”, #p < 0.05 “Stressed HEC” vs “Stressed AGO”, one way ANOVA followed by Newman-Keuls multiple comparisons test (HDAC2 and MLL3) or Kruskal-Wallis test followed by Dunn's multiple comparisons test (HDAC6).

Discussion

The effects of chronic social defeat stress and chronic agomelatine treatment on mouse behavior and plasticity gene expression reported here showed that agomelatine failed to reverse stress-induced alterations on anxiety- and social-like behaviors, but was able to prevent CSDS deleterious effects on cognitive performances and hippocampal gene expression.

Krishnan et al.10 initially demonstrated that a population of C57BL/6J subjected to the CSDS paradigm could be separated into susceptible mice, with behavioral and molecular alterations, and resilient mice that were less prone to develop such deficits. Those two groups could be separated using social avoidance test, based on the natural propensity of mice to interact with unfamiliar mouse34. Here, the social avoidance test, performed 24 h after the end of the last defeat session, revealed that 59% of total mice subjected to the stress protocol were susceptible and 41% were resilient, as expected from data in the literature10. We assessed the effect of chronic agomelatine treatment on stress-induced behavior and molecular modifications on susceptible mice only. To this aim, social avoidance test was replicated on D29, after a 3-week treatment with either agomelatine or its vehicle in CSDS-exposed mice, or vehicle in control mice. However, after this 3-week treatment delay, no statistical difference in the social avoidance test could be found between the 3 groups of mice. The loss of the stress effect, which was clearly present on D11, could result from a marked decrease in the interaction ratio mean of control group on D29 (−42%). According to previous work3, mice habituation or disinterest to arena exploration is unlikely to explain this behavior. Animal isolation11 and daily repeated vehicle injections35 which are known to be meaningful stressors may have contributed to this confounding effect. However, the fact that the interaction ratio of CSDS-exposed mice treated with agomelatine did not differ from that of vehicle-treated mice suggest that this antidepressant was ineffective to reverse a depression-related behavior in the present social avoidance test. Those results did not meet previous results since agomelatine after chronic administration at the same dose ranges has been shown to display significant efficacy in various preclinical depression-like models related to stress including the transgenic GR1 mouse36,37, the corticosterone-treated mouse38, the chronic mild stress in rats39 and the prenatally stressed rat40,41, but for most of them, the stress procedure was different and based on much milder stressors than those applied here. As expected from CSDS experiments, susceptible mice displayed a strong decrease in the interaction ratio mean of control mice, which was clearly present on D11, could result from a marked decrease in the interaction ratio mean of control group on D29 (−42%). According to previous work3, mice habituation or disinterest to arena exploration is unlikely to explain this behavior. Animal isolation11 and daily repeated vehicle injections35 which are known to be meaningful stressors may have contributed to this confounding effect. However, the fact that the interaction ratio of CSDS-exposed mice treated with agomelatine did not differ from that of vehicle-treated mice suggest that this antidepressant was ineffective to reverse a depression-related behavior in the present social avoidance test. Those results did not meet previous results since agomelatine after chronic administration at the same dose ranges has been shown to display significant efficacy in various preclinical depression-like models related to stress including the transgenic GR1 mouse36,37, the corticosterone-treated mouse38, the chronic mild stress in rats39 and the prenatally stressed rat40,41, but for most of them, the stress procedure was different and based on much milder stressors than those applied here. As expected from CSDS experiments, susceptible mice displayed a strong decrease in the interaction ratio mean of control mice, which was clearly present on D11, could result from a marked decrease in the interaction ratio mean of control group on D29 (−42%).
have been observed in mild stress paradigms, was not able to reverse the behavioral deficits induced by CSDS, in contrast to antidepressants of different families such as imipramine or fluoxetine\(^8^,47\).

However, and although it had no significant efficacy on anxiety- and depressive-like behaviors in the present study, agomelatine showed clear cut effects on CSDS-induced memory impairments measured with the NOR test, a well-validated paradigm for evaluating episodic memory in rodents\(^8^,32\). When testing the mice on D30, no deficit in the short-term memory of CSDS-subjected animals was observed in ITI1 conditions, but the memory performances of stressed-HEC mice were significantly altered in the ITI24 ones. These data corroborated previous studies that also described impaired cognition in social-defeated mice using the NOR test\(^8^,41\) with mnesic alterations in that case recorded at both short and long term, but measured 5 days only after the last defeat session. Interestingly, we showed that long term memory was still affected 3 weeks after CSDS. In addition, chronic agomelatine treatment reversed CSDS-induced cognitive dysfunction in this behavior, as previously demonstrated in various memory-like models. Indeed, agomelatine was shown to produce memory facilitating effects in the NOR task in naïve rats\(^25^,26\) or to improve mnesic alteration in mice exposed to unpredictable mild stress\(^37\). Agomelatine was also effective in enhancing animal performances in other cognition tests such as the radial-arm water maze\(^28\) and the Morris water maze\(^47\) and it improves social memory in prenatal stressed rats\(^29\). The positive profile of agomelatine on memory processes makes it stands out from classical antidepressant drugs, for which the effect on memory is still matter of debate (for exhaustive review see ref. 5).

The involvement of the neurotrophic factor BDNF in mnesic processes has been clearly demonstrated\(^40,51\). Lesion studies and inactivation models have shown that in the NOR test object recognition memory was hippocampus-dependent\(^52^,53\) when using long ITI (24 h), as we did presently, and not a short one, further supporting a delay-dependent role for the hippocampus in object recognition memory. Therefore, we quantified mRNA levels of Bdnf gene in this structure, and also two of its exons which have been shown to be reduced in CSDS, the Bdnf exons IV and VI\(^47\). In our conditions, CSDS failed to induce any modification in total Bdnf gene expression. This does not meet previous results that demonstrated a clear downregulation of total Bdnf gene in the hippocampus of social defeated mice, at both short and long term\(^47^,54^–^57\), although one study reported no change in the same conditions\(^58\). While Bdnf exon VI mRNA gene expression was also unchanged, Bdnf exon IV mRNA was significantly downregulated in CSDS stressed mice. Interestingly, this effect was abolished after a 3-week treatment with agomelatine. These data could be related to those published using imipramine, where a 10 day social defeat stress paradigm downregulated hippocampal Bdnf exon IV and a 4-week imipramine treatment restored basal mRNA level\(^47\).

Previous studies had also demonstrated the enhancing effect of chronic agomelatine (40–50 mg/kg i.p.) on hippocampal Bdnf gene expression both in naïve rodents\(^49^,41\) and in depressive like-models\(^27,37\). The particular regulation of the Bdnf IV transcript had already been proposed to be crucial in the mechanism of action of antidepressant drugs\(^37\). Interestingly, this transcript is known to selectively stimulate proximal dendrites growth in primary cultures of rat hippocampal neurons\(^8\). Therefore, it can be proposed that in CSDS, the decrease in Bdnf exon IV gene expression might led to a reduced synaptic plasticity leading to the cognitive impairments observed in the NOR test.

To further evaluate this hypothesis, we then quantified hippocampal mRNA levels of synaptic plasticity markers, especially those of genes that have already been associated with cognitive alterations\(^36^–^39\). While PSD-95, Synaptophysin, Spinaophilin and Synapsin I mRNA levels remained unchanged whatever the conditions, CSDS decreased MAP-2 gene expression and this effect was prevented by agomelatine treatment. MAP-2 is a protein that regulates microtubules and dendritic remodeling\(^28\). Long-term reduced hippocampal expression of the latter gene has been previously described in mice subjected to stress protocols\(^48\), but to our knowledge, this is the first study revealing social stress-induced MAP-2 gene expression modifications. Ladurelle et al.\(^26\) have shown that a 22-day agomelatine treatment was able to strongly increase the expression of MAP-2 protein in the hippocampus of naïve rats. These last data supported our present results that showed that chronic agomelatine administration also prevented the alterations on MAP-2 gene expression induced by CSDS, although in our conditions, changes in MAP-2 gene expression induced by CSDS are rather mild, and should be supported by further morphological studies, to address its functional relevance. However, those changes suggested that social stress-induced down-regulation of MAP-2 along with Bdnf exon IV might has provoked dendritic or synaptic alterations, leading to cognitive impairments and that chronic agomelatine could exert a protective effect on mice mnemonic functions by preventing Bdnf IV and MAP-2 gene expression deficits.

Whether the alterations in Bdnf and MAP-2 gene expression were supported by alterations in epigenetic regulations was assessed by studying hippocampal gene expression of various epigenetic modifying enzymes. RT-qPCR experiments revealed a downregulation of HDAC2, and HDAC6 gene expression in vehicle-treated CSDS mice. Interestingly, chronic agomelatine reversed these alterations. Since these epigenetic markers are a marker of active transcription\(^9\), it appears that they were probably not related to the downregulation of Bdnf exon IV and MAP-2 expression observed after CSDS. However, the effect of agomelatine on HDAC2 mRNA is consistent with the work of Boulle et al.\(^39\), wherein a 25-day agomelatine treatment (50 mg/kg/d i.p.) was able to prevent hippocampal HDAC2 downregulation observed in C57BL/6 mice exposed to chronic ultra mild stress, but had no effect by itself in non-stressed mice. Moreover, HDAC2 expression downregulation in the hippocampus was also observed just after a chronic restraint stress\(^68\), in a maternal separation model\(^80\) and in a genetic model of depression based on impaired glucocorticoid receptor expression\(^70\), suggesting that HDAC2 alteration is a long lasting phenomenon. In CSDS, Covington et al.\(^71\) also evidenced a reduced HDAC2 mRNA and protein levels in the nucleus accumbens 24 h and 2 weeks after the last defeat episode. HDAC6 mRNA level has already been shown to be decreased in the dorsal raphe of CSDS-exposed mice\(^72\), but to our knowledge, our study is the first which demonstrated such regulation in the hippocampus. In addition, we also described a stress-induced modification in the expression of MLL3. MLL3 is a histone H3K4-specific methyltransferase\(^81\), and hypermethylation at this site is known to have a positive impact on gene transcription\(^74^,75\). Consequently, a reduced MLL3 mRNA
level in this structure might lead to a general decrease in histone H3K4 methylation, therefore inhibiting gene transcription, which could explain CSDS-induced Bdnf exon IV and MAP-2 downregulation. Chronic agomelatine, normalizing MLL3 mRNA level, could prevent the deleterious stress effect on the latter gene expression. Nevertheless, no formal link can be found in the literature between MLL3 and these two genes. Gupta et al. showed that in rats, memory formation was associated with increased global hippocampal H3K4 methylation and data suggested that this hypermethylation was set at Bdnf exon I, but not exon IV, promoter. However, it is interesting to note that MLL3 has also been involved in genome-scale circadian transcription further linking the antidepressant efficacy of agomelatine to its circadian effect.

In conclusion, we showed that agomelatine could reverse the cognitive and gene-related effects of social defeat stress in mice. In our conditions, CSDS induced long lasting deleterious effects on C57BL/6J social behavior, anxiety- and memory-related behaviors as well as hippocampal gene expression. While chronic agomelatine treatment was ineffective in social and anxiety-like behaviors, it significantly reversed the memory impairments observed in the NOR test. These data provide evidences that agomelatine could be a relevant therapy to alleviate depression-associated cognitive disorders. CSDS-induced mnesic deficits might be related to Bdnf exon IV and MAP-2 gene expression alteration. Chronic agomelatine prevented these effects, possibly in part through epigenetic-related mechanisms. However, further studies are needed to evaluate the direct impact of CSDS and agomelatine on epigenetic marks and homeostasis at the hippocampus and to clarify through which mechanisms this compounds could achieve its effects.

References
1. Wood, A. M., Boyce, C. L., Moore, S. C. & Brown, G. D. An evolutionary based social rank explanation of why low income predicts mental distress: a 17 year cohort study of 30,000 people. J. Affect. Disord. 136, 882–888 (2012).
2. Bjorkqvist, K. Social defeat as a stressor in humans. Physiol. Behav. 73, 435–442 (2001).
3. Chaouloff, F. Social stress models in depression research: what do they tell us? Cell Tissue Res. 354, 179–190 (2013).
4. Nurius, P. S., Uehara, E. & Zatzick, D. F. Intersection of Stress, Social Disadvantage, and Life Course Processes: Reframing Trauma and Mental Health. Am. J. Psychiatr. Rehabil. 16, 91–114 (2013).
5. Athanassiadou, A., Han M. H., Graham D. L., Berton O., Renthal W., Russo S. J. et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391–404 (2007).
6. Conradi, H. J., Ormel, J. & de Jonge, P. Presence of individual (residual) symptoms during depressive episodes and periods of remission: a 3-year prospective study. Psychol. Med. 41, 1165–1174 (2011).
7. Gonda, X. et al. The role of cognitive dysfunction in the symptoms and remission from depression. Ann. Gen. Psychiat. 14, 27 (2015).
8. Miczek, K. A., Yap, J. J. & Covington, H. E. 3rd. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. Pharmacol. Ther. 120, 102–128 (2008).
9. Berton, O. et al. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864–866 (2006).
10. Zhao, T., Krishnan V., Han M. H., Graham D. L., Berton O., Renthal W., Russo S. J. et al. MLL3: a gene for individual differences in the stress response. Proc. Natl. Acad. Sci. USA 100, 753, 19–31 (2003).
11. Venzala, E., Garcia-Garcia, A. L., Elizalde, N. & Tordera, R. M. Social vs. environmental stress models of depression from a neurobehavioral perspective. Neuropsychopharmacol. 23, 697–708 (2003).
12. Barik, J. et al. Chronic stress triggers social aversion via glucocorticoid receptor in dopaminergic neurons. Science 339, 332–335 (2013).
13. Iniguez, S. D. et al. Social defeat stress induces a depression-like phenotype in adolescent male C57BL/6 mice. Stress 17, 247–255 (2014).
14. Wang, X. D. et al. Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendiritic remodeling. Neurobiol. Dis. 42, 300–310 (2011).
15. Yu, T. et al. Cognitive and neural correlates of depression-like behaviour in socially defeated mice: an animal model of depression with cognitive dysfunction. Int. J. Neuropsychopharmacol. 14, 303–317 (2011).
16. Zhao, T. et al. Effects of chronic social defeat stress on behavior and choline acetyltransferase, 78-kDa glucose-regulated protein, and CCAAT/enhancer-binding protein (C/EBP) homologous protein in adult mice. Psychopharmacology 228, 217–230 (2013).
17. Frankland, P. W. & Bontempi, B. The organization of recent and remote memories. Nat Rev. Neurosci. 6, 119–130 (2005).
18. Buvvala, B. et al. Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning. Neurosci. Biobehav. Rev. 29, 83–97 (2005).
19. Bondi, C. O., Rodriguez, G., Gould, G. G., Frazer, A. & Morilak, D. A. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmacol. 33, 320–331 (2008).
20. Elizalde, N. et al. Long-lasting behavioural effects on recognition memory deficit induced by chronic mild stress in mice: effect of antidepressant treatment. Psychopharmacol. 199, 1–14 (2008).
21. Naudon, L., Hotte, M. & Tay, T. M. Effects of acute and chronic antidepressant treatments on memory performance: a comparison between paroxetine and imipramine. Psychopharmacology 191, 353–64 (2007).
22. Valluzzi, J. A. & Chan, K. Effects of fluoxetine on hippocampal-dependent and hippocampal independent learning tasks. Behav. Pharmacol. 18, 507–13 (2007).
23. de Bodinat, C. et al. Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. Nat. Rev. Drug Discov. 9, 628–642 (2010).
24. Guardiola-Lemaître, B. et al. Agomelatine: mechanism of action and pharmacological profile in relation to antidepressant properties. Br. J. Pharmacol. 171, 3601–3619 (2014).
25. Bertram, A. et al. Agomelatine blocks the adverse effects of stress on memory and enables spatial learning to rapidly increase neural cell adhesion molecule (NCAM) expression in the hippocampus of rats. Int. J. Neuropsychopharmacol. 12, 329–341 (2009).
26. Barik, J. et al. The effects of antidepressant treatment in prenatally stressed rats support the glutamatergic hypothesis of stress-related disorders. J. Neurosci. 34, 2015–2024 (2014).
30. Bouille, E. et al. Hippocampal and behavioral dysfunctions in a mouse model of environmental stress: normalization by agomelatine. *Transl. Psychiatry* 4, e485 (2014).

31. Golden, S. A., Covington, H. E. 3rd, Berton, O. & Russo, S. J. A standardized protocol for repeated social defeat stress in mice. *Nat. Protoc.* 6, 1183–1191 (2011).

32. Chuang, J. C. et al. A β3-adrenergic-leptin-melanocortin circuit regulates behavioral and metabolic changes induced by chronic stress. *Biol. Psychiatry* 67, 1075–1082 (2010).

33. Stragier, E. et al. Brain plasticity and cognitive functions after ethanol consumption in C57BL/6j mice. *Transl. Psychiatry* 5, e696 (2015).

34. Vialou, V. et al. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat. Neurosci.* 13, 745–752 (2010).

35. Appleyard, M. E., Taylor, S. C. & Little, H. J. Acetylcholinesterase activity in regions of mouse brain following acute and chronic treatment with a benzodiazepine inverse agonist. *Br. J. Pharmacol.* 101, 599–604 (1990).

36. Paizanis, E. et al. Behavioural and neuroplastic effects of the new-generation antidepressant agomelatine compared to fluoxetine in glucocorticoid receptor-impaired mice. *Int. J. Neuropsychopharmacol.* 13, 759–774 (2010).

37. Bouille, F. et al. Behavioral and neurochemical characterization of TrkB-dependent mechanisms of agomelatine in glucocorticoid receptor-impaired mice. *Eur. Neuropsychopharmacol.* 26, 65–77 (2016).

38. Rainer, Q. et al. Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety. *Int. J. Neuropsychopharmacol.* 15, 321–332 (2012).

39. Papp, M., Gruca, P., Boyer, P. A. & Mocaer, E. Effect of agomelatine in the chronic mild stress model of depression in the rat. *Neuropsychopharmacology* 28, 694–703 (2003).

40. Morley-Fletcher, S. et al. Chronic agomelatine treatment corrects behavioral, cellular, and biochemical abnormalities induced by prenatal stress in rats. *Psychopharmacology* 217, 301–313 (2011).

41. Jin, H. M. et al. The effects of social defeat on behavior and dopaminergic markers in mice. *Neuroscience* 288, 167–177 (2015).

42. Liu, B. et al. Icaritin exerts an antidepressant effect in an unpredictable chronic mild stress model of depression in rats and is associated with the regulation of hippocampal serotonin innervation. *Neuroscience* 294, 193–205 (2015).

43. Millan, M. J. Serotonin 5-HT2C receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. *Therapie* 60, 441–460 (2005).

44. Papp, M., Lütwa, E., Gruca, P. & Mocaer, E. Anxiolytic-like activity of agomelatine and melatonin in three animal models of anxiety. *Behav. Pharmacol.* 17, 9–18 (2006).

45. Tuma, J. et al. Anxiolytic-like action of the antidepressant agomelatine (S 20098) after a social defeat requires the integrity of the scn. *Eur. Neuropsychopharmacol.* 15, 545–553 (2005).

46. Koolhaas, J. M. et al. Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.* 35, 1291–301 (2011).

47. Tsankova, N. M. et al. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat. Neurosci.* 9, 519–525 (2006).

48. Hutchinson, A. N., Deng, J. V., Cohen, S. & West, A. E. Phosphorylation of MeCP2 at Ser421 contributes to chronic antidepressant action. *J. Neurosci.* 32, 14355–14363 (2012).

49. Leger, M. et al. Object recognition test in mice. *Nat. Protoc.* 8, 2531–2537 (2013).

50. Andero, R., Choi, D. C. & Ressler, K. J. BDNF-TrkB receptor regulation of distributed adult neural plasticity, memory formation, and psychiatric disorders. *Prog. Mol. Biol. Transl. Sci.* 122, 169–192 (2014).

51. Kuhn, M., Popovic, A. & Pezawas, L. Neuroplasticity and memory formation in major depressive disorder: an imaging genetics perspective on serotonin and BDNF. *Restor. Neurol. Neurosci.* 32, 25–49 (2014).

52. Hammond, R. S., Tull, L. E. & Stackman, R. W. On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol. Learn. Mem.* 82, 26–34 (2004).

53. Broadbent, N. J., Gaskin, S., Squire, L. R. & Clark, R. E. Object recognition memory and the rodent hippocampus. *Learn. Mem.* 17, 5–11 (2010).

54. Wu, X., Wu, J., Xia, S., Li, B. & Dong, J. Icaritin opposes the development of social aversion after defeat stress via increases of GR mRNA and BDNF mRNA in mice. *Behav. Brain Res.* 256, 602–608 (2013).

55. Haenisch, B., Bilkei-Gorzo, A., Caron, M. G. & Bonisch, H. Knockout of the norepinephrine transporter and pharmacologically diverse antidepressants prevent behavioral and brain neurotrophin alterations in two chronic stress models of depression. *J. Neurochem.* 111, 403–416 (2009).

56. Komatsu, H. et al. Decreased response to social defeat stress in mu-opioid-receptor knockout mice. *Pharmacol. Biochem. Behav.* 99, 676–682 (2011).

57. Jiang, B., Huang, C., Zhu, Q., Tong, L. J. & Zhang, W. WY14643 produces anti-depressant-like effects in mice via the BDNF signaling pathway. *Psychopharmacology* 232, 1629–1642 (2015).

58. Brown, R. W. et al. Eszopiclone facilitation of the antidepressant efficacy of fluoxetine using a social defeat stress model. *Pharmacol. Biochem. Behav.* 99, 648–658 (2011).

59. Banasr, M., Soumier, A., Hery, M., Mocaer, E. & Daxzuta, A. Agomelatine, a new antidepressant, induces regional changes in hippocampal neurogenesis. *Biol. Psychiatry* 59, 1087–1096 (2006).

60. Soumier, A. et al. Mechanisms contributing to the phase-dependent regulation of neurogenesis by the novel antidepressant, agomelatine, in the adult rat hippocampus. *Neuropsychopharmacology* 34, 2390–2403 (2009).

61. Calabrese, F. et al. Modulation of neuroplastic molecules in selected brain regions after chronic administration of the novel antidepressant agomelatine. *Psychopharmacology* 215, 267–275 (2011).

62. Baj, G. et al. Physical exercise and antidepressants enhance BDNF targeting in hippocampal CA3 dendrites: further evidence of a spatial code for BDNF splice variants. *Neuropsychopharmacology* 37, 1600–1611 (2012).

63. Bianchi, M. et al. Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. *Eur. J. Neurosci.* 24, 2894–2902 (2006).

64. Biala, Y. N., Bogoch, Y., Bejar, C., Linial, M. & Weinstock, M. Prenatal stress diminishes gender differences in behavior and in expression of hippocampal synaptic genes and proteins in rats. *Hippocampus* 21, 1114–1125 (2011).

65. Wadhwa, M. et al. Caffeine and modafinil given during 48 h sleep deprivation modulate object recognition memory and synaptic proteins in the hippocampus of the rat. *Behav. Brain Res.* 294, 95–101 (2015).

66. Yang, C. R. et al. Enhanced aggressive behaviour in a mouse model of depression. *Neurotox. Res.* 27, 129–142 (2015).

67. Puchalski, M. et al. The potential use of histone deacetylase inhibitors in the treatment of depression. *Prog. Neurobiol.* 94, 320–324 (2016).

68. Han, A., Sung, Y. B., Chung, S. Y. & Kwon, M. S. Possible additional antidepressant-like mechanism of sodium butyrate: targeting the hippocampus. *Neuropharmacology* 81, 292–302 (2014).

69. Suri, D., Bhattacharya, A. & Vaidya, V. A. Early stress evokes temporally distinct consequences on the hippocampal transcriptome, anxiety and cognitive behaviour. *Int. J. Neuropsychopharmacol.* 17, 289–301 (2014).

70. Massar, R., Mongeau, R. & Lanfumey, L. Beyond the monoaminergic hypothesis: neuroplasticity and epigenetic changes in a transgenic mouse model of depression. *Phil. Trans. R. Soc. B.* 367, 2483–2494 (2012).

71. Covington, H. E. 3rd. et al. Antidepressant actions of histone deacetylase inhibitors. *J. Neurosci.* 29, 11451–11460 (2009).
72. Espallergues, J. et al. HDAC6 regulates glucocorticoid receptor signaling in serotonin pathways with critical impact on stress resilience. *J. Neurosci.* **32**, 4400–4416 (2012).
73. Lee, S. et al. Crucial roles for interactions between MLL3/4 and INI1 in nuclear receptor transactivation. *Mol. Endocrinol.* **23**, 610–619 (2009).
74. Santos-Rosa, H. et al. Active genes are tri-methylated at K4 of histone H3. *Nature* **419**, 407–411 (2002).
75. Zegerman, P., Canas, B., Pappin, D. & Kouzarides, T. Histone H3 lysine 4 methylation disrupts binding of nucleosome remodeling and deacetylase (NuRD) repressor complex. *J. Biol. Chem.* **277**, 11621–11624 (2002).
76. Gupta, S. et al. Histone methylation regulates memory formation. *J. Neurosci.* **30**, 4400–4416 (2012).
77. Valekunja, U. K. et al. Histone methyltransferase MLL3 contributes to genome-scale circadian transcription. *Proc. Natl. Acad. Sci. USA* **110**, 1554–1559 (2013).

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V.M., L.L., N.A., P.F., S.L., E.M. and C.G. designed the study. V.M., L.L., M.E., T.M., A.R., and B.F. contributed to data collection. V.M., L.L. and M.E. analyzed experimental data, N.A., P.F., S.L., E.M. and C.G. were involved in discussing data content. V.M. and L.L. wrote the manuscript, P.F., S.L., E.M. and C.G. participated in its reviewing.

**Additional Information**
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**Competing Interests:** E.M. and C.G. are employed by IRIS, Suresnes, France. The other authors declare no potential conflict of interest.

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