Early life stress induces age-dependent epigenetic changes in p11 gene expression

Mi Kyoung Seo

Paik Institute for Clinical Research, Inje University

Jung Goo Lee

Inje University Haeundae Paik Hospital

Sung Woo Park (✉ swpark@inje.ac.kr)

Inje University, College of Medicine

Research Article

Keywords: Depression, DNA methylation, early life stress, histone modification, maternal separation, p11

Posted Date: December 9th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-118558/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Early life stress (ELS) causes long-lasting changes in depression-like behaviors through epigenetic mechanisms. However, little is known about the effects of ELS in adulthood, specifically across different age groups. In this study, the epigenetic modifications of p11 expression in adult mice subjected to ELS were investigated in different stages of adulthood. Pups experienced maternal separation (MS) for 3 h daily from postnatal day 1 to 21. At young and middle adulthood, behavior phenotypes, hippocampal p11 expression levels, and levels of histone acetylation and methylation and DNA methylation at the hippocampal p11 promoter were measured. Middle-aged, but not young adult, MS mice exhibited depression-like behavior in the forced swimming test. Concurrent with reduced hippocampal p11 levels, mice in both age groups showed decreases in histone acetylation and activating histone methylation as well as increases in repressive histone methylation at the p11 promoter. The extent of the reduction in gene expression and histone acetylation was much higher in middle than in young adulthood. Moreover, DNA methylation analysis of the p11 promoter revealed increased CpG methylation in middle-aged MS mice only. The results highlight the age-dependent deleterious effects of ELS on depression-like behavior and on the epigenetic modifications of p11 transcription.

Introduction

Children exposed to early life stress (ELS) such as neglect and abuse have a significantly increased risk of developing depression^{1,2}. In human and animal studies, ELS has been reported to induce a depression-like phenotype in adulthood^{3,4}. These studies are focused on the epigenetic mechanisms, for example, DNA methylation and histone modification, by which ELS may alter the expression of genes involved in the stress response, including brain-derived neurotrophic factor (BDNF), glucocorticoid receptor (GR; *NR3C1*), and corticotrophin-releasing factor^{3,4}. The emphasis of these papers is mainly on the detrimental effects of ELS. However, little is known regarding the effects of ELS on behaviors and epigenetic mechanisms over the lifespan, especially among different adult age groups.

DNA methylation and histone modification, two representative epigenetic mechanisms, control gene transcription by affecting chromatin remodeling⁵. DNA methylation at the 5'-C-phosphate-G-3' (CpG) dinucleotide is classically associated with transcriptional repression⁶. Regions with a high density of CpGs are known as CpG islands; they are often found in the gene regulatory regions of promoters. Methylated CpGs in a promoter can induce gene silencing by blocking transcription factor binding or by attracting proteins that cause chromatin remodeling⁶. Histone modifications affect chromatin structure via post-translational modification, for example, acetylation and methylation, of the lysine (K) residues in the histone tails⁷. Acetylation of histone H3 or H4 relaxes the interaction between DNA and histone, allowing the transcriptional machinery access to the promoter, thereby activating transcription⁷. K4 and K19 on histone H3 are commonly modified in gene transcription activation⁸. Histone methylation, in contrast, is associated with both gene activation (H3K4 and H3K36) and repression (H3K9, H3K27, and H4K20), depending on the K residue methylated and its valence state (i.e., mono-, di-, or tri-methylation)⁹.

In animals that had experienced ELS, those in young adulthood exhibit decreased H3K9 dimethylation (me2) at the BDNF IV promoter and, accordingly, increased BDNF IV expression, whereas those in middle adulthood show increased repressive H3K9me2 at the BDNF IV promoter and concurrent decreased BDNF IV expression¹⁰. Additionally, ELS was found to be associated with an age-dependent decline in cognition. In another study, ELS was demonstrated to be associated with long-term deleterious effects on the regulation of GR expression via histone acetylation and methylation as well as on depression-like behavior¹¹. The effects of ELS on the epigenetic regulation of other stress-related genes besides BDNF and GR are less well-known and warrant further investigation.

P11 (*S100A10*) plays an important role in depression and antidepressant action¹². Patients with depression show reduced p11 levels in the brain, and p11 knockout mice display a depression-like phenotype^{13–16}. Antidepressant therapy, including selective serotonin reuptake inhibitors, enhances p11 expression^{15,17,18}. Furthermore, p11 gene transfer therapy effectively reverses depression-like behavior in mice¹⁴. In addition, p11 is critical in the antidepressant actions of BDNF¹⁸. However, whether epigenetic regulation of the p11 gene following ELS is altered across the life span is unknown. Maternal separation (MS) is widely used as a model to examine the effects of ELS¹⁹. In this study, the effects of MS on histone modification and DNA methylation at the p11 promoter in the hippocampus was investigated as the animals aged.

Results

Effects of MS on depression-like behavior and hippocampal p11 expression in young adult and middle-aged mice

FST was used to evaluate depression-like phenotypes. Control and MS animals in young adulthood did not significantly differ in immobility time. Conversely, middle aged-MS animals were significantly more immobile than controls, indicating a depression-like phenotype (control = 31.03 ± 7.86 sec, MS = 78.18 ± 10.82 sec; $t = 3.411$, $p = 0.003$; Fig. 3A).

Hippocampal p11 mRNA expression levels between control and MS animals in young and middle adulthood were assessed. MS animals showed a significant reduction in p11 mRNA in young (control = 1.00 ± 0.13 , MS = 0.76 ± 0.12 ; $t = 2.277$, $p = 0.034$) and middle (control = 1.00 ± 0.11 , MS = 0.33 ± 0.14 ; $t = 8.644$, $p < 0.001$) adulthood (Fig. 3B). Moreover, the reduction was about 43% higher in middle-aged than in young adult MS animals.

Effects of MS on histone acetylation and methylation at the hippocampal p11 promoter of young adult and middle-aged mice

Epigenetic histone modifications at the p11 promoter were examined in control and MS mice in young and middle adulthood. Histone acetylation at the hippocampal p11 promoter was reduced in both young adult (control = 1.00 ± 0.11 , MS = 0.73 ± 0.09 ; $t = 3.172$, $p = 0.005$) and middle-aged (control = 1.00 ± 0.09 ,

MS = 0.38 ± 0.13 ; $t = 8.378$, $p < 0.001$) MS mice (Fig. 4A). The reduction was about 45% greater in middle-aged MS animals compared to those in young adulthood.

Similarly, H3K4 trimethylation, a marker of histone modification activation, of the p11 promoter was reduced in MS mice in young (control = 1.00 ± 0.08 , MS = 0.32 ± 0.17 ; $t = 8.063$, $p < 0.001$) and middle adulthood (control = 1.00 ± 0.13 , MS = 0.57 ± 0.15 ; $t = 3.997$, $p = 0.001$; Fig. 4B). In contrast, H3K27 trimethylation, a marker of histone modification repression, was increased greatly in the MS group of both young (control = 1.00 ± 0.12 , MS = 1.54 ± 0.13 ; $t = 3.422$, $p = 0.003$) and middle adulthood (control = 1.00 ± 0.14 , MS = 1.68 ± 0.13 ; $t = 3.882$, $p = 0.001$; Fig. 4C).

Effects of MS on DNA methylation at the hippocampal p11 promoter of young adult and middle-aged mice

CpG methylation at the p11 promoter was examined in the hippocampus of young adult and middle-aged MS and control animals. While DNA methylation at the p11 promoter did not differ between MS and control mice in young adulthood, it was significantly increased in the middle-aged MS group (control = $100 \pm 11.36\%$, MS = $141.20 \pm 7.89\%$; $t = 3.051$, $p = 0.006$; Fig. 5).

Discussion

This paper reports that MS in early life exerts persistent effects on depression-like behavior and on epigenetic mechanisms associated with decreased p11 expression in adulthood, and these effects become more pronounced with age. MS had no effect on behavior in young adult mice, but depression-like behavior appeared in middle age. The distinct behavioral changes observed between young adult and middle-aged MS mice are likely to be due to long-term deleterious effects associated with changes in hippocampal p11 expression via histone modification and DNA methylation at its promoter.

In a past study, young adult (2 months) MS mice did not exhibit depression-like behavior in the FST; however, MS mice in middle adulthood (8 months) showed increased immobility time¹¹, a finding replicated here. Most MS studies report depression-like behaviors in the FST in young adult animals²⁰, although some papers are inconsistent. The findings seem to depend on the strain of mice used in the study. Similar to the MS model used here, Ruiz *et al.* (2018) found that MS (3 h daily from PND 1 to PND 14) in rats increased immobility time at two ages; furthermore, middle-aged (10 months) rats had longer immobility times than young adult (4 months) rats²¹. Taken together, early MS seems to induce more severe depressive-like behaviors in middle than in young adulthood.

In this study, MS is associated with a reduction of hippocampal p11 expression at two time points in adulthood. Previous studies have demonstrated that chronic stress in adults induces p11 loss, as well as depression-like behaviors^{22,23}. This study is the first to show an association between ELS and p11 levels. In a study using yeast two-hybrid screening, p11 was initially identified as a binding protein to the serotonin 1B (5-HT_{1B}) receptor; the 5-HT_{1B} receptor regulates serotonin neurotransmission¹⁵. p11

interacts with the 5-HT_{1B} receptor by increasing its trafficking to the cell surface, where it binds to serotonin released from presynaptic neurons. Consequently, 5-HT_{1B} receptor signaling efficacy is enhanced. In mice, p11 overexpression increases 5-HT_{1B} receptor function and is associated with reduced immobility in the tail suspension test. In contrast, in p11 knockout mice, the number of 5-HT_{1B} receptors is reduced at the cell membrane. Notably, MS (3 h daily from PND 2 to PND 13) in a rat ELS model reduced hippocampal 5-HT_{1B} receptor binding when assayed through [¹²⁵I] cyanopindolol autoradiography²⁴. The reduced binding may result from ELS-induced reductions of p11 levels.

Reduced hippocampal p11 expression in young adult and middle-aged MS animals was associated with altered histone modifications (i.e., significant decreases in H3 acetylation and H3K4me3 and a significant increase in H3K27me3) at the p11 promoter region. Moreover, p11 expression levels and H3 acetylation after MS were more severely perturbed in middle adulthood. Suri *et al.* (2014) demonstrated that MS induces opposing age-dependent effects on the expression of histone modifying enzymes such as histone deacetylase (*Hdac1* to *Hdac11*) and histone methyltransferase (*G9a* and *Suv391*) in young adulthood (2 months) versus middle adulthood²⁵. Nonetheless, the altered expression of these enzymes did not affect overall H3 acetylation, H3K9me2, and H3K9me3 at either of the ages. Although histone modifying enzymes may not globally change histone modification across the lifespan, these enzymes may differentially regulate histone modifications at the promoters of stress-related genes during aging. A loss of balance between epigenetic modifications during aging is referred to as “epigenetic drift”²⁶. The global distribution of many histone acetylation and methylation modifications (i.e., H3K27me3, H3K56ac, and H4K16ac) has been found to be altered during aging in various organisms^{27,28}. Accordingly, MS animals in middle age, but not young adulthood, show histone modifications associated with decreased BDNF IV expression concurrent with impairments in hippocampal-dependent cognition¹⁰. A similar age-dependent effect on histone acetylation and methylation at the GR exon I₇ promoter has been observed¹¹. Taken together, middle-aged MS animals may exhibit more severe changes in histone modification than young adult MS animals due to age-related epigenetic drift.

Increased DNA methylation in p11 promoter regions following MS were only present in middle-aged mice, not those in young adulthood. The increased DNA methylation, along with the altered histone modifications, could account for the reduced p11 expression in middle-aged MS animals. In accordance, a negative correlation between p11 gene transcription and CpG DNA methylation at the p11 promoter has been reported²⁹. Moreover, reduced p11 levels in a genetic rodent model of depression were associated with higher DNA methylation, while escitalopram treatment elevated p11 levels and reduced DNA methylation of the p11 promoter.

In rodents, the absence of maternal care such as licking, grooming, and arched-back nursing, is associated with hypothalamic-pituitary-adrenal (HPA) axis dysregulation as identified by increased glucocorticoid levels³⁰. The lack of maternal care modifies DNA methylation, resulting in decreased hippocampal GR expression³¹. Accordingly, adults with histories of childhood maltreatment show

increased DNA methylation of the GR exon I_F promoter and, consequently, increased HPA activity^{32,33}. GR acts as a ligand-activated transcription factor. When activated by glucocorticoids, GR translocates to the nucleus and binds to GR binding sites within the promoters of target genes, thereby activating or repressing their expression³⁴. p11 is a target gene for GRs. Putative GR binding sites were identified using the web-based tools described in the Materials and Methods section; this site includes one CpG site (Fig. 2). Zhang *et al.* (2008) reported that GR increases p11 promoter activity via the interaction of glucocorticoid-bound GR with GR binding sites³⁵. Thus, elevated GR levels may activate p11 transcription. Hippocampal GR expression levels have been found to be reduced in young adult and middle-aged MS animals and, furthermore, the reduction in middle adulthood was remarkably higher than in young adulthood¹¹. Thus, the differential reduction in p11 levels in middle-aged MS animals could be due to CpG methylation of the GR binding site, which prevents GR from easily accessing the p11 promoter, resulting in reduced p11 transcriptional activity. Nevertheless, further experiments are needed to address whether MS causes an age-dependent decrease in nuclear GR levels.

In conclusion, this study demonstrates that ELS induces long-term deleterious effects on depression-like behavior and hippocampal p11 expression through altered epigenetic modifications of the p11 promoter during adulthood. Furthermore, the deleterious effects are more severe with age.

Methods

Animals

The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the College of Medicine Inje University (approval no. 2016-053) and performed in accordance with the IACUC and the ARRIVE³⁶ guidelines. All animals were anesthetized with carbon dioxide (CO₂) according to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020 Edition). Pregnant C57BL/6J mice (Daehan Biolink, Chungbuk, Korea) arrived at the Inje Medical College animal facility on gestation day 15. Each dam and its litter were housed in standard cages under standard laboratory conditions (21 °C, 12 h/12 h light/dark cycle, food and water available ad libitum).

Maternal separation (MS)

Litters were divided randomly into control and MS groups. Pups in the MS group were separated from their mothers for 3 h daily starting on postnatal day (PND) 1 to PND 21 as described previously³⁷. Pups in the control group were left undisturbed except during routine animal facility handling. Male control and MS mice were assayed at either 2 months old (young adulthood) or at 8 months old (middle adulthood; Fig. 1).

Forced swimming test (FST)

To assess depression-like behavior, the FST was performed in control and MS mice at either young ($n = 10-12/\text{group}$) or middle ($n = 11-13/\text{group}$) adulthood as described previously³⁷. Mice were individually placed in transparent plastic cylinders (25 cm height $\times$ 10 cm diameter) containing 12 cm of water (23–25 °C) for 7 min and recorded on video. After an initial 2-min habituation period, the time spent immobile during the remaining 5 min was analyzed.

Measurement of mRNA levels using quantitative real-time polymerase chain reaction (qRT-PCR)

Following the FST, whole brains were extracted ($n = 10-12/\text{group}$, young adulthood; $n = 11-13/\text{group}$, middle adulthood). The hippocampus was dissected from the brain; RNA isolation, cDNA synthesis, and qRT-PCR were performed on the hippocampal tissue as described previously³⁷. Gene-specific primers (Table 1) for p11 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used under the following conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec, 55 °C for 35 sec, and 72 °C for 35 sec. The cycle threshold (Ct) values were calculated automatically. Relative quantification was performed using the $2^{-\Delta\Delta\text{Ct}}$ comparative Ct method, where $\Delta\text{Ct} = \text{Ct}_{\text{target gene}} - \text{Ct}_{\text{GAPDH}}$. Final values are expressed relative to the control group.

Table 1
Primers used in the study.

Primer Sequence (5'–3')	
quantitative real-time polymerase chain reaction (qRT-PCR) for mRNA	
P11 mRNA ^{44*}	Forward TGCTCATGGAAAGGGAGTTC
	Reverse CCCC GCCACTATTGATAGAA
GAPDH mRNA ^{45#}	Forward AACAGCAACTCCCATTCTTC
	Reverse TGGTCCAGGGTTTCTTACTC
qRT-PCR for histone modification (chromatin immunoprecipitation [ChIP] assay)	
P11 promoter [†]	Forward CGTTCCTCCTGCTTATCTAG
(189 base-pair [bp]; base 93554641–93554829)	Reverse GCTCTTAGTATTTTCAGGGCA
qRT-PCR for DNA methylation (methylation-specific polymerase chain reaction [MSP] analysis)	
P11 promoter [†]	
Methylation-Specific:	Forward TTTGGTTATTGTGTTTTTCGAGAC
(150 bp; base 93554710–93554859)	Reverse ACCCTATTATAAACGTCCCTACGA
Unmethylation-Specific:	Forward TTTTGGTTATTGTGTTTTTTGAGAT
(155 bp; 93554609–93554863)	Reverse AACAACCCTATTATAAACATCCCTACA
* <i>Mus musculus</i> S100 calcium binding protein A10 (calpactin; S100a10), mRNA; NCBI Reference Sequence: NM_009112.2	
# <i>Mus musculus</i> glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pseudogene 14 (Gapdh-ps14) on chromosome 8; NCBI Reference Sequence: NG_007829.2	
† <i>Mus musculus</i> strain C57BL/6J chromosome 3, GRCm38.p4 C57BL/6J; NCBI Reference Sequence: NC_000069.6 (GenBank Assembly ID: GCF_000001635.24)	

Chromatin immunoprecipitation (ChIP) assays

Chromatin was extracted from the isolated hippocampus using a standard protocol (SimpleChIP® Plus Enzymatic Chromatic IP Kit; Cell Signaling, Beverly, MA, USA) as described previously ($n = 10–12$ /group, young adulthood; $n = 11–13$ /group, middle adulthood)³⁷. Primers were designed around a putative p11

promoter region (Table 1 and Fig. 2). Because the p11 promoter is not well characterized in mice, the region of interest was created based on previous data on epigenetic alterations in the rat p11 promoter^{29,38}. The putative proximal promoter of the p11 gene (~ 700 base-pair upstream region) includes transcription factor binding sites generated by PROMO^{39,40} and MotifMap^{41,42}, two freely available web-based tools that identify presumptive transcription factor binding sites (Fig. 2).

Chromatin was immunoprecipitated with antibodies against histone H3 acetylated at K9 and K14 (AcH3, 06-599; Millipore Sigma, Billerica, MA, USA)³⁷, histone H3 trimethylated at K4 (H3K4me3, ab8580; Abcam, Cambridge, MA, USA)¹¹, and histone H3 trimethylated at K27 (H3K27me3, ab6002; Abcam)¹¹ using a SimpleChIP® Plus Enzymatic Chromatic IP Kit. To confirm antibody specificity, chromatin samples were immunoprecipitated with ChIP antibodies and normal rabbit IgG (#2729; Cell Signaling). qRT-PCR was performed on purified DNA using a control primer set (SimpleChIP® Mouse RPL30 Intron 2 Primers #7015; Cell Signaling) and p11 promoter primers (Figure S1). Ct values were normalized to input DNA. The $2^{-\Delta\Delta Ct}$ comparative Ct method was used for relative quantification, where $\Delta Ct = Ct_{\text{immunoprecipitation}} - Ct_{\text{input}}$. Final values are expressed relative to control group.

Methylation-specific polymerase chain reaction (MSP) analysis

Genomic DNA was extracted from the hippocampus using a QIAGEN DNA prep kit (51036; Valencia, CA, USA) and treated with bisulfite using an EpiTect® Bisulfite kit (59104; QIAGEN). To determine the DNA methylation status of CpG in the p11 promoter region, a qRT-PCR was performed on the same amount of bisulfite-treated DNA using an EpiScope® MSP kit (#R100A; TaKaRa, Otsu, Japan) containing SYBR green (TaKaRa). Specific primers for methylated or unmethylated p11 promoters were designed using MethPrimer⁴³. Primers include either a 150 base-pair (methylation-specific) or 155 base-pair (unmethylation-specific) region with 5 CpGs sites in the p11 promoter region (Fig. 2). Primer sequences are listed in Table 1. The p11 promoter region was confirmed to be amplified with methylation- and unmethylation-specific primers (Figure S2). The qPCR reaction conditions were as follows: initial denaturation at 95 °C for 30 sec, followed by 40 cycles of denaturation at 98 °C for 5 sec, annealing at 53 °C for 30 sec, and extension at 72 °C for 1 min. Ct values were normalized to GAPDH, and differences in methylation and unmethylation between control and MS groups ($n = 10-12/\text{group}$, young adulthood; $n = 11-13/\text{group}$, middle adulthood) were calculated using the $2^{-\Delta\Delta Ct}$ comparative Ct method. Relative levels of methylated DNA (%) were calculated according to the following formula: methylated rate (%) = methylated DNA / (methylated DNA + unmethylated DNA) $\times$ 100.

Statistical analysis

GraphPad Prim 8.0 (La Jolla, USA) was used for statistical analysis. All data are presented as the mean $\pm$ standard error of the mean (SEM). Because control and MS groups were compared in young *or* middle adulthood, the data were analyzed using unpaired Student's t-tests.

Data Availability

The data are available from the corresponding author upon request.

Declarations

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R1D1A1B07049481 to J.G. Lee and NRF-2020R1I1A3060731 to M.K. Seo) & the Korea government (MIST) (NRF-2020R1A2C1010148 to S.W. Park).

Author Contributions

Conceptualization, S.W.P. and J.G.L.; Experiments and Analysis, M.K.S., S.W.P. and J.G.L.; Writing – Original Draft, S.W.P.; Writing-Assistance, M.K.S.; Supervision, S.W.P. and J.G.L.

Competing Interests

The authors declare no conflict of interest.

References

1. Bernet, C.Z. & Stein, M.B. Relationship of childhood maltreatment to the onset and course of major depression in adulthood. *Depress. Anxiety* **9**, 169–174 (1999).
2. Burgess, R.L. & Conger, R.D. Family interaction in abusive, neglectful, and normal families. *Child Dev.* **49**, 1163–1173 (1978).
3. Lutz, P.E. & Turecki, G. DNA methylation and childhood maltreatment: from animal models to human studies. *Neuroscience* **264**, 142–156 (2014).
4. Zannas, A.S. & West, A.E. Epigenetics and the regulation of stress vulnerability and resilience. *Neuroscience* **264**, 157–170 (2014).
5. Jaenisch, R. & Bird, A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* **33**, 245–254 (2003).
6. Jones, P.A. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* **13**, 484–492 (2012).
7. Kouzarides, T. Chromatin modifications and their function. *Cell* **128**, 693–705 (2007).
8. Turner, B.M. Cellular memory and the histone code. *Cell* **111**, 285–291 (2002).
9. Izzo, A. & Schneider, R. Chatting histone modifications in mammals. *Brief Funct. Genomics* **9**, 429–443 (2010).

10. Suri, D., *et al.* Early stress evokes age-dependent biphasic changes in hippocampal neurogenesis, BDNF expression, and cognition. *Biol. Psychiatry* **73**, 658–666 (2013).
11. Seo, M.K., *et al.* Effects of early life stress on epigenetic changes of the glucocorticoid receptor 1₇ promoter during adulthood. *Int. J. Mol. Sci.* **21**, 6331; 10.3390/ijms21176331 (2020).
12. Svenningsson, P., Kim, Y., Warner-Schmidt, J., Oh, Y.S. & Greengard, P. p11 and its role in depression and therapeutic responses to antidepressants. *Nat. Rev. Neurosci.* **14**, 673–680 (2013).
13. Anisman, H., *et al.* Serotonin receptor subtype and p11 mRNA expression in stress-relevant brain regions of suicide and control subjects. *J. Psychiatry Neurosci.* **33**, 131–141 (2008).
14. Alexander, B., *et al.* Reversal of depressed behaviors in mice by p11 gene therapy in the nucleus accumbens. *Sci. Transl. Med.* **2**, 54ra76; 10.1126/scitranslmed.3001079 (2010).
15. Svenningsson, P., *et al.* Alterations in 5-HT_{1B} receptor function by p11 in depression-like states. *Science* **311**, 77–80 (2006).
16. Zhang, L., *et al.* P11 (S100A10) as a potential biomarker of psychiatric patients at risk of suicide. *J. Psychiatr. Res.* **45**, 435–441 (2011).
17. Egeland, M., Warner-Schmidt, J., Greengard, P. & Svenningsson, P. Neurogenic effects of fluoxetine are attenuated in p11 (S100A10) knockout mice. *Biol. Psychiatry* **67**, 1048–1056 (2010).
18. Warner-Schmidt, J.L., *et al.* A role for p11 in the antidepressant action of brain-derived neurotrophic factor. *Biol. Psychiatry* **68**, 528–535 (2010).
19. Levine, S. Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* **30**, 939–946 (2005).
20. Tractenberg, S.G., *et al.* An overview of maternal separation effects on behavioural outcomes in mice: Evidence from a four-stage methodological systematic review. *Neurosci. Biobehav. Rev.* **68**, 489–503 (2016).
21. Ruiz, R., *et al.* Early life stress accelerates age-induced effects on neurogenesis, depression, and metabolic risk. *Psychoneuroendocrinology* **96**, 203–211 (2018).
22. Seo, J.S., *et al.* Cellular and molecular basis for stress-induced depression. *Mol. Psychiatry* **22**, 1440–1447 (2017).
23. Seo, M.K., Lee, J.G. & Park, S.W. Effects of escitalopram and ibuprofen on a depression-like phenotype induced by chronic stress in rats. *Neurosci. Lett.* **696**, 168–173 (2019).
24. Shrestha, S.S., *et al.* Antidepressant effects on serotonin 1A/1B receptors in the rat brain using a gene x environment model. *Neurosci. Lett.* **559**, 163–168 (2014).
25. 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).
26. Li, Y. & Tollefsbol, T.O. Age-related epigenetic drift and phenotypic plasticity loss: implications in prevention of age-related human diseases. *Epigenomics* **8**, 1637–1651 (2016).

27. Maures, T.J., Greer, E.L., Hauswirth, A.G. & Brunet, A. The H3K27 demethylase UTX-1 regulates *C. elegans* lifespan in a germline-independent, insulin-dependent manner. *Aging Cell* **10**, 980–990 (2011).
28. Dang, W., *et al.* Histone H4 lysine 16 acetylation regulates cellular lifespan. *Nature* **459**, 802–807 (2009).
29. Melas, P.A., *et al.* Antidepressant treatment is associated with epigenetic alterations in the promoter of P11 in a genetic model of depression. *Int. J. Neuropsychopharmacol.* **15**, 669–679 (2012).
30. Liu, D., *et al.* Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* **277**, 1659–1662 (1997).
31. Weaver, I.C., *et al.* Epigenetic programming by maternal behavior. *Nat. Neurosci.* **7**, 847–854 (2004).
32. McGowan, P.O., *et al.* Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat. Neurosci.* **12**, 342–348 (2009).
33. Perroud, N., *et al.* Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl. Psychiatry* **1**, e59; 10.1038/tp.2011.60 (2009).
34. Newton, R. & Holden, N.S. Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor? *Mol. Pharmacol.* **72**, 799–809 (2007).
35. Zhang, L., *et al.* p11 is up-regulated in the forebrain of stressed rats by glucocorticoid acting via two specific glucocorticoid response elements in the p11 promoter. *Neuroscience* **153**, 1126–1134 (2008).
36. Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. & Altman, D.G. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol.* **8**, e1000412; 10.1371/journal.pbio.1000412. (2010).
37. Seo, M.K., *et al.* Early life stress increases stress vulnerability through BDNF gene epigenetic changes in the rat hippocampus. *Neuropharmacology* **105**, 388–397 (2016).
38. Theilmann, W., *et al.* Behavioral differences of male Wistar rats from different vendors in vulnerability and resilience to chronic mild stress are reflected in epigenetic regulation and expression of p11. *Brain Res.* **1642**, 505–515 (2016).
39. Farré, D., *et al.* Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. *Nucleic Acids Res.* **31**, 3651–3653 (2003).
40. Messeguer, X., *et al.* PROMO: detection of known transcription regulatory elements using species-tailored searches. *Bioinformatics* **18**, 333–334 (2002).
41. Daily, K., Patel, V.R., Rigor, P., Xie, X. & Baldi, P. MotifMap: integrative genome-wide maps of regulatory motif sites for model species. *BMC Bioinformatics* **12**, 495; 10.1186/1471-2105-12-495 (2011).
42. Xie, X., Rigor, P. & Baldi, P. MotifMap: a human genome-wide map of candidate regulatory motif sites. *Bioinformatics* **25**, 167–174 (2009).

43. Li, L.C. & Dahiya, R. MethPrimer: designing primers for methylation PCRs. *Bioinformatics* **18**, 1427–1431 (2002).
44. Brkic, Z., *et al.* Distinct modifications of hippocampal glucocorticoid receptor phosphorylation and FKBP5 by lipopolysaccharide in depressive female and male rats. *J. Psychopharmacol.* **31**, 1234–1249 (2017).
45. Schmidt, H.D., *et al.* Increased brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area during cocaine abstinence is associated with increased histone acetylation at BDNF exon I-containing promoters. *J Neurochem.* **120**, 202–209 (2012).

Figures

Figure 1

Experimental design timeline. Pups were separated from their mothers for 3 h daily from postnatal day (PND) 1 to PND 21. When the pups reached young adulthood (2 months) or middle age (8 months), they were subjected to the forced swimming test (FST). Following the FST, the mice were sacrificed for the p11 molecular analysis.

androgen receptor binding site, and the second encircled region is the putative glucocorticoid receptor binding site. CpG sites investigated in this study are underlined and bolded.

A.

B.

Figure 3

Changes in depression-like behavior and hippocampal p11 levels in young adult and middle-aged mice following maternal separation (MS) exposure. (A) Immobility time in the FST was measured at 2 months (young adulthood) or 8 months (middle adulthood). (B) p11 mRNA levels in the hippocampus were measured using quantitative real-time polymerase chain reaction (qRT-PCR). All quantities were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data are expressed as values relative to the control group using the $2^{-\Delta\Delta C_t}$ method and are presented as mean $\pm$ standard error of the mean (SEM; $n = 10-12$ /group, young adulthood; $n = 11-13$ /group, middle adulthood; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, unpaired Student's t-test).

A.

B.

C.

Figure 4

Alterations in histone acetylation and methylation of the hippocampal p11 promoter in young adult and middle-aged mice following MS exposure. The levels of histone H3 acetylation (AcH3, A), histone H3 trimethylation at lysine residue 4 (H3K4me3, B), and histone H3 trimethylation at lysine residue 27 (H3K27me3, C) at the p11 promoter in the hippocampus were measured using a chromatin immunoprecipitation assay with antibodies to AcH3, H3K4me3, and H3K27me3. Data were normalized to input DNA and are expressed as values relative to the control group using the $2^{-\Delta\Delta Ct}$ method. The data are presented as mean $\pm$ SEM (n = 10–12/group, young adulthood; n = 11–13/group, middle adulthood; **p < 0.01, ***p < 0.001, unpaired Student's t-test).

Figure 5

Alterations in DNA methylation at the hippocampal p11 promoter in young adult and middle-aged mice following MS exposure. DNA methylation levels at the hippocampal p11 promoter were measured using methylation-specific polymerase chain reaction (MSP). All quantities were normalized to GAPDH. Data are expressed as values relative to the control group using the $2^{-\Delta\Delta Ct}$ method and are represented as mean $\pm$ SEM ($n = 10-12$ /group, young adulthood; $n = 11-13$ /group, middle adulthood; ** $p < 0.01$, unpaired Student's t-test).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalInformation201207.docx](#)