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RESEARCH ARTICLE

A comparison of stress reactivity between BTBR and C57BL/6J mice: an impact of early-life stress

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Abstract

Early-life stress (ELS) is associated with hypothalamic–pituitary–adrenal (HPA) axis dysregulation and can increase the risk of psychiatric disorders later in life. The aim of this study was to investigate the influence of ELS on baseline HPA axis functioning and on the response to additional stress in adolescent male mice of strains C57BL/6J and BTBR. As a model of ELS, prolonged separation of pups from their mothers (for 3 h once a day: maternal separation [MS]) was implemented. To evaluate HPA axis activity, we assessed serum corticosterone levels and mRNA expression of corticotropin-releasing hormone (*Crh*) in the hypothalamus, of steroidogenesis genes in adrenal glands, and of an immediate early gene (*c-Fos*) in both tissues at baseline and immediately after 1 h of restraint stress. HPA axis activity at baseline did not depend on the history of ELS in mice of both strains. After the exposure to the acute restraint stress, C57BL/6J-MS mice showed less pronounced upregulation of *Crh* and of corticosterone concentration as compared to the control, indicating a decrease in stress reactivity. By contrast, BTBR-MS mice showed stronger upregulation of *c-Fos* in the hypothalamus and adrenal glands as compared to controls, thus pointing to greater activation of these organs in response to the acute restraint stress. In addition, we noted that BTBR mice are more stress reactive (than C57BL/6J mice) because they exhibited greater upregulation of corticosterone, *c-Fos*, and *Cyp11a1* in response to the acute restraint stress. Taken together, these results indicate strain-specific and situation-dependent effects of ELS on HPA axis functioning and on *c-Fos* expression.

Keywords Early-life stress · Stress reactivity · BTBR · HPA

Introduction

Early-life stress (ELS) can have detrimental effects by increasing the risk of many psychopathologies (anxiety disorders, schizophrenia, major depressive disorder, and bipolar disorder) including autism spectrum disorders (Kessler et al. 2010; Singletary. 2015). ELS is considered a potent developmental risk factor likely acting through a number of mechanisms (Malave et al. 2022), including changes in stress reactivity (Agorastos et al. 2018; Reshetnikov et al. 2021b), epigenetics (Ershov et al. 2018; Torres-Berrio et al. 2019), and brain morphology alterations (Teicher et al. 2016; Antontseva et al. 2020; Reshetnikov et al. 2020). Nonetheless, the molecular mechanisms by which the risk of psychopathologies increases are not fully understood.

The early postnatal period is a stress hyporesponsive period (SHRP). This period is necessary to complete normal processes of neuro- and synaptogenesis and brain development (Sapolsky et al. 1985). It has been argued that the biological function of the SHRP is to protect the developing organism, specifically the brain, from an excess of circulating glucocorticoids. In rodents, it lasts from postnatal day (PND) 2 to PND15 (Schmidt et al. 2003, 2007; Enthoven et al. 2010), whereas in humans, the similar period corresponds to the first year of life (Gunnar and Donzella 2002). ELS, such as maternal deprivation,

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disrupts the programmed brain development, thereby yielding a myriad of deviations in brain circuitry, stress responsivity, cognitive function, and general health. This is true for both humans and animal models. Evidence indicates a causal relation between nonsexual maltreatment of a child and a range of mental disorders in the adult human (Heim et al. 2012). According to rodent studies, exposure to ELS increases anxiety and negatively affects behavior, including locomotor and exploratory activities, in adolescent and adult animals (Bondar et al. 2018; Orso et al. 2020; Sharma et al. 2022) and leads to social impairments (Usui et al. 2021). The observed alterations of behavior after exposure to ELS during the SHRP seem to be due to the ability of ELS to alter baseline and stress-induced activities of the HPA axis (Reshetnikov et al. 2018, 2021b; Suchecki 2018; Rajan et al. 2019; Nishi 2020; Orso et al. 2020). Nonetheless, in some meta-analyses, behavioral data are contradictory among ELS studies, and some of them indicate that mice exposed to maternal separation show no alteration of anxiety-like behavior (Tractenberg et al. 2016; Wang et al. 2020). One possible reason for the inconsistency of the results may be dissimilarities in the effects of ELS on mice of different strains. Investigation into the effects of early postnatal stress on different rodent strains should elucidate the influence of a genetic background on consequences of stress.

Strains such as BALB/cJ and DBA/2J mice and F344 rats are more sensitive to stress, and the delayed effects of ELS on rodents of these strains may be more pronounced (Novais et al. 2017). The genetic background also affects sensitivity to stress in humans (Assary et al. 2018).

Strains of mice with predisposition to certain diseases can be sensitive to stress too, and the latter may be a provoking factor for the development of a disease. In the past few years, the attention of researchers was focused on mice of the BTBR strain, which is promising for investigation into the pathogenesis of autism spectrum disorders and demonstrates the same behavior disturbances: decreased social preferences (Moy et al. 2007; Yang et al. 2007; McFarlane et al. 2008; Pobbe et al. 2010), anomalies of ultrasonic vocalizations (Scattoni et al. 2011; Wohr et al. 2011), and repetitive behaviors (Silverman et al. 2010; Amodeo et al. 2012). It has been hypothesized that the development and expression of the unique autism-like BTBR phenotype may be a consequence of a stress-reactive phenotype. This strain demonstrates increased anxiety (comorbid cognitive and emotional impairments in autism spectrum disorders) as compared to the control C57BL/6 strain (Pobbe et al. 2011; Langley et al. 2015; Chao et al. 2018). In addition, a few studies have revealed an elevated level of corticosterone in blood plasma of these animals in adulthood (Frye and Llaneza 2010) and a significant increase in this parameter after stress as compared with other mouse strains (Gould et al. 2014). These observations may indicate dysfunction of the HPA axis and sensitivity to stress in the BTBR mouse strain, but the exact mechanisms of the observed high stress reactivity are still unclear. Here, we hypothesized that ELS can modulate the HPA axis of BTBR mice both at baseline and after exposure to acute stress.

Materials and methods

Mice and housing

The animals were kept under standard conditions of a specific pathogen-free (SPF) animal facility (RFMEFI62119×0023) in triangular cages with dimensions 34.3 cm (L) × 29.2 cm (W) × 15.5 cm (H) (Optimice, Animal Care Systems, Inc.) and containing bedding (birch chips) and nesting material. Feed (pellets) and water were available ad libitum. In the area where the mice were kept, a photoperiod standard for an SPF animal facility was implemented: 14 h of light and 10 h of darkness, 40 lx, with a daybreak at 01:00 h and a sunset at 15:00 h. All procedures were approved by the Ethics Committee of the ICG SB RAS (Protocol number 25, December 2014) in conformity with EU Directive 2010/63/EU for animal experiments.

Maternal separation (MS)

Thirty adult B6 females and 30 female BTBR (BTBR T + Itpr3tf/J) mice (11–13 weeks old) were mated with a naïve male of their strain (1 male for 2–3 females). After 19 days, the females were moved to separate cages at least 2 days before they gave birth. The day of delivery was designated as PND0. Litters were randomly assigned to either an MS group or a control (normal rearing conditions, NC) group. Pups in the MS group were subjected to separation from their mothers (3 h once a day) from PND3 to PND15. This procedure was performed as described previously (Reshetnikov et al. 2021a).

The experimental design

Four groups of mice were set up: control C57BL/6J males (B6-NC), control BTBR males (BTBR-NC), maternally separated C57BL/6J males (B6-MS), and maternally separated BTBR males (BTBR-MS). The groups consisted of 89 males in total (21 B6-NC mice, 19 B6-MS mice, 24 BTBR-NC mice, and 25 BTBR-MS mice). On PND23, mice from each group were randomly assigned to two types of behavioral test batteries as described in our previous work (Reshetnikov et al. 2021a). On PND41, mice from each group either were assigned to acute restraint stress for 1 h or stayed in their home cage [1st and 2nd subgroups, respectively; the groups

were divided equally ($n = 9$–13) (Reshetnikov et al. 2021a). Mice were subjected to acute restraint stress via placement for 1 h into 50 mL conical tubes (Eppendorf, Germany) containing holes for ventilation. Immediately after that, the two subgroups, i.e., the mice subjected to acute restraint stress (the animals were decapitated after 1 h of restraint stress) and the animals without exposure to this stress were killed by decapitation (Fig. 1A). Adrenal glands were excised, and the following formula was used for adrenal index calculation: adrenal weight (g)/body weight (g). Brains were excised, and the hypothalamus was dissected. Adrenal glands, brains, and the hypothalamus were snap-frozen in liquid nitrogen in 1.5 mL plastic tubes, followed by storage at − 80 °C for subsequent analysis of expression of some genes by a TaqMan quantitative PCR (qPCR) assay.

The corticosterone assay

Trunk blood was collected, left at room temperature for 1 h, and then centrifuged twice at $3000 \times g$ for 10 min, and the precipitates were discarded. The resultant blood serum was stored at − 80 °C until analysis. Corticosterone levels were measured in plasma by the Competitive Enzyme Immunoassay (EIA) (Immunodiagnostic Systems, AC-15F1) according to the manufacturer's instructions. There were two technical replicates. Eight to ten animals from each group were analyzed.

Gene expression

RNA was extracted from a frozen tissue with PureZol (Bio-Rad, USA) in accordance with the manufacturer's protocol. The obtained samples of RNA were purified on Agencourt RNAClean XP beads (Beckman Coulter, Germany) and were diluted in double-distilled water. RNA quality and quantity were evaluated using a NanoDrop 2000 spectrophotometer. To obtain cDNA, the RNA was reverse-transcribed by means of a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher).

Gene expression was assessed by quantitative PCR involving fluorescent TaqMan probes on a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). Forward and reverse primers were chosen manually so that they matched different exons to prevent amplification of genomic DNA (Table 1). The PCR conditions were as follows: 95 °C for 5 min followed by 39 cycles at 95 °C for 15 s and at 60 °C for 20 s. We assayed the expression of genes *c-Fos*, *Crh*, *Cyp11b1*, *Cyp11a1*, *Mc2r*, *Star*, and *Hsd11b* (Fig. 3). Each reaction was carried out in a mixture of a template and BioMaster HS-qPCR. *Pik* and *Rab* served as housekeeping genes, because according to prefrontal cortex (PFC), transcriptomic data obtained in our laboratory (unpublished data), the expression of these genes is unchanged in animals with a history of early postnatal stress. There were three technical replicates in this experiment. Each group consisted of 7 to 11 animals.

Statistical analysis

This analysis was performed in STATISTICA 6.0 software. Normality of data distributions was evaluated by the Kolmogorov–Smirnov test, and Levene's test was performed to assess the equivalence of variances. For statistical analysis of the adrenal index, two-way analysis of variance (ANOVA; the stress and the strain of mice served as factors) was conducted because it was performed only on mice not subjected to acute restraint stress. To assess differences in the expression of genes, three-way ANOVA for other parameters (MS, the strain of animals, and restraint served as factors) was carried out with Fisher's least significant difference (LSD) test as a post hoc analysis. Differences between mouse groups were considered statistically significant at $p < 0.05$. Data are reported as the mean ± standard error of the mean (SEM). The presence of correlations was assessed by means of the Pearson coefficient for each strain of mice separately.

Results

The adrenal index

Two-way ANOVA uncovered a significant effect of factor "strain" [$F(1,75) = 44.535$, $p < 0.001$] and of an interaction of factors "strain × stress" [$F(3,72) = 4.309$, $p < 0.05$] on the adrenal index. Mice of the BTBR strain had a lower adrenal index as compared to the B6 strain ($p < 0.001$). Early postnatal stress affected only B6 mice: B6-MS mice had a lower adrenal index than B6-NC mice did ($p = 0.032$), whereas BTBR-MS mice had no significant differences from BTBR-NC mice (Fig. 1B).

Acute restraint stress increases the serum corticosterone level and expression of stress-related genes in the hypothalamus

Plasma corticosterone levels and mRNA expression of *c-Fos* and *Crh* in the hypothalamus were assessed on PND41 both at baseline and after exposure to acute restraint stress. We detected effects of factors "strain" [*c-Fos*: $F(1,53) = 34.698$, $p < 0.001$; corticosterone levels: $F(1,53) = 23.274$, $p < 0.001$], "restraint" [*c-Fos*: $F(1,53) = 230.785$, $p < 0.001$; corticosterone levels: $F(1,53) = 388.227$, $p < 0.001$], and "stress × restraint" [*c-Fos*: $F(3,50) = 44.869$, $p < 0.001$; corticosterone levels: $F(3,50) = 25.469$, $p < 0.001$] on *c-Fos* mRNA and corticosterone levels. There were effects of the

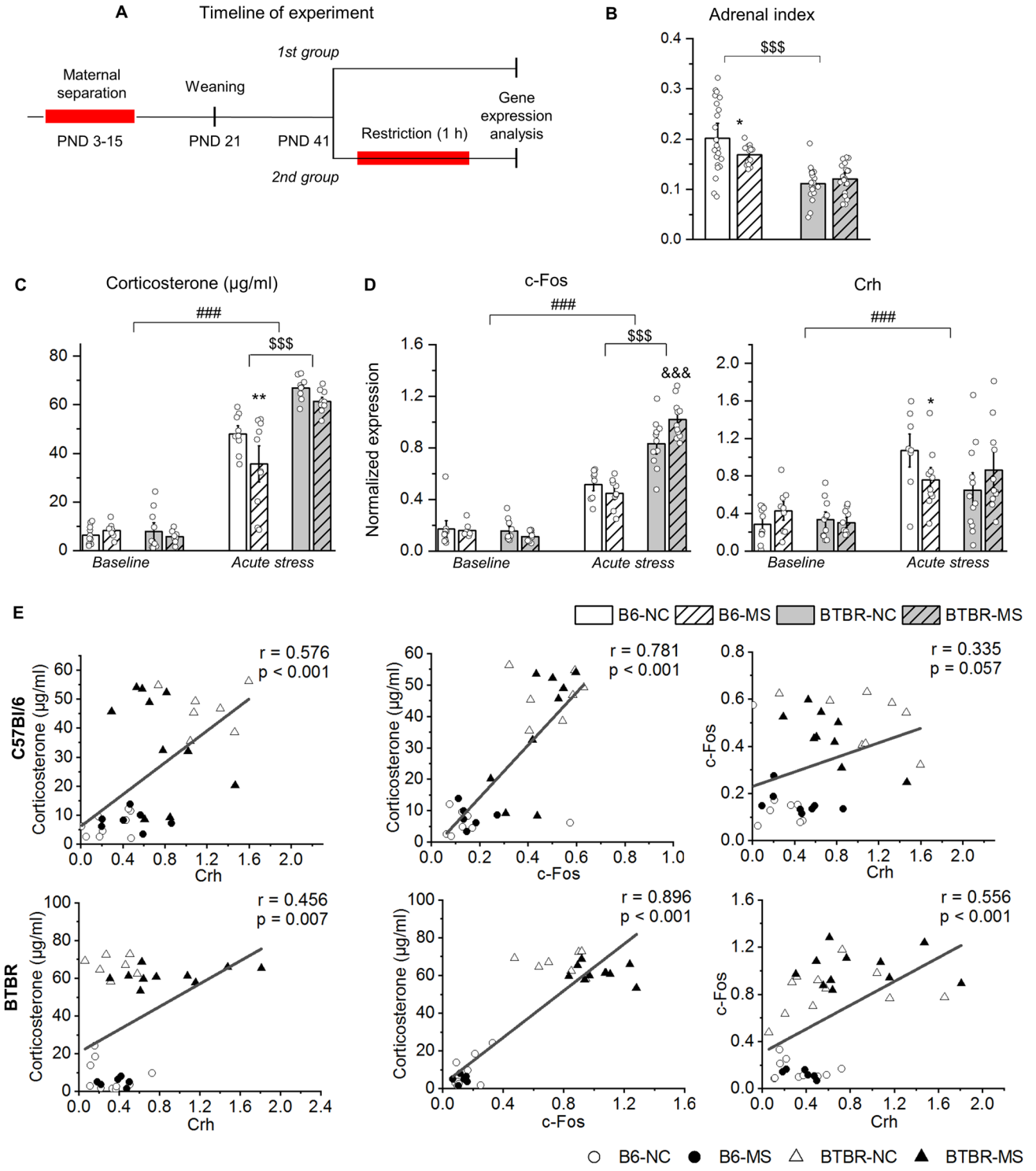

Fig. 1 The impact of ELS on stress reactivity. **A** The design of the experiment: mice of both strains in the MS group were subjected to maternal separation from PND3 to PND15. NC groups were not stressed in the early postnatal period. On PND41, some animals from both groups were subjected to acute restraint stress lasting for 1 h. **B** The adrenal index. **C** Plasma concentration of corticosterone in animals at baseline and after the acute stress. **D** mRNA expression of genes *c-Fos* and *Crh* in the hypothalamus of mice. **E** Analysis of correlations between mRNA expression levels of genes *c-Fos* and *Crh* in the hypothalamus and corticosterone concentration in blood plasma. The data are presented as the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ for the comparison B6-MS vs. B6-NC; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, and $^{\&\&\&}p < 0.001$ for the comparison BTBR-MS vs. BTBR-NC; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, and $^{\$\$\$}p < 0.001$ for the comparison BTBR vs. B6; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ for the comparison acute restraint stress vs. baseline

Table 1 Sets of primers

Gene name		Sequences 5′-3′	
c-Fos	Proto-oncogene c-Fos	For	CGGGTTTCAACGCCGACTA
		Rev	TTGGCACTAGAGACGGACAGA
		Probe	ROX-AGTCCTGTGTGTTGACAGG BHQ2
Crh	Corticotropin-releasing hormone	For	GGCATCCTGAGAGAAGTCC
		Rev	GGCTGCAAGAAATTCAAGGG
		Probe	ROX-ATGCTGCTGGTGGCTCTGTCGTCC BHQ2
Mc2r	Melanocortin 2 receptor	For	CTTGCCGAGAAAGATCCTAC
		Rev	GCCTTGGAAGCAGCAGAATC
		Probe	ROX-CTGAAGCCAGCAAGCCTGCC-BHQ2
Cyp11a1	Cytochrome P450 family 11 subfamily A member 1	For	GCTGAAGCAGAGCAATGGCAG
		Rev	GTGATGGACACGTTGACCTTGG
		Probe	ROX-CCTTGGCTGGGAAAATGACCC-BHQ2
Star	Steroidogenic acute regulatory protein	For	AAACTCACTTGGCTGCTCAGTA
		Rev	TGCGATAGGACCTGGTTGAT
		Probe	ROX-TGAAGGGGTGGCTGCCGAAG-BHQ2
Cyp11b1	Cytochrome P450 family 11 subfamily B member 1	For	GCAGAGGCAGAGATGATGC
		Rev	ACAGGCCTGAAAGTGAGGAG
		Probe	ROX-CACCATGTGCTGAAATCCTTCC-BHQ2/
Pik3c3	Phosphatidylinositol 3-kinase catalytic subunit type 3	For	GGATTGGCTGGACAGATT
		Rev	CTCCTTGTCATCGCACTT
		Probe	HEX-ACTTGATGGTTGAGTTTCGCTGTGT-BHQ1
Rab22a	RAB22A, member of RAS oncogene family	For	GATACGGGTGTGGGTAAATC
		Rev	CTGGACAGTCTTGGTCATAAA
		Probe	Cy5-AGCATCGTGTGGCGGTTTGTG-BHQ2

"restraint" [$F(1,53) = 30.046$, $p < 0.001$] factor and of interaction "strain × MS × restraint" [$F(9,44) = 7.816$, $p < 0.01$] on mRNA expression of *Crh*. The acute stress caused an increase in serum corticosterone levels and overexpression of *Crh* and *c-Fos* mRNA in the hypothalamus of mice of both strains compared to the mice not subjected to acute restraint stress ($p < 0.001$, Fig. 1C). An interstrain comparison showed that BTBR mice did not have significant differences from B6 mice at baseline; however, after acute restraint stress, they exhibited a greater increase in corticosterone levels and stronger upregulation of *c-Fos* mRNA ($p < 0.001$); meanwhile, upregulation of *Crh* mRNA in response to acute restraint stress was comparable between the strains. Thus, our results suggested that BTBR mice are more stress reactive than B6 mice.

Early postnatal stress (MS) did not cause significant changes in any of the tested parameters in both strains at baseline ($p > 0.05$). Unexpectedly, in response to acute restraint stress, B6-MS mice showed a less pronounced increase in corticosterone levels ($p < 0.01$) and in mRNA expression of *Crh* ($p < 0.05$) as compared to the B6-NC group. Such effects of early postnatal stress were not detectable in BTBR mice; on the contrary, BTBR-MS mice experienced more pronounced upregulation of *c-Fos* mRNA in the hypothalamus after acute restraint stress in comparison with BTBR-NC mice ($p < 0.001$).

Next, we analyzed how mRNA expression of *Crh* and *c-Fos* and serum corticosterone levels is related to one another. Analysis of a correlation between the key gene of the core mechanism of HPA axis regulation (*Crh*) and plasma concentration of corticosterone in the animals revealed a stable positive association between *Crh* mRNA and corticosterone levels (B6 mice: $r = 0.576$, $p < 0.001$; BTBR mice: $r = 0.456$, $p = 0.007$, Fig. 1E) and between *c-Fos* mRNA and corticosterone levels (B6 mice: $r = 0.781$, $p < 0.001$; BTBR mice: $r = 0.896$, $p < 0.001$) in mice of both strains. In addition, there was a correlation between mRNA expression levels of *c-Fos* and *Crh* in BTBR mice ($r = 0.556$, $p < 0.001$).

Acute restraint stress induces expression of genes involved in steroidogenesis and stress-related genes in adrenal glands

There were effects of factors "strain" [*Cyp11b1*: $F(1,59) = 7.275$, $p < 0.01$; *Cyp11a1*: $F(1,59) = 10.152$, $p < 0.01$] and "restraint" [*Cyp11b1*: $F(1,59) = 96.546$, $p < 0.001$; *Cyp11a1*: $F(1,59) = 23.062$, $p < 0.01$] and of their

interaction [*Cyp11b1*: F(3,56) = 5.488, $p < 0.05$; *Cyp11a1*: F(3,56) = 4.695, $p < 0.05$] on mRNA expression of genes *Cyp11b1* and *Cyp11a1*. Post hoc analysis revealed that after acute restraint stress, there was upregulation of *Cyp11b1* and *Cyp11a1* mRNA in adrenal glands of mice of both strains (Fig. 2). A comparison of mRNA expression of *Cyp11b1* between BTBR and B6 mice showed that the expression of this gene was lower in BTBR mice at baseline ($p < 0.001$), but this difference disappeared after exposure to the acute stress. On the contrary, mRNA expression of *Cyp11a1* was not different between the strains at baseline; however, after exposure to acute restraint stress, this parameter increased more significantly in BTBR mice ($p > 0.001$).

mRNA expression of genes *Mc2r* and *Star* was affected only by the "restraint" factor [*Mc2r*: F(1,67) = 19.808, $p < 0.001$; *Star*: F(1,67) = 81.841, $p < 0.001$]. Acute restraint stress enhanced the expression of these genes in adrenal glands of the animals with or without the history of early postnatal stress. There were no interstrain differences both at baseline and after exposure to the acute stress. Furthermore, early postnatal stress did not affect mRNA expression of *Cyp11b1*, *Cyp11a1*, *Star*, and *Mc2r* both at baseline and after acute restraint stress (Fig. 3).

Finally, we evaluated mRNA expression of *c-Fos* as a key early response gene. The expression of *c-Fos* in adrenal glands of the animals was influenced by factors "strain" [F(1,59) = 40.329, $p < 0.001$] and "restraint" [F(1,59) = 45.535, $p < 0.001$] and by interactions "strain × restraint" [F(3,56) = 34.367, $p < 0.001$] and "strain × restraint × MS" [F(1,59) = 4.956, $p < 0.05$]. Post hoc analysis revealed that acute restraint stress upregulated *c-Fos* mRNA in the adrenal glands ($p < 0.001$). In BTBR mice, mRNA expression of *c-Fos* went up almost threefold after acute restraint stress, whereas in B6 mice, this increase was less than twofold ($p < 0.001$). In addition, early postnatal stress had an impact on the mRNA level of *c-Fos* in BTBR mice: BTBR-MS mice showed more pronounced upregulation of *c-Fos* in adrenal glands in response to acute restraint stress as compared to BTBR-NC mice ($p < 0.05$); this finding is similar to the effects registered in the hypothalamus. Thus, most of the observed changes were detected after exposure to acute restraint

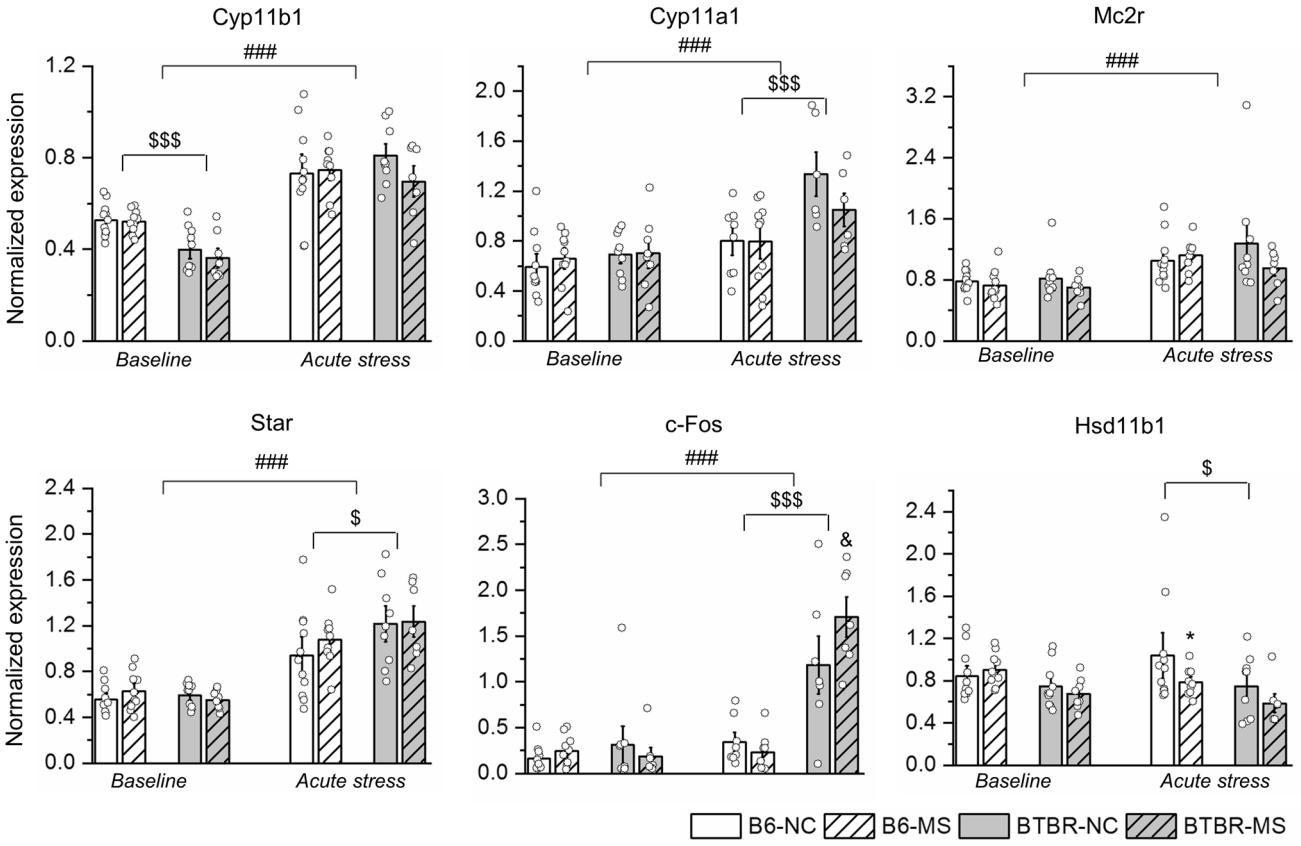

Fig. 2 Levels of mRNA expression of key genes of steroidogenesis in adrenal glands of the animals of both strains at baseline and after acute restraint stress. The data are presented as the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ for the comparison B6-MS vs. B6-NC; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, and $^{\&\&\&}p < 0.001$ for the comparison BTBR-MS vs. BTBR-NC; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, and $^{\$\$\$}p < 0.001$ for the comparison BTBR vs. B6; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ for the comparison acute restraint stress vs. baseline

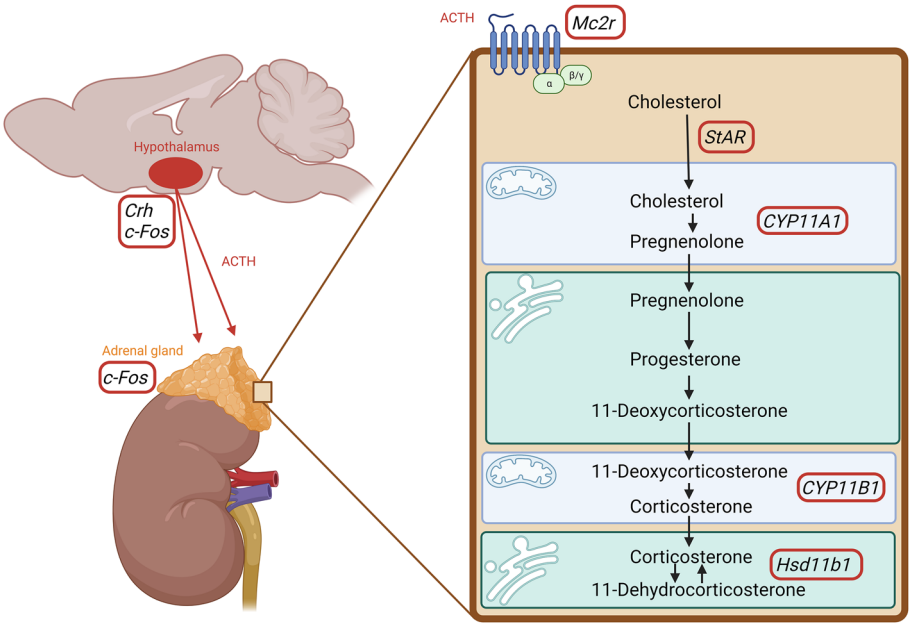

Fig. 3 Schematic representation of the functioning of the HPA axis and adrenal glucocorticoid synthesis and of early response gene *c-Fos* after exposure to stress. Stress induces a release of corticotropin-releasing hormone (CRH) secreted in paraventricular neurons of the hypothalamus. CRH stimulates a release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland; this hormone then binds to melanocortin 2 receptor (MC2R) on steroidogenic cells of the adrenal fasciculus. The triggered cascade of reactions leads to the phosphorylation of steroidogenic acute regulation protein (StAR), which stimulates steroidogenesis. The participation of these organ tissues in the response to stress was tested here by assays of mRNA expression of *c-Fos* in the hypothalamus and adrenal glands

stress. Similar to our finding of strong elevation of blood corticosterone concentration or of *c-Fos* mRNA expression in the hypothalamus, BTBR mice showed upregulation of *Cyp11a1*, which encodes a key enzyme of corticosterone synthesis.

Next, we decided to compare how expression levels of the genes assayed in the adrenal glands correlate with the concentration of corticosterone in the blood and *Crh* and *c-Fos* mRNA expression levels in the hypothalamus (Fig. 4). We noticed that in both strains of mice, adrenal expression of genes correlated either with hypothalamic expression of stress-related genes or with serum corticosterone levels.

Discussion

Our results indicate that early postnatal stress does not cause a pronounced change in HPA axis activity at baseline in either B6 or BTBR mice. The restriction of mobility for 60 min resulted in stronger growth of the corticosterone level and an increase in mRNA expression of *c-Fos* and of some genes of steroidogenesis in BTBR mice than in B6 mice, thereby pointing to elevated stress reactivity of the BTBR strain. Unexpectedly, the impact of early postnatal stress (MS) had opposite directions between the two strains of mice after acute restraint stress. Namely, B6 mice with the history of early postnatal stress showed a less pronounced increase in corticosterone and *Crh* mRNA levels in comparison with control B6 mice. On the other hand, BTBR mice with the history of early postnatal stress exhibited stronger upregulation of *c-Fos* in both the hypothalamus and adrenal glands as compared to control BTBR mice. Altogether, these data suggest that early postnatal stress can have different effects on animals of various strains, by reducing stress reactivity in B6 mice and enhancing certain parameters of stress reactivity in BTBR mice.

Literature data about effects of early postnatal stress on subsequent stress reactivity in adolescence and adulthood are inconsistent (Table 2). Most studies on rats and mice show an increase in stress reactivity (Kember et al. 2012; Wang et al. 2012; Sachs et al. 2013; Liu et al. 2016; McIlwrick et al. 2016; Biggio et al. 2018; Dandi et al. 2018; Bonapersona et al. 2019). Other research articles point to a reduction of stress reactivity in rodents having a history of early postnatal stress (Hsiao et al. 2016; Fuentes et al. 2017; Odeon et al. 2017; Marrocco et al. 2019). Differences in corticosteroid levels—reported in the literature—after the experience of maternal separation may be partially explained by the type of early postnatal stress, its duration, and by age at the time of an HPA axis assay (Rice et al. 2008; Korosi et al. 2010; van Bodegom et al. 2017). Activation of the HPA axis is limited by glucocorticoid negative feedback

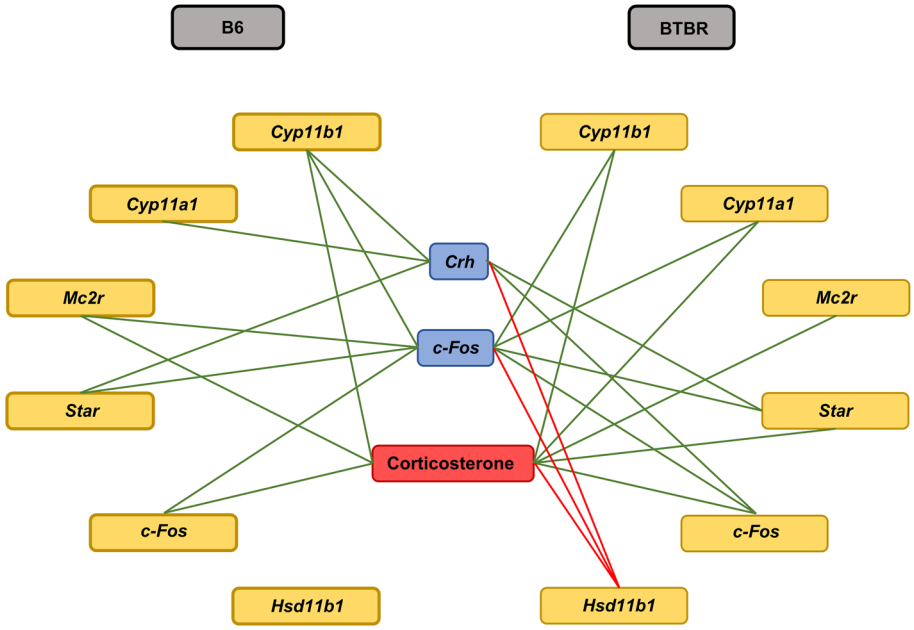

Fig. 4 Correlation between activity parameters of the HPA axis in B6 and BTBR mice. The mouse strains showed a significant ($p < 0.01$) positive correlation between some parameters. In B6 mice, an increase in mRNA expression of *Crh* was found to significantly correlate with upregulation of *Cyp11b1*, *Cyp11a1*, and *Star* mRNA. An increase in mRNA expression of *c-Fos* in the hypothalamus correlated with enhanced transcription of peripheral regulatory genes of the HPA axis (such as *Cyp11b1*, *Star*, and *Mc2r*) and with an increase in mRNA expression of *c-Fos* in the adrenal glands. *Cyp11b1* and *Mc2r* mRNA data also showed a significant association with corticosterone concentration in blood plasma of the mice. Moreover, an increase in the level of this hormone significantly correlated with *c-Fos* upregulation in adrenal glands of the animals. In assays of activity parameters of the HPA axis in BTBR mice, it was revealed that an increase in the mRNA level of *Crh* in the hypothalamus correlated only with two adrenal genes: *c-Fos* and *Star*. mRNA expression of *c-Fos* in the hypothalamus of this strain correlated with mRNA levels of *Cyp11b1*, *Cyp11a1*, *Star*, and *c-Fos*. An increase in the concentration of corticosterone in BTBR mice was associated with upregulation of all the tested genes, except for *Hsd11b1*. *Hsd11b1* significantly ($p < 0.01$) negatively correlated with upregulation of hypothalamic *c-Fos* and *Crh* and of corticosterone concentration in blood plasma of the mice. A positive correlation is highlighted in green, and a negative correlation is red

(inhibition) (de Kloet and Herman 2018). The impairment of stress reactivity may be caused by dysregulation of the expression of glucocorticoid receptors and mineralocorticoid receptors and their cochaperones—immunophilins FKBP4 and FKBP5—in the hippocampus and PFC of animals with a history of early postnatal stress. Evidence from both mice and rats suggests that prolonged maternal separation early in life leads to reduced glucocorticoid receptor expression in the hippocampus and frontal cortex and an increased MR/GR mRNA ratio (Ladd et al. 2004; Navailles et al. 2010, Reshetnikov 2018) and mRNA underexpression of *Fkbp5* in the frontal cortex (van der Doelen et al. 2014). Traumatic life events in childhood also result in multidirectional effects on the HPA axis in humans (van Bodegom et al. 2017; Huang et al. 2021; Juruena et al. 2021).

The increase in stress reactivity in BTBR mice that we documented here is in good agreement with other reports, which show a decline of exploratory activity and of social behavior (Meyza et al. 2013; Scattoni et al. 2013; Reshetnikov et al. 2021a). Of note, data on anxiety levels of BTBR mice are still a matter of debate because some studies show an increase in anxiety, whereas others suggest that anxiety levels do not differ from those in standard C57BL/6 mice (Meyza et al. 2013). These specific behavioral characteristics, just as the elevated stress reactivity, may be caused by neuroanatomical features and molecular or immune abnormalities in BTBR mice (Mutovina et al. 2022; Kisaretova et al. 2023). Aside from the most striking neuroanatomical feature—the absence of the corpus callosum (Meyza et al. 2013)—BTBR mice possess a smaller brain as a whole and have a deficient dorsal hippocampal commissure and smaller hippocampal volume (Dodero et al. 2013; Faraji et al. 2018), not to mention dramatically smaller thickness and volume of the cortex, particularly in the PFC (Faraji et al. 2018; Reshetnikov et al. 2021a). Parts of the PFC such as infralimbic and prelimbic cortices and the hippocampus mediate trans-synaptic inhibition of stress responses of the HPA axis (Herman et al. 2020). Thus, we propose that one of possible reasons for the increase in stress reactivity of BTBR mice is impaired regulation of the PFC–hippocampus–HPA system.

Even though we previously reported that ELS does not worsen autism-related behavior in juvenile and adolescent

Table 2 Effects of different paradigms of ELS on stress reactivity

Timing of ELS	Type of ELS	Acute stress	Age of corticosterone measurement	Baseline corticosterone	Stress-induced corticosterone	Species, strain	References
PND 2–9	LNB	Restraint 10 min	Adult 10, 20, 30 min after restraint	♂: NA ♀: NA	♂: ↑, ELS ↑ vs. control (trend) ♀: ↑, ELS ↓ vs. control	Mice, MRcamkcre/wt; MRflox/flox	(Bonapersona et al. 2019)
PND 2–12	LNB	Acute forced-swim stress	Adult 1 h after swim	♂: NA	♂: ↑, ELS ↓ vs. control	Mice, Gprin3-Bac-TRAP	(Marrocco et al. 2019)
PND 2–9	LNB	Restraint 30 min	PND 32–35 Immediately after restraint	♂: ELS ↑ vs. control	♂: ↑, ELS ↓ vs. control	Mice, C57BL/6	(Hsiao et al. 2016)
PND 1–21	MS 3 h/day	Rotating disk for 15 min	Adult Immediately after rotating	♂: NA	♂: ↑, ELS ↑ vs. control	Rats, Wistar	(Dandi et al. 2018)
PND 2–9	LNB	Restraint 15 min	Adult Immediately and 60 min after restraint	♂: ELS ↑ vs. control	♂: ↑, ELS ↑ vs. control	Mice, high HPA axis stress reactivity model	(Mcilwrick et al. 2016)
PND 1–14	MS 3 h/day	Restraint 15 min	Adult Immediately after restraint	♂: NA	♂: ↑, ELS ↑ vs. control	Mice, Tph2 R439H KI	(Sachs et al. 2013)
PND 2–9	LNB	Restraint 5 min	Adult 30, 90 min after restraint	♂: NA	♂: ↑, ELS ↑ vs. control	Mice, Crhr1 loxP/loxP-Camk2aCre	(Wang et al. 2012)
PND 2–14	MS 3 h/day	Acute forced-swim stress	Adult 30 min after swim	♂: ELS ↑ vs. control	♂: ↑, ELS ↑ vs. control	Mice, C57bl/6	(Liu et al. 2016)
PND 2	MS 24 h	Acute forced-swim stress	Adult 30 min after swim	♂: NA ♀: NA	♂: ↑, ELS C57bl/6 ↑ vs. control C57bl/6 ♀: ↑,	Mice, C57bl/6, DBA/2 j	(Kember et al. 2012)
PND 1–21	LNB 3 h/day	Water avoidance stress	Adult Immediately after stress	♂: ELS ↓ vs. control	♂: NA	Mice, C57bl/6	(Fuentes et al. 2017)
PND 3–15	Maternal separation 3 h/day	2-min cold swim stress	Adult 5, 15, and 60 min after swim	♂: ELS ↑ vs. control	♂: ↑, ELS = . control	Rats, Sprague–Dawley	(Vargas et al. 2016)
PND 3–15	Maternal separation 3 h/day	Foot-shock stress	Adult 25 min after the end of the foot-shock	♂: ELS ↓ vs. control	♂: ↑, ELS ↑ vs. control	Rats, Sprague–Dawley	(Biggio et al. 2018)
PND 2–22	Maternal separation plus cold stress 1 h/day	Immobilization for 2 h	Adult Immediately after acute stress	♂: ELS ↓ vs. control	♂: ↑, ELS ↓ vs. control	Rats, Wistar	(Odeon et al. 2017)

NA no available data, *LNB* limited nesting and bedding, *MS* maternal separation

BTBR mice (Reshetnikov et al. 2021a), the specific features of HPA axis activity observed in the present study may affect the behavioral phenotype. Disturbances of HPA axis functioning are seen in a variety of mental disorders, e.g., in patients with autism spectrum disorders (Muscatello et al. 2021). For instance, children with this disorder show an atypical response to social stress (Corbett et al. 2019). Some researchers attribute this phenomenon to elevated sensitivity of patients with autism spectrum disorders to stress owing to hyper-reactivity of the HPA axis (Spratt et al. 2012). As compared to their peers without such disorders, children with autism spectrum disorders are more likely to experience

stress, anxiety, and depression (Simonoff et al. 2008; van Steensel et al. 2011); furthermore, these pediatric patients have a high concentration of cortisol in blood plasma and saliva both at baseline (Taylor and Corbett 2014) and after exposure to various stressors, such as a school environment (Spratt et al. 2012). Nonetheless, data about the abnormality of basal functioning of the HPA axis are contradictory (Albantakis et al. 2021; Bakker-Huvenaars et al. 2018). On the other hand, it is unclear whether ELS can worsen the observed HPA axis dysregulation in children with autism spectrum disorders. The available data are incomplete and contradictory (Makris et al. 2022), thereby necessitating further research into HPA axis functioning at baseline, after stress exposure, and after exposure to additional stress in different age periods, for example, in animal models.

In summary, our study allowed us to investigate the functioning of the HPA axis in C57BL/6J and BTBR mice in a steady state (at baseline) and after acute stress exposure (stress reactivity). On the basis of these data, we cannot propose a complete theory describing the differences in HPA axis activities between C57BL/6J and BTBR mice; however, the uncovered unique features of BTBR mice should improve our understanding of the behavioral and molecular abnormalities of this strain. We believe that comprehensive studies on different strains of mice with contrasting behavioral phenotypes will elucidate the molecular mechanisms underlying the delayed effects of postnatal stress.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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