




Early-Life Adversity Induces Epigenetically Regulated Changes in Hippocampal Dopaminergic Molecular Pathways

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Abstract

Early-life adversity (ELA) represents a major risk factor for the development of behavioral dysfunctions and mental disorders later in life. On the other hand, dependent on type, time point, and duration, ELA exposure can also induce adaptations, which result in better stress coping and resilience later in life. Guided by the hypothesis that chronic exposure to ELA results in dysfunctional brain and behavior, whereas short exposure to ELA may result in resilience, the behavioral and neurobiological consequences of long-term separation stress (LTSS) and short-term separation stress (STSS) were compared in a mouse model for ELA. In line with our hypothesis, we found that LTSS induced depressive-like behavior, whereas STSS reduced depressive-like behavioral symptoms. We then tested the hypothesis that the opposite behavioral outcomes of the two stress paradigms may be mediated by functional, epigenetically regulated changes of dopaminergic modulation in the hippocampal formation. We found that STSS exposure elevated dopamine receptor D1 (DRD1) gene expression and decreased gene expression of its downstream modulator DARPP-32 (32-kDa dopamine- and cAMP-regulated phosphoprotein), which was paralleled by decreased H3 acetylation at its gene promoter region. In contrast, LTSS elevated DARPP-32 gene expression, which was not paralleled by changes in histone acetylation and DRD1 gene expression. These findings indicate that short- and long-term neonatal exposure to ELA induces changes in dopaminergic molecular pathways, some of which are epigenetically regulated and which either alleviate or aggravate depressive-like symptoms later in life.

Keywords Dopamine receptor 1 · DARPP-32 · Depression · Histone modification · Resilience

Introduction

Early-life adversity (ELA) affects brain development and has been identified as a major risk factor contributing to the etiology of mental disorders, including depression, anxiety and ADHD [1–8]. The particular outcome of ELA exposure has been shown to depend on the type, intensity, and duration of

the stressor [9–12]. While the majority of experimental studies focused on stress vulnerability and pathological behavioral outcomes [13–16], evidence is accumulating that acute, brief, or intermittent ELA can promote flexibility and adaptation of behavioral strategies to changing environments [17–20] through activating cellular mechanisms mediating stress resilience [21–24]. So far, the mechanisms determining stress vulnerability or resilience are not well understood. Key mechanisms conferring an individual's adaptation to environmental challenges include transient or lasting epigenetic alterations as has been shown in a variety of studies in animals and humans [3, 20, 25–30]. Clinical studies revealed that epigenetic modifications are involved in the development of various psychopathologies, such as bipolar disorder and schizophrenia, which are associated with changes in neurotransmitter functions [31, 32]. Since depressive symptoms include anhedonia and despair, dopaminergic modulation of brain regions, which are part of the “reward system,” is particularly affected [33–35]. Animal studies have shown that exposure to ELA as well as variations in maternal care interferes with the maturation of catecholaminergic innervation and dopaminergic

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function in prefronto-limbic circuits [36–42] and induces sex-specific changes in dopamine receptor D1 (DRD1) density [43] as well as epigenetic modifications regulating the expression of the DRD1 receptor [44, 45]. A key downstream regulator of dopaminergic signaling and mediator of actions and interactions of dopamine with other neurotransmitters is DARPP-32 (32-kDa dopamine- and cAMP-regulated phosphoprotein), which is phosphorylated upon activation of DRD1 receptors. DARPP-32 has been suggested to be involved in neuropsychiatric and neurodegenerative disorders associated with dopaminergic dysfunction [46–49]. For example, it was shown that specific polymorphisms in *PPP1R1B*, the gene encoding DARPP-32 in the human brain, were associated with the risk for schizophrenia [50] and may predict autism susceptibility [51]. Although such polymorphisms are not clearly evident in depression, evidence from human studies indicates a role for DARPP-32 in mood disorders [52]. An animal study described alterations of DARPP-32 in the prefronto-limbic system in mice developing depressive-like symptoms in response to psychosocial stress or social defeat [53, 54].

Based on this evidence, the aims of this study were to compare the behavioral and neurobiological consequences of short-term early-life separation stress (STSS) and long-term separation stress (LTSS) in a mouse model for ELA and to assess changes in DRD1 and DARPP-32 mediated via histone modifications.

Material and Methods

Animals

C57BL/6 mice were housed on a 12-h light-dark cycle with food and water provided ad libitum. During pregnancy, the home cages were cleaned once a week. After delivery of the pups (day of birth = postnatal day, PND 0), the home cages were not cleaned for the first 16 postnatal days to minimize stress for the mother and her pups. Males from litters of approximately the same size (five to eight pups) were randomly assigned to the stress groups (see below) and the control groups. The experimental protocols were approved by the ethics committee of the government of the state of Saxony-Anhalt according to the German guidelines for the care and use of animals in laboratory research (§8 TSchG; AZ: 42502–2-1272).

Stress Paradigms

“Mild” Short-Term Separation Stress (STSS) Pups of this group were separated from their mother at PND 14–16 using the same separation conditions as described for the LTSS group (see below). After the last separation session on PND 16, the

pups remained undisturbed until weaning on PND 21. On PND 21, the animals were reared in groups with a maximum of five individuals until the onset of the experiments.

Control (CON) for STSS Paradigm Animals of this control group lived undisturbed with their mother and littermates. After weaning at PND 21, they were group housed with a maximum of five same-sex individuals until the time of the respective experiment.

“Chronic” Long-Term Separation Stress (LTSS) Pups of this group were exposed to daily maternal separation from PND 1 to PND 21 by removing them from the home cage and individually placed in isolation boxes (13 × 13 cm, covered with paper bedding) for 3 h each day (9:00–12:00), which allowed olfactory and auditory but no visual or body contact with their separated siblings. The dam remained undisturbed in the home cage. Prior to the return of the pups, fresh nesting material was provided. After weaning on PND 21, the animals were housed individually until the time of the respective experiment.

Control (CON) for LTSS Paradigm Animals of this control group lived undisturbed with their mother and littermates. After weaning at PND 21, they were group housed with a maximum of five same-sex individuals until the time of the respective experiment.

Distribution of Animals

In the STSS experiments, 75 control and 67 stressed animals derived from 24 and 24 litters, respectively, were used. The LTSS experiments included 80 control animals from 24 litters and 50 stressed animals from 20 litters.

Forced Swim Test

To test for depressive-like behavioral traits, the STSS and their respective control group were subjected to the forced swim test (FST) on PND 62 and 63. On the first day, the animals were habituated to the test situation by transferring them for 15 min to a glass container filled with 22 °C tempered water. On the subsequent test day, the behavior was videotaped during a 15-min trial. Active swimming and passive floating behavior during the first 5 min of the FST were quantified with the “Observer” (Noldus, Wageningen, Netherlands) software. Results for the LTSS group have been published in a previous paper [15].

For the FST in the STSS experiments, 21 control animals and 20 stressed animals, each derived from 6 litters, were used. For the LTSS experiments, 45 control animals derived from 11 litters and 21 stressed animals from 7 litters were used. For the statistical analysis, N = number of animals was used.

Tissue Preparation

Animals of all experimental and control groups were decapitated on PND 64. Brain tissue of the hippocampal formation was collected and frozen on liquid nitrogen and stored at -80°C . Tissue for gene expression and western blot analysis was derived from the same animals, i.e., the hippocampus of the left hemisphere was used for protein extraction, and tissue from the right hippocampus underwent RNA extraction. Tissue for native chromatin immunoprecipitation (nChIP) was derived from additional animals. Hippocampal tissue of the LTSS group from our previous study [15] was collected and analyzed in the same way.

Gene Expression

Expression of DRD1 and DARPP-32 mRNA was quantified by real-time quantitative PCR (qPCR). RNA extraction was performed using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany). Genomic DNA was removed utilizing the RNase-free DNase Kit (Qiagen GmbH, Hilden, Germany). Gene expression analysis was carried out with the Rotor-Gene Multiplex RT-PCR Kit (Qiagen GmbH, Hilden, Germany), which allows the execution of simultaneous one-step quantitative real-time PCR of two to five genes using TaqMan gene expression assays. Commercially available assays for the dopamine D1 receptor (DRD1; Mm01353211_m1_Drd1a) and the dopamine- and cAMP-regulated neuronal phosphoprotein 32-kDa protein (DARPP-32; Mm00454892_m1_Ppp1r1b) were used. As a reference gene, hypoxanthine phosphoribosyltransferase I (Hprt I; Mm01545399_m1; VIC) was used. Gene expression of DRD1, DARPP-32, and Hprt was calculated utilizing the delta-delta CT method [55]. The samples were normalized to their respective control groups.

For gene expression analysis for the STSS experiments, hippocampal tissue of 41 control animals and 30 stressed animals, each derived from 9 litters, was used, and for the LTSS experiments, 24 control animals derived from 7 litters and 23 stressed animals derived from 6 litters were used. For statistical analysis, N = number of animals was used.

Histone Acetylation

Acetylation of H3 and H4 was assessed by quantitative western blot (WB) analysis. Tissue samples were homogenized in extraction buffer (0.1 M Tris/HCl pH 8.0; 0.01 M EDTA; 10% SDS; $1\times$ Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, Waltham, MA, USA)) using ultrasonic vibration. After centrifugation, the supernatant was removed and protein concentration was measured using the Bio-Rad DCTM Universal Protein Assay Kit II (Bio-Rad, Hercules, CA, USA). SDS-PAGE was performed using a 20- μl reaction

volume per lane, which contained 50 μg protein mixed with loading buffer and Bio-Rad Tris-Glycine Mini-PROTEAN TGX Precast Gels (Bio-Rad, Hercules, CA, USA). The prestained protein molecular weight marker PeqGOLD Protein Marker V (PeqLab/VWR International GmbH, Darmstadt, Germany) was used to monitor the progress of SDS-PAGE and to assess transfer efficiency onto the membrane during western blot. After gel electrophoresis, the samples were blotted onto a nitrocellulose membrane (GE Healthcare, Chalfont St Giles, UK), and the proteins were visualized by Red Alert staining (Novagen, brand by Merck KGaA, Darmstadt, Germany). The blots were blocked by using Roti-Block (Carl Roth, Karlsruhe, Germany) working solution followed by overnight incubation with primary antibodies anti-acetyl H3 (#06-599, 1:10,000; Merck Millipore, Billerica, MA, USA), anti-acetyl H4 (#06-866, 1:4000; Merck Millipore, Billerica, MA, USA), and antihypoxanthine phosphoribosyltransferase I (#ab10479, 1:500; Abcam, Cambridge, UK) at 4°C . The blots were then incubated for 1 h at room temperature with a secondary antibody (horseradish peroxidase conjugated anti-rabbit) (#12-348, 1:4000; Novagen, brand by Merck Millipore, Billerica, MA, USA). Horseradish peroxidase was detected by using Luminata Crescendo Western HRP substrate (Merck Millipore, Billerica, MA, USA), and signals were detected by using Syngene G:Box system (Syngene Europe, Cambridge, UK). Western blot data were analyzed using Gene Tools software (Syngene Europe, Cambridge, UK).

Histone acetylation analysis for the STSS experiments was conducted in hippocampal tissue from 48 control animals and 41 stressed animals, each derived from 12 litters. For the LTSS experiments, 29 control animals and 23 stressed animals, each derived from 7 litters, were used. For statistical analysis, N = number of litters.

Histone Acetylation at the DARPP-32 Promoter

H3 and H4 acetylation associated to DARPP-32 expression was quantified by nChIP. Tissue samples were homogenized in 500 μl MNase Reaction Buffer (10 mM Tris pH 8.8, 1 mM CaCl_2). After adding 10 units MNase (Affymetrix, Cleveland, OH, USA), the samples were incubated for 2 min at 37°C . The enzymatic reaction was blocked by adding 10 μl 0.5 M EDTA. The samples were mixed with 5 ml 0.02 mM EDTA and 6 μl 100 mM PMSF and were incubated for 1 h on ice while mixing them every 10 min. After the incubation period, 9 μl 1 M DTT was added and the samples were centrifuged at $4000\times g$ for 10 min at 4°C . The supernatant was divided into four parts: 500 μl input and $3\times 1500\ \mu\text{l}$ H3, H4, and control. The three latter samples (H3, H4, control) were incubated for 2 h at room temperature with the respective antibodies: anti-acetyl H3 (#06-599, 1:10,000; Merck Millipore, Billerica, MA, USA) and anti-acetyl H4 (#06-866, 1:4000; Merck

Millipore, Billerica, MA, USA). Protein A/G Magnetic Beads (Thermo Fisher Scientific, Waltham, MA, USA) were used for immunoprecipitation. Before use, the beads were washed twice with TBS wash buffer containing 0.05% Tween-20 and 0.5 M NaCl. The beads and the ChIP-antibody samples were incubated overnight at 4 °C while rotating. Beads were separated from unbound sample fraction and washed twice using TBS wash buffer and once with dH₂O. The DNA was then eluted in 100 µl low pH elution buffer (0.1 M glycine pH 2.0). After incubating for 10 min, the eluates were separated from the beads and pH was neutralized with 15 µl 1 M Tris pH 7.5. Subsequently, the samples were subjected to protein digestion by adding 400 µl Tris-HCl pH 7.5, 20 µl Tris-HCl pH 6.8, 20 µl 5 M NaCl, 10 µl 0.5 mM EDTA pH 8.0, and 1 µl 20 mg/ml Proteinase K (Roche Diagnostics, Rotkreuz, Switzerland) and incubated overnight.

DNA was extracted from the sample by adding 500 µl phenol/chloroform/isoamylalcohol (Carl Roth GmbH, Karlsruhe, Germany). DNA purification was performed using the MinElute Reaction CleanUp Kit (Qiagen GmbH, Hilden, Germany). ChIP quantitative real-time PCR was carried out using the Rotor-Gene Multiplex PCR Kit (Qiagen, Hilden, Germany). A custom-made FAM-coupled TaqMan assay targeting the DARPP-32 promoter region was used (Life Technologies, Carlsbad, CA, USA).

In both the STSS and the LTSS experiments, histone acetylation at the DARPP-32 promoter was analyzed in hippocampal tissue from six control animals and six stressed animals, each derived from six litters. For statistical analysis, *N* = number of animals was used.

Statistical Analyses

The data for each experiment were tested for normal distribution using the D'Agostino and Pearson omnibus normality test. If normally distributed, data were analyzed for significance with a two-sided unpaired *t* test. Non-normally distributed data were tested for significance with a Mann-Whitney *U* test. Data of the gene expression analysis were normalized to controls. All data are presented as means ± SEM. Significance was set at **p* ≤ 0.05 and ***p* ≤ 0.01 for all data sets. Statistical analysis was performed and graphs were produced using GraphPad Prism 6.0 software (GraphPad, La Jolla, CA, USA).

Results

Behavior

STSS resulted in reduced depressive-like behavior in adulthood, measured as lower time of immobility (floating) compared to controls (*p* = 0.005, Table 1; *N*: CON = 21, STSS = 20). In contrast, LTSS (PND 1–21) induced enhanced

Table 1 Duration of immobility in the forced swim test

Treatment	<i>N</i>	Duration of immobility (mean ± SEM) (s)	<i>p</i> value
STSS	Stressed	20 68.67 ± 9.76	0.005
	Control	21 124.1 ± 14.7	
LTSS	Stressed	21 195.6 ± 10.69 ^a	0.015
	Control	45 161.8 ± 7.73 ^a	

^a Published in [15]

depressive-like behavior measured as elevated duration of immobility as published in a previous study [15].

Gene Expression

In the hippocampus of the STSS group, increased DRD1 expression was found compared to the control group (*p* = 0.023, Fig. 1a; *N*: CON = 39, STSS = 28), whereas in LTSS animals, DRD1 expression remained unchanged compared to controls (*p* = 0.433, Fig. 1a; *N*: CON = 24, LTSS = 22). Expression of DARPP-32 was decreased in the STSS group (*p* = 0.049, Fig. 1b; *N*: CON = 41, STSS = 30), whereas it was increased in the LTSS group (*p* = 0.033, Fig. 1b; *N*: CON = 24, LTSS = 22).

Histone Acetylation

STSS decreased histone H3 acetylation in the hippocampus compared to control animals (*p* = 0.0147, Fig. 2a; *N*: CON = 11, STSS = 11), and no significant changes were detected in histone H4 acetylation (*p* = 0.248, Fig. 2c; *N*: CON = 12, STSS = 12). LTSS did not affect histone H3 and H4 acetylation (*p* = 0.459, Fig. 2a; and *p* = 0.497, Fig. 2c; *N*: CON = 7, LTSS = 7).

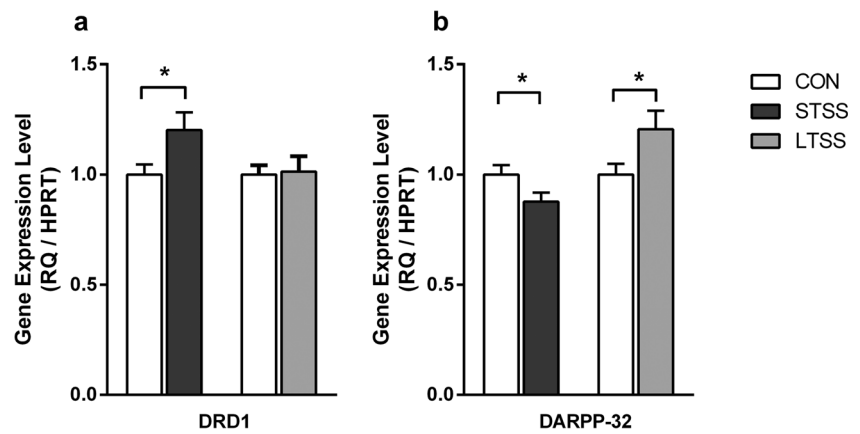
Histone Acetylation at the DARPP-32 Promoter

nChIP-qPCR revealed that the reduced expression of DARPP-32 was associated with reduced acetylation of H3 at the promoter region of its gene (*p* = 0.0252, Fig. 2b; *N*: CON = 6, STSS = 5), while H4 acetylation at the promoter region of DARPP-32 was not altered (*p* = 0.5414, Fig. 2d; *N*: CON = 6, STSS = 6). In the LTSS group, H3 and H4 acetylation at the DARPP-32 promoter region was unchanged (*p* = 0.285, Fig. 2b; *p* = 0.264, Fig. 2d; *N*: CON = 7, LTSS = 7).

Discussion

In line with our working hypothesis, we show that exposure to “mild” STSS reduces depressive-like behavioral symptoms in adulthood, whereas a previous study [15] demonstrated that “chronic” LTSS increased depressive-like symptoms. We further found that the opposing

Fig. 1 ELA induces changes in DRD1 and DARPP-32 gene expression. **a** Short-term separation stress (STSS) induced an increase in DRD1 gene expression, whereas long-term separation stress (LTSS) had no effect on the expression of the DRD1 gene ($*p \leq 0.05$). **b** STSS induced reduced gene expression of DARPP-32; in contrast, LTSS induced increased DARPP-32 expression ($*p \leq 0.05$)

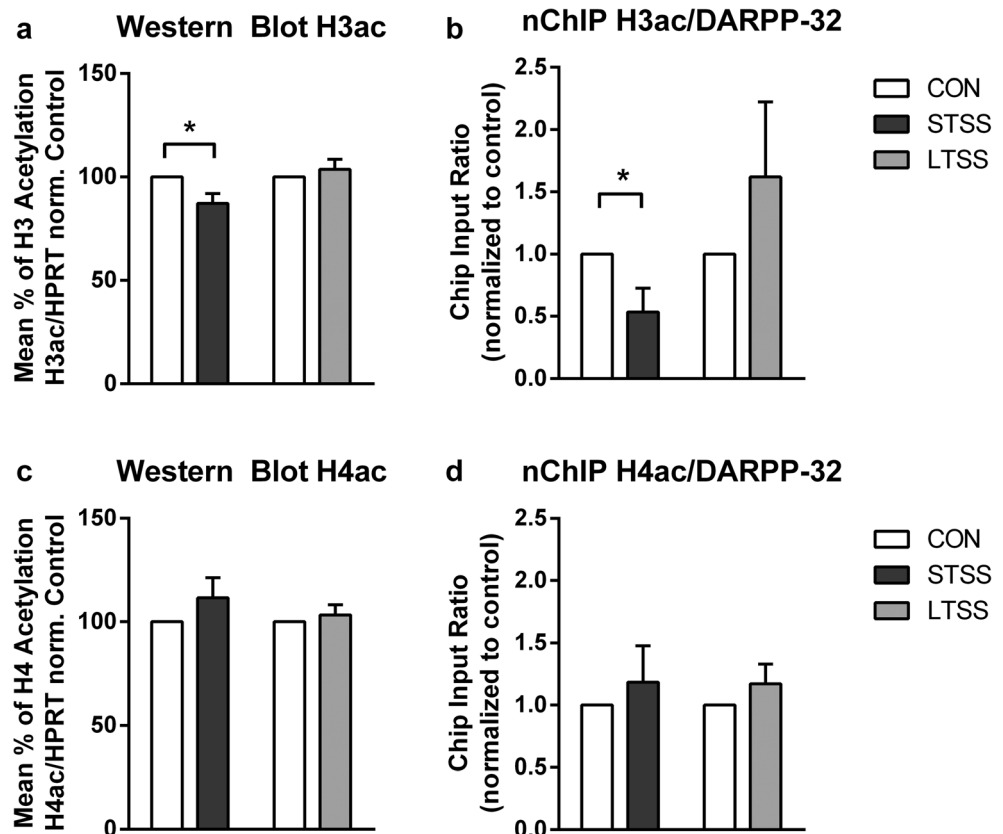


behavioral outcomes were paralleled by partly opposite changes in dopaminergic cellular pathways in the hippocampal formation. STSS elevated DRD1 gene expression and decreased gene expression of its downstream regulator DARPP-32, the latter of which is regulated via reduced H3 acetylation at its promoter region. In contrast, LTSS did not alter DRD1 gene expression and elevated DARPP-32 gene expression, which was not associated with changes in histone acetylation. Global hippocampal histone H3 acetylation was reduced after STSS but not after LTSS.

Methodological Considerations

With respect to our STSS paradigm, it is important to point out that this manipulation not only represents a mild, short-term stress experience but is also restricted to a defined developmental time window, i.e., after the end of the so-called stress hyporesponsive period (SHRP) of the HPA axis. In mice and rats, the SHRP is characterized by low levels of circulating corticosterone and a relative hyporesponsiveness to mild environmental stressors [56, 57]. Our previous studies in rats revealed that the extent and direction of stress-induced

Fig. 2 ELA-induced changes in histone acetylation. **a** STSS induced a reduction of H3 acetylation in the hippocampus, whereas LTSS had no effect ($*p \leq 0.05$). **b** STSS resulted in reduced H3 acetylation at the promoter region of DARPP-32, whereas LTSS had no effect ($*p \leq 0.05$). **c**, **d** Global H4 acetylation as well as DARPP-32 promoter-specific H4 acetylation was affected neither by STSS nor by LTSS



neuromorphological outcomes are dependent on the time point of the respective stress exposure (maternal separation), i.e., prior, during, or after the SHRP [58, 59]. We specifically selected a time window after the SHRP for this experiment since our previous study in mice demonstrated that stress exposure during this time window increases dendritic complexity and the number of excitatory spine synapses of hippocampal CA3 neurons [19], which we predicted to reflect positive behavioral outcome. Comparing the behavioral assessment during the FST, it is obvious that there are significant differences between the immobility times of the two separate control groups, i.e., the control group for the present study and the control group for the previous study [15]. The animals of the respective control groups were derived from completely different cohorts, and it is not unusual that basal behavioral parameters may differ between such separate samples. Hence, this finding emphasizes the importance of running parallel control animals for every experiment so that they can be internally compared to the respective experimental group. With respect to our findings, the difference between the two separate control groups does not affect the results, since the STSS animals show lower immobility levels than their respective control group and the LTSS animals display higher levels than their respective control group.

ELA-Induced Changes of Depressive-like Behavior Depend on Chronicity of Stress Experience

As stated above, the results of the present and previous studies [15] revealed opposite behavioral outcomes. First of all, it is important to state that due to the large spectrum of different maternal separation (MS) paradigms (procedure, timing, duration, etc.) used by different research groups, it is difficult to compare the specific outcomes that have been described [60–62]. Various studies have reported an increase in depressive-like behavior as a consequence of repeated MS [63–67], whereas evidence is accumulating from clinical and animal studies that stress experience early in life may also promote adaptive effects that are beneficial to emotional and cognitive development. Rats exposed to MS displayed reduced immobility time in the FST [68], and a combination of MS with reduced bedding material (maternal neglect paradigm) increased active behaviors in the FST [69]. A recent study in mice revealed that exposure to ELA induced by limited nesting and bedding material leads to better coping with challenging environments in adulthood [18]. These findings can be interpreted within the concept of the “stress-inoculation/induced resilience” hypothesis [70–81]. Along the same line, the present study revealed that STSS results in reduced “behavioral despair” symptoms (less floating), indicating better stress coping in this group, in contrast to animals exposed to LTSS, which displayed increased “behavioral despair” [15].

ELA “Reprograms” Dopaminergic System Development

The dopaminergic system is involved in the modulation of a number of important brain functions such as motor control, control of emotional behavior, reward, cognition, and decision-making. Consequently, dysfunctions in the dopaminergic system are implicated in a number of pathologies such as Parkinson’s disease, schizophrenia, depression, and ADHD. Studies in a variety of species and stress paradigms revealed that the development of the dopaminergic system is particularly sensitive toward ELA such as prenatal stress [4, 25] and neonatal stress experience [26, 36, 40, 43, 82–84]. With regard to changes of dopamine receptors, it has been reported in rats that MS (24 h on PND 9) increases the expression of DRD1 (and DRD2) receptors [16, 85]. Similarly, a study in male mandarin voles reports that early deprivation leads to an increase of D1 and D2 receptors [86]. Evidence for dysfunctional development of the dopaminergic system is also revealed by a series of studies in the biparental rodent *Octodon degus*, which show that exposure to repeated brief parental separation stress induces an increase in DRD1 receptor (and other receptors) expression in the hippocampal formation [43] and the amygdala (only in females). In addition, chronic, repeated exposure to parental separation resulted in decreased dopaminergic innervation in the hilus of the dentate gyrus and increased innervation in the stratum granulosum and subgranular layer, and changes in dopaminergic innervation were also observed in prefrontal and other limbic regions [38, 40, 87].

The reduced “behavioral despair” symptoms observed in STSS animals during the FST might be mediated by elevated expression of DRD1 in the hippocampal formation. This view is supported by pharmacological analyses in mice, which revealed that passive behavioral traits in the forced swim test induced by MS are specifically mediated by DRD1 [63]. Dopamine D1 receptor agonists were found to induce anti-immobility effects, and the “antidepressant” effect of imipramine can be antagonized by SCH 23390, a selective dopamine D1 receptor blocker [88, 89].

Exposure to STSS resulted in a downregulation of DARPP-32, a key mediator of dopaminergic transmission [46, 49, 90], whereas it was upregulated in the LTSS group. It is tempting to speculate that changes in hippocampal DARPP-32 gene expression may alter short-term and long-term hippocampal synaptic plasticity. DARPP-32 is required for LTP induction in the hippocampal-PFC pathway [91], and DRD1 activation is involved in late LTP [92–94]. Moreover, it was reported that LTP induction in hippocampal afferents into the PFC, which depends on DRD1 activation [95], was facilitated after short stress exposure, whereas prolonged exposure to stress impaired LTP induction [96].

ELA-Induced Downregulation of DARPP-32 Expression Is Mediated by Specific Epigenetic Histone Modification

First, the present study revealed a global reduction in H3 acetylation in the STSS but not in the LTSS group, which is in line with the concept that gene \times environment interactions are mediated by specific epigenetic modifications. With respect to ELA, a number of studies revealed an influence on epigenetic mechanisms that interfere with preprogrammed developmental processes resulting in adaptive or maladaptive neuronal and behavioral alterations [4, 97–104]. Epigenetic adaptations in response to early environmental challenges are dynamic multistep events starting with rapid and transient alterations, some of which eventually may become permanent epigenetic marks [75, 105]. Our results are in line with this dynamic concept as we observed a rapid increase in H3 as well as H4 acetylation in the hippocampus of juvenile STSS-exposed animals [19], while the present study demonstrates a lasting reduction of hippocampal H3 acetylation in adult STSS-exposed animals.

Second, we also show that the STSS-induced reduction in DARPP-32 gene expression is mediated by a reduction of H3 acetylation at the promoter region of the DARPP-32 gene, whereas elevation of DARPP-32 gene expression observed in the LTSS group appears not to be related to histone modifications; hence, it remains to be further investigated whether other epigenetic mechanisms, such as DNA methylation, may be involved.

In conclusion, this study revealed differential behavioral, molecular, and epigenetic changes in response to exposure to “mild” STSS and chronic LTSS, which are potentially related to improved (STSS exposure) or impaired (LTSS exposure) adaptability and coping toward environmental adversities later in life.

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Compliance with Ethical Standards

The experimental protocols were approved by the ethics committee of the government of the state of Saxony-Anhalt according to the German guidelines for the care and use of animals in laboratory research (§8 TSchG; AZ: 42502–2-1272).

Conflict of Interest The authors declare that they have no conflict of interest.

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References

- Babenko O, Kovalchuk I, Metz GAS (2015) Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. *Neurosci Biobehav Rev* 48:70–91. <https://doi.org/10.1016/j.neubiorev.2014.11.013>
- Bahari-Javan S, Varbanov H, Halder R, Benito E, Kaurani L, Burkhardt S, Anderson-Schmidt H, Angheliescu I et al (2017) HDAC1 links early life stress to schizophrenia-like phenotypes. *Proc Natl Acad Sci* 114:E4686–E4694. <https://doi.org/10.1073/pnas.1613842114>
- Bock J, Rether K, Gröger N, Xie L, Braun K (2014) Perinatal programming of emotional brain circuits: an integrative view from systems to molecules. *Front Neurosci* 8:1–16. <https://doi.org/10.3389/fnins.2014.00011>
- Braun K, Bock J, Wainstock T, Matas E, Gaisler-Salomon I, Fegert J, Ziegenhain U, Segal M (2017) Experience-induced transgenerational (re-)programming of neuronal structure and functions: impact of stress prior and during pregnancy. *Neurosci Biobehav Rev*:0–1. <https://doi.org/10.1016/j.neubiorev.2017.05.021>
- Burkholder AR, Koss KJ, Hostinar CE, Johnson AE, Gunnar MR (2016) Early life stress: effects on the regulation of anxiety expression in children and adolescents. *Soc Dev* 25:777–793. <https://doi.org/10.1111/sode.12170>
- Cirulli F, Berry A, Panetta P, Bellisario V, Capoccia S, Luoni A, Riva MA (2015) Prenatal stress as a risk factor for major depression: an investigation on therapeutic intervention with antipsychotics and the role of social stimuli during periadolescence. *Eur Psychiatry* 30:185. [https://doi.org/10.1016/S0924-9338\(15\)30149-8](https://doi.org/10.1016/S0924-9338(15)30149-8)
- Heim C, Nemeroff CB (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* 49:1023–1039. [https://doi.org/10.1016/S0006-3223\(01\)01157-X](https://doi.org/10.1016/S0006-3223(01)01157-X)
- Walker C-D, Bath KG, Joels M, Korosi A, Larauche M, Lucassen PJ, Morris MJ, Rainecki C et al (2017) Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress* 20:421–448. <https://doi.org/10.1080/10253890.2017.1343296>
- Branchi I, Santarelli S, D’Andrea I, Alleva E (2013) Not all stressors are equal: early social enrichment favors resilience to social but not physical stress in male mice. *Horm Behav* 63: 503–509. <https://doi.org/10.1016/j.yhbeh.2013.01.003>
- Gröger N, Matas E, Gos T, Lesse A, Poeggel G, Braun K, Bock J (2016) The transgenerational transmission of childhood adversity: behavioral, cellular, and epigenetic correlates. *J Neural Transm* 123:1037–1052. <https://doi.org/10.1007/s00702-016-1570-1>
- Liu H, Atrooz F, Salvi A, Salim S (2017) Behavioral and cognitive impact of early life stress: insights from an animal model. *Prog Neuro-Psychopharmacology Biol Psychiatry* 78:88–95. <https://doi.org/10.1016/j.pnpbp.2017.05.015>
- Molet J, Maras PM, Avishai-Eliner S, Baram TZ (2014) Naturalistic rodent models of chronic early-life stress. *Dev Psychobiol* 56:1675–1688. <https://doi.org/10.1002/dev.21230>
- de Azeredo LA, Wearick-Silva LE, Viola TW, Tractenberg SG, Centeno-Silva A, Orso R, Schröder N, Bredy TW et al (2017) Maternal separation induces hippocampal changes in cadherin-1 (CDH-1) mRNA and recognition memory impairment in adolescent mice. *Neurobiol Learn Mem* 141:157–167. <https://doi.org/10.1016/j.nlm.2017.04.006>
- Kuniishi H, Ichisaka S, Yamamoto M, Ikubo N, Matsuda S, Futora E, Harada R, Ishihara K et al (2017) Early deprivation increases high-leaning behavior, a novel anxiety-like behavior, in the open

- field test in rats. *Neurosci Res* 123:27–35. <https://doi.org/10.1016/j.neures.2017.04.012>
15. Lesse A, Rether K, Gröger N, Braun K, Bock J (2017) Chronic postnatal stress induces depressive-like behavior in male mice and programs second-hit stress-induced gene expression patterns of OxtR and AvpR1a in adulthood. *Mol Neurobiol* 54:4813–4819. <https://doi.org/10.1007/s12035-016-0043-8>
 16. Rentesi G, Antoniou K, Marselos M, Syrrou M, Papadopoulou-Daifoti Z, Konstandi M (2013) Early maternal deprivation-induced modifications in the neurobiological, neurochemical and behavioral profile of adult rats. *Behav Brain Res* 244:29–37. <https://doi.org/10.1016/j.bbr.2013.01.040>
 17. Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. *Nature* 455:894–902. <https://doi.org/10.1038/nature07455>
 18. Santarelli S, Zimmermann C, Kalideris G, Lesuis SL, Arloth J, Uribe A, Doumes C, Balsevich G et al (2017) An adverse early life environment can enhance stress resilience in adulthood. *Psychoneuroendocrinology* 78:213–221. <https://doi.org/10.1016/j.psyneuen.2017.01.021>
 19. Xie L, Korkmaz KS, Braun K, Bock J (2013) Early life stress-induced histone acetylations correlate with activation of the synaptic plasticity genes Arc and Egr1 in the mouse hippocampus. *J Neurochem* 125:457–464. <https://doi.org/10.1111/jnc.12210>
 20. Zannas AS, West AE (2014) Epigenetics and the regulation of stress vulnerability and resilience. *Neuroscience* 264:157–170. <https://doi.org/10.1016/j.neuroscience.2013.12.003>
 21. McEwen BS (2016) In pursuit of resilience: stress, epigenetics, and brain plasticity. *Ann N Y Acad Sci* 1373:56–64. <https://doi.org/10.1111/nyas.13020>
 22. Ricon T, Toth E, Leshem M, Braun K, Richter-Levin G (2012) Unpredictable chronic stress in juvenile or adult rats has opposite effects, respectively, promoting and impairing resilience. *Stress* 15:11–20. <https://doi.org/10.3109/10253890.2011.572207>
 23. Russo SJ, Murrough JW, Han M-H, Charney DS, Nestler EJ (2012) Neurobiology of resilience. *Nat Neurosci* 15:1475–1484. <https://doi.org/10.1038/nn.3234>
 24. Wang X-D, Schmidt MV (2016) Editorial: Molecular mechanisms for reprogramming hippocampal development and function by early-life stress. *Front Mol Neurosci* 9:9–11. <https://doi.org/10.3389/fnmol.2016.00006>
 25. Bock J, Wainstock T, Braun K, Segal M (2015) Stress in utero: prenatal programming of brain plasticity and cognition. *Biol Psychiatry* 78:315–326. <https://doi.org/10.1016/j.biopsych.2015.02.036>
 26. Matas E, Bock J, Braun K (2016) The impact of parent-infant interaction on epigenetic plasticity mediating synaptic adaptations in the infant brain. *Psychopathology* 49:201–210. <https://doi.org/10.1159/000448055>
 27. McClelland S, Korosi A, Cope J, Ivy A, Baram TZ (2011) Emerging roles of epigenetic mechanisms in the enduring effects of early-life stress and experience on learning and memory. *Neurobiol Learn Mem* 96:79–88. <https://doi.org/10.1016/j.nlm.2011.02.008>
 28. Roth TL (2013) Epigenetic mechanisms in the development of behavior: advances, challenges, and future promises of a new field. *Dev Psychopathol* 25:1279–1291. <https://doi.org/10.1017/S0954579413000618>
 29. Vaiserman AM (2015) Epigenetic programming by early-life stress: evidence from human populations. *Dev Dyn* 244:254–265. <https://doi.org/10.1002/dvdy.24211>
 30. Zucchi FCR, Yao Y, Ward ID, Ilnytsky Y, Olson DM, Benzie K, Kovalchuk I, Kovalchuk O et al (2013) Maternal stress induces epigenetic signatures of psychiatric and neurological diseases in the offspring. *PLoS One* 8:e56967. <https://doi.org/10.1371/journal.pone.0056967>
 31. Li Y, Camarillo C, Xu J, Arana TB, Xiao Y, Zhao Z, Chen H, Ramirez M et al (2015) Genome-wide methylome analyses reveal novel epigenetic regulation patterns in schizophrenia and bipolar disorder. *Biomed Res Int* 2015:1–15. <https://doi.org/10.1155/2015/201587>
 32. Ni X, Trakalo JM, Mundo E, Macciardi FM, Parikh S, Lee L, Kennedy JL (2002) Linkage disequilibrium between dopamine D1 receptor gene (DRD1) and bipolar disorder. *Biol Psychiatry* 52:1144–1150. [https://doi.org/10.1016/S0006-3223\(02\)01433-6](https://doi.org/10.1016/S0006-3223(02)01433-6)
 33. Belujon P, Grace AA (2015) Regulation of dopamine system responsivity and its adaptive and pathological response to stress. *Proc R Soc B Biol Sci* 282:20142516–20142516. <https://doi.org/10.1098/rspb.2014.2516>
 34. Gold MS, Blum K, Febo M, Baron D, Modestino EJ, Elman I, Badgaiyan RD (2018) Molecular role of dopamine in anhedonia linked to reward deficiency syndrome (RDS) and anti-reward systems. *Front Biosci (Schol Ed)* 10:309–325
 35. Grace AA (2016) Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nat Rev Neurosci* 17:524–532. <https://doi.org/10.1038/nrn.2016.57>
 36. Bock J, Breuer S, Poeggel G, Braun K (2017) Early life stress induces attention-deficit hyperactivity disorder (ADHD)-like behavioral and brain metabolic dysfunctions: functional imaging of methylphenidate treatment in a novel rodent model. *Brain Struct Funct* 222:765–780. <https://doi.org/10.1007/s00429-016-1244-7>
 37. Braun K, Bock J, Metzger M, Jiang S, Schnabel R (1999) The dorsocaudal neostriatum of the domestic chick: a structure serving higher associative functions. *Behav Brain Res* 98:211–218. [https://doi.org/10.1016/S0166-4328\(98\)00086-2](https://doi.org/10.1016/S0166-4328(98)00086-2)
 38. Gos T, Becker K, Bock J, Malecki U, Bogerts B, Poeggel G, Braun K (2006) Early neonatal and postweaning social emotional deprivation interferes with the maturation of serotonergic and tyrosine hydroxylase-immunoreactive afferent fiber systems in the rodent nucleus accumbens, hippocampus and amygdala. *Neuroscience* 140:811–821. <https://doi.org/10.1016/j.neuroscience.2006.02.078>
 39. Jezierski G, Zehle S, Bock J, Braun K, Gruss M (2007) Early stress and chronic methylphenidate cross-sensitize dopaminergic responses in the adolescent medial prefrontal cortex and nucleus accumbens. *J Neurochem* 103:2234–2244. <https://doi.org/10.1111/j.1471-4159.2007.04927.x>
 40. Kunzler J, Braun K, Bock J (2015) Early life stress and sex-specific sensitivity of the catecholaminergic systems in prefrontal and limbic regions of Octodon degus. *Brain Struct Funct* 220:861–868. <https://doi.org/10.1007/s00429-013-0688-2>
 41. Peña CJ, Neugut YD, Calarco CA, Champagne FA (2014) Effects of maternal care on the development of midbrain dopamine pathways and reward-directed behavior in female offspring. *Eur J Neurosci* 39:946–956. <https://doi.org/10.1111/ejn.12479>
 42. Schäble S, Poeggel G, Braun K, Gruss M (2007) Long-term consequences of early experience on adult avoidance learning in female rats: role of the dopaminergic system. *Neurobiol Learn Mem* 87:109–122. <https://doi.org/10.1016/j.nlm.2006.07.005>
 43. Ziabreva I, Poeggel G, Schnabel R, Braun K (2003) Separation-induced receptor changes in the hippocampus and amygdala of Octodon degus: influence of maternal vocalizations. *J Neurosci* 23:5329–5336
 44. Azogu I, de la Tremblaye PB, Dunbar M, Lebreton M, LeMarec N, Plamondon H (2015) Acute sleep deprivation enhances avoidance learning and spatial memory and induces delayed alterations in neurochemical expression of GR, TH, DRD1, pCREB and Ki67 in rats. *Behav Brain Res* 279:177–190. <https://doi.org/10.1016/j.bbr.2014.11.015>
 45. Sasagawa T, Horii-Hayashi N, Okuda A, Hashimoto T, Azuma C, Nishi M (2017) Long-term effects of maternal separation coupled

- with social isolation on reward seeking and changes in dopamine D1 receptor expression in the nucleus accumbens via DNA methylation in mice. *Neurosci Lett* 641:33–39. <https://doi.org/10.1016/j.neulet.2017.01.025>
46. Beaulieu J-M, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63:182–217. <https://doi.org/10.1124/pr.110.002642>
 47. Gould TD, Manji HK (2005) DARPP-32: a molecular switch at the nexus of reward pathway plasticity. *Proc Natl Acad Sci* 102:253–254. <https://doi.org/10.1073/pnas.0408700102>
 48. Nishi A, Shuto T (2017) Potential for targeting dopamine/DARPP-32 signaling in neuropsychiatric and neurodegenerative disorders. *Expert Opin Ther Targets* 21:259–272. <https://doi.org/10.1080/14728222.2017.1279149>
 49. Svenningsson P, Nishi A, Fisone G, Girault J-A, Nairn AC, Greengard P (2004) DARPP-32: an integrator of neurotransmission. *Annu Rev Pharmacol Toxicol* 44:269–296. <https://doi.org/10.1146/annurev.pharmtox.44.101802.121415>
 50. Meyer-Lindenberg A, Straub RE, Lipska BK, Verchinski BA, Goldberg T, Callicott JH, Egan MF, Huffaker SS et al (2007) Genetic evidence implicating DARPP-32 in human frontostriatal structure, function, and cognition. *J Clin Invest* 117:672–682. <https://doi.org/10.1172/JCI30413>
 51. Hettinger JA, Liu X, Hudson ML, Lee A, Cohen IL, Michaelis RC, Schwartz CE, Lewis SM et al (2012) DRD2 and PPP1R1B (DARPP-32) polymorphisms independently confer increased risk for autism spectrum disorders and additively predict affected status in male-only affected sib-pair families. *Behav Brain Funct* 8:19. <https://doi.org/10.1186/1744-9081-8-19>
 52. Amare AT, Schubert KO, Klingler-Hoffmann M, Cohen-Woods S, Baune BT (2017) The genetic overlap between mood disorders and cardiometabolic diseases: a systematic review of genome wide and candidate gene studies. *Transl Psychiatry* 7:e1007. <https://doi.org/10.1038/tp.2016.261>
 53. Azzinnari D, Sigrist H, Staehli S, Palme R, Hildebrandt T, Leparo G, Hengerer B, Seifritz E et al (2014) Mouse social stress induces increased fear conditioning, helplessness and fatigue to physical challenge together with markers of altered immune and dopamine function. *Neuropharmacology* 85:328–341. <https://doi.org/10.1016/j.neuropharm.2014.05.039>
 54. Jin HM, Shrestha Muna S, Bagalkot TR, Cui Y, Yadav BK, Chung YC (2015) The effects of social defeat on behavior and dopaminergic markers in mice. *Neuroscience* 288:167–177. <https://doi.org/10.1016/j.neuroscience.2014.12.043>
 55. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta(C)T)} method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
 56. Rosenfeld P, Suchecki D, Levine S (1992) Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. *Neurosci Biobehav Rev* 16:553–568
 57. Levine S (2001) Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. *Physiol Behav* 73:255–260. [https://doi.org/10.1016/S0031-9384\(01\)00496-6](https://doi.org/10.1016/S0031-9384(01)00496-6)
 58. Bock J, Gruss M, Becker S, Braun K (2005) Experience-induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlation with developmental time windows. *Cereb Cortex* 15:802–808. <https://doi.org/10.1093/cercor/bhh181>
 59. Gos T, Bock J, Poeggel G, Braun K (2008) Stress-induced synaptic changes in the rat anterior cingulate cortex are dependent on endocrine developmental time windows. *Synapse* 62:229–232. <https://doi.org/10.1002/syn.20477>
 60. Nishi M, Horii-Hayashi N, Sasagawa T (2014) Effects of early life adverse experiences on the brain: implications from maternal separation models in rodents. *Front Neurosci* 8:1–6. <https://doi.org/10.3389/fnins.2014.00166>
 61. Pryce CR, Rüedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, Feldon J (2005) Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. *Neurosci Biobehav Rev* 29:649–674. <https://doi.org/10.1016/j.neubiorev.2005.03.011>
 62. Schmidt MV, Wang XD, Meijer OC (2011) Early life stress paradigms in rodents: potential animal models of depression? *Psychopharmacology* 214:131–140. <https://doi.org/10.1007/s00213-010-2096-0>
 63. Amiri S, Haj-Mirzaian A, Amini-khoei H, Momeny M, Shirzadian A, Rahimi-Balaei M, Zarrinrad G, Ghazi-Khansari M et al (2016) NMDA receptor antagonists attenuate the proconvulsant effect of juvenile social isolation in male mice. *Brain Res Bull* 121:158–168. <https://doi.org/10.1016/j.brainresbull.2016.01.013>
 64. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM (2000) Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res* 122:81–103
 65. MacQueen GM, Ramakrishnan K, Ratnasingan R, Chen B, Young LT (2003) Desipramine treatment reduces the long-term behavioural and neurochemical sequelae of early-life maternal separation. *Int J Neuropsychopharmacol* 6:391–396. <https://doi.org/10.1017/S1461145703003729>
 66. Millstein RA, Holmes A (2007) Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. *Neurosci Biobehav Rev* 31:3–17. <https://doi.org/10.1016/j.neubiorev.2006.05.003>
 67. Rüedi-Bettschen D, Zhang W, Russig H, Ferger B, Weston A, Pedersen EM, Feldon J, Pryce CR (2006) Early deprivation leads to altered behavioural, autonomic and endocrine responses to environmental challenge in adult Fischer rats. *Eur J Neurosci* 24:2879–2893. <https://doi.org/10.1111/j.1460-9568.2006.05158.x>
 68. Kwak HR, Lei JW, Kwon KJ, Kang CD, Cheong Y, Chun W, Kim SS, Lee HJ (2009) Maternal social separation of adolescent rats induces hyperactivity and anxiolytic behavior. *Korean J Physiol Pharmacol* 13:79–83. <https://doi.org/10.4196/kjpp.2009.13.2.79>
 69. Fuentes IM, Walker NK, Pierce AN, Holt BR, Di Silvestro ER, Christianson JA (2016) Neonatal maternal separation increases susceptibility to experimental colitis and acute stress exposure in male mice. *IBRO Reports* 1:10–18. doi: <https://doi.org/10.1016/j.ibror.2016.07.001>
 70. Abraham A, Gruss M (2010) Stress inoculation facilitates active avoidance learning of the semi-precocial rodent *Octodon degus*. *Behav Brain Res* 213:293–303. <https://doi.org/10.1016/j.bbr.2010.05.018>
 71. Boyce WT, Ellis BJ (2005) Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Dev Psychopathol* 17:271–301
 72. Oitzl MS, Champagne DL, van der Veen R, de Kloet ER (2010) Brain development under stress: hypotheses of glucocorticoid actions revisited. *Neurosci Biobehav Rev* 34:853–866. <https://doi.org/10.1016/j.neubiorev.2009.07.006>
 73. Parker KJ, Maestriepieri D (2011) Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neurosci Biobehav Rev* 35:1466–1483. <https://doi.org/10.1016/j.neubiorev.2010.09.003>
 74. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER (2013) The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* 38:1858–1873. <https://doi.org/10.1016/j.psyneuen.2013.06.008>
 75. Dudley KJ, Li X, Kobor MS, Kippin TE, Bredy TW (2011) Epigenetic mechanisms mediating vulnerability and resilience to

- psychiatric disorders. *Neurosci Biobehav Rev* 35:1544–1551. <https://doi.org/10.1016/j.neubiorev.2010.12.016>
76. Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci* 10:446–457. <https://doi.org/10.1038/nrn2649>
 77. Gunnar MR, Wewerka S, Frenn K, Long JD, Griggs C (2009) Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: normative changes and associations with puberty. *Dev Psychopathol* 21:69–85. <https://doi.org/10.1017/S0954579409000054>
 78. Karatsoreos IN, McEwen BS (2013) Annual research review: the neurobiology and physiology of resilience and adaptation across the life course. *J Child Psychol Psychiatry* 54:337–347. <https://doi.org/10.1111/jcpp.12054>
 79. Katz M, Liu C, Schaer M, Parker KJ, Ottet M-C, Epps A, Buckmaster CL, Bammer R et al (2009) Prefrontal plasticity and stress inoculation-induced resilience. *Dev Neurosci* 31:293–299. <https://doi.org/10.1159/000216540>
 80. Lyons DM, Parker KJ (2007) Stress inoculation-induced indications of resilience in monkeys. *J Trauma Stress* 20:423–433. <https://doi.org/10.1002/jts.20265>
 81. Macri S, Zoratto F, Laviola G (2011) Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer. *Neurosci Biobehav Rev* 35:1534–1543. <https://doi.org/10.1016/j.neubiorev.2010.12.014>
 82. Kasanova Z, Hernaes D, Vaessen T, van Amelsvoort T, Winz O, Heinzl A, Pruessner J, Mottaghy FM et al (2016) Early-life stress affects stress-related prefrontal dopamine activity in healthy adults, but not in individuals with psychotic disorder. *PLoS One* 11:e0150746. <https://doi.org/10.1371/journal.pone.0150746>
 83. Majcher-Maślanka I, Solarz A, Wędzony K, Chocyk A (2017) The effects of early-life stress on dopamine system function in adolescent female rats. *Int J Dev Neurosci* 57:24–33. <https://doi.org/10.1016/j.ijdevneu.2017.01.001>
 84. Shin S, Pribiag H, Lilascharoen V, Knowland D, Wang X-Y, Lim BK (2018) Drd3 signaling in the lateral septum mediates early life stress-induced social dysfunction. *Neuron* 97:195–208.e6. <https://doi.org/10.1016/j.neuron.2017.11.040>
 85. Zamberletti E, Prini P, Speziali S, Gabaglio M, Solinas M, Parolaro D, Rubino T (2012) Gender-dependent behavioral and biochemical effects of adolescent delta-9-tetrahydrocannabinol in adult maternally deprived rats. *Neuroscience* 204:245–257. <https://doi.org/10.1016/j.neuroscience.2011.11.038>
 86. Yu P, An S, Tai F, Wang J, Wu R, Wang B (2013) Early social deprivation impairs pair bonding and alters serum corticosterone and the NAcc dopamine system in mandarin voles. *Psychoneuroendocrinology* 38:3128–3138. <https://doi.org/10.1016/j.psyneuen.2013.09.012>
 87. Braun K, Lange E, Metzger M, Poeggel G (2000) Maternal separation followed by early social deprivation affects the development of monoaminergic fiber systems in the medial prefrontal cortex of *Octodon degus*. *Neuroscience* 95:309–318. [https://doi.org/10.1016/S0306-4522\(99\)00420-0](https://doi.org/10.1016/S0306-4522(99)00420-0)
 88. D'Aquila PS, Brain P, Willner P (1994) Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. *Physiol Behav* 56:861–867. [https://doi.org/10.1016/0031-9384\(94\)90316-6](https://doi.org/10.1016/0031-9384(94)90316-6)
 89. Hirano S, Miyata S, Onodera K, Kamei J (2007) Involvement of dopamine D1 receptors and alpha1-adrenoceptors in the antidepressant-like effect of chlorpheniramine in the mouse tail suspension test. *Eur J Pharmacol* 562:72–76. <https://doi.org/10.1016/j.ejphar.2007.01.063>
 90. Greengard P, Allen PB, Nairn AC (1999) Beyond the dopamine receptor. *Neuron* 23:435–447. [https://doi.org/10.1016/S0896-6273\(00\)80798-9](https://doi.org/10.1016/S0896-6273(00)80798-9)
 91. Hotte M, Thuault S, Dineley KT, Hemmings HC, Nairn AC, Jay TM (2007) Phosphorylation of CREB and DARPP-32 during late LTP at hippocampal to prefrontal cortex synapses in vivo. *Synapse* 61:24–28. <https://doi.org/10.1002/syn.20339>
 92. Frey U, Schroeder H, Matthies H (1990) Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. *Brain Res* 522:69–75
 93. Huang YY, Kandel ER (1995) D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. *Proc Natl Acad Sci* 92:2446–2450. <https://doi.org/10.1073/pnas.92.7.2446>
 94. Navakkode S, Sajikumar S, Frey JU (2007) Synergistic requirements for the induction of dopaminergic D1/D5-receptor-mediated LTP in hippocampal slices of rat CA1 in vitro. *Neuropharmacology* 52:1547–1554. <https://doi.org/10.1016/j.neuropharm.2007.02.010>
 95. Gurden H, Takita M, Jay TM (2000) Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. *J Neurosci* 20:RC106
 96. Goto Y, Grace AA (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat Neurosci* 8:805–812. <https://doi.org/10.1038/nm1471>
 97. Doherty TS, Roth TL (2016) Insight from animal models of environmentally driven epigenetic changes in the developing and adult brain. *Dev Psychopathol* 28:1229–1243. <https://doi.org/10.1017/S095457941600081X>
 98. Gröger N, Bock J, Goehler D, Blume N, Lisson N, Poeggel G, Braun K (2016) Stress in utero alters neonatal stress-induced regulation of the synaptic plasticity proteins Arc and Egr1 in a sex-specific manner. *Brain Struct Funct* 221:679–685. <https://doi.org/10.1007/s00429-014-0889-3>
 99. Hoffmann A, Spengler D (2012) The lasting legacy of social stress on the epigenome of the hypothalamic-pituitary-adrenal axis. *Epigenomics* 4:431–444. <https://doi.org/10.2217/epi.12.34>
 100. Kundakovic M, Lim S, Gudsnuk K, Champagne FA (2013) Sex-specific and strain-dependent effects of early life adversity on behavioral and epigenetic outcomes. *Front Psychiatry* 4:1–13. <https://doi.org/10.3389/fpsy.2013.00078>
 101. Lutz P-E, Turecki G (2014) DNA methylation and childhood maltreatment: from animal models to human studies. *Neuroscience* 264:142–156. <https://doi.org/10.1016/j.neuroscience.2013.07.069>
 102. Mychasiuk R, Metz GAS (2016) Epigenetic and gene expression changes in the adolescent brain: what have we learned from animal models? *Neurosci Biobehav Rev* 70:189–197. <https://doi.org/10.1016/j.neubiorev.2016.07.013>
 103. Provençal N, Binder EB (2015) The effects of early life stress on the epigenome: from the womb to adulthood and even before. *Exp Neurol* 268:10–20. <https://doi.org/10.1016/j.expneurol.2014.09.001>
 104. Szyf M (2013) DNA methylation, behavior and early life adversity. *J Genet Genomics* 40:331–338. <https://doi.org/10.1016/j.jgg.2013.06.004>
 105. Meaney MJ, Ferguson-Smith AC (2010) Epigenetic regulation of the neural transcriptome: the meaning of the marks. *Nat Neurosci* 13:1313–1318. <https://doi.org/10.1038/nn1110-1313>