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ORIGINAL RESEARCH

Social Behaviour and Epigenetic Status in Adolescent and Adult Rats: The Contribution of Early-Life Stressful Social Experience

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Abstract

Early-life experiences have been linked to individual's epigenetic status and social behaviour. Therefore, the present study aims to test whether the presence of mother suppress the early-life stressful social experience (SSE)-induced effect on social behaviour of adolescent and adult rats, and associated epigenetic changes. To test this, experimental groups [maternally separated pups (MSP)/pups with their mother (M+P)] were allowed to experience the presence of a stranger (ST), and then their social behaviour was compared with the maternal separated (MS) and control (Con) group. We observed that MS, MSP-ST group showed less social interaction with the unknown conspecifics than known conspecifics compared to other groups. Subsequently, we found that SSE elevated the level of DNA methyltransferases (*Dnmt3a*), ten-eleven translocation (*Tet3*), methyl-CpG-binding protein-2 (MeCP2) and Repressor Element-1 Silencing Transcription Factor (REST) in amygdala of adolescent and adult MS, MSP-ST groups compared to other groups. As expected, SSE altered the histone (H3) lysine (K14/K9) acetylation (ac) and H3K4/K9 methylation (me2/me3). SSE decreased the level of H3K14ac and H3K9ac in adolescents and then increased in adults. Interestingly, H3K4me2/me3 levels were elevated in adolescent and adults. Whereas H3K9me2/me3 shows contrasting pattern in adolescent, but H3K9me2/me3 levels were increased in adults. In addition, the expression of brain-derived neurotrophic factor (BDNF) was reduced in MS, MSP-ST groups' adolescent and adult rats. Observed correlation between epigenetic changes and social behaviour possibly contributed by early-life SSE in the absence of mother, but mother's presence suppresses the effect of early-life SSE.

Keywords Early-life stress · Social behaviour · Amygdala · Epigenetics · Histone methylation · Histone acetylation

Introduction

Early-life stress (ELS) has marked effect on social, non-social behaviour and irreversible changes in gene expression that persist into their adulthood (Holmes et al. 2005; Roth et al. 2009; Moriceau et al. 2009; McGowan et al. 2011). In rodent models, maternal separation (MS) has been used to induce the programming effects like ELS and genome-wide analysis correlates epigenetic changes and behavioural responses to challenging stressful stimuli in later life (Covington et al. 2009). ELS induced long-term changes in gene expression possibly mediated via aberrant DNA methylation. The 5-hydroxymethylcytosine (5-hmC) has been recognized as a key epigenetic DNA modification in the brain (Hahn et al. 2013). The ten-eleven translocation (TET) hydroxylase isoforms (TET 1–3) generate 5hmC from 5-methylcytosine (5-mC) (Tahiliani et al. 2009), and its accumulation linked positively with active gene expression (Hahn et al. 2013; Colquitt et al. 2013). On the other side, DNA methyltransferases (DNMTs) repress gene expression through methylation at cytosine base of CpG dinucleotides and often correlated with behavioural disorders (Murgatroyd et al. 2009; Elliot et al. 2016). Further, methyl CpG binding protein-2 (MeCP2) and Repressor Element-1 Silencing Transcription Factor (REST) has been known to binds with methylated DNA and provide signals to alter chromatin structure, which is considered as a core component of the repressive system and silence the gene expression for a long-time (Bruce et al. 2004; Guy et al. 2010).

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Studies have been augmented that stressful environmental stimuli remodelling the chromatin structure and epigenetic landscape through histone modifications (acetylation/ methylation). However, depending on the histone methylation at specific residue and the number (mono, di-, tri- methylation) of methyl groups facilitate either positively or negatively regulate gene expression (Berger 2007; Covington et al. 2011). Genome-wide, di- and tri-methylation of histone-3 at lysine-4 (H3K4 me2/me3) and histone-3 at lysine-9 (H3K9 me2/ me3) (Hunter et al. 2009; Gupta et al. 2011; Sailaja et al. 2012; Wang et al. 2017; Peterson and Laniel 2004; Liu et al. 2014) are known to facilitate transcriptional activation or inactivation in different brain region according to the environmental stimulation to regulate synaptic plasticity, resilience the stress or stress related behavioural disorders. Further, in rodent models, several studies demonstrated that stressful life events alter the epigenetic status of brain-derived neurotrophic factor (BDNF) at *exon IV* and expression level, which is a core mechanism for the development of many behavioural disorders (Mitchelmore and Gede 2014; Boersma et al. 2014; Doherty et al. 2016).

Earlier, MS has been used as an animal model of ELS (Roth et al. 2009; Bian et al. 2015) known to alter the epigenetic status of genes that lead to the development of behavioural disorders/ impairment of behavioural responses to stressful challenges later in life (Kao et al. 2012; Wang et al. 2017; Feifel et al. 2017). Thus, we hypothesize that the presence of the mother may resilience; the early-life stressful social experience (SSE) induced changes in epigenetic signatures that can alter the gene expression and social behaviour. To test this, experimental group rat pups (maternally separated pups/ pups with their mother) were allowed to experience the presence of a stranger during their postnatal days, and then their social interactions with conspecifics was tested during adolescent and adulthood. Later on, the experimental groups' behavioural profile, epigenetic status compared with the maternal separated and control group.

Materials and Methods

Subject Animals

Pregnant Wistar rats (*Rattus norvegicus*) housed individually in a standard rectangular laboratory cage (43 × 27 × 15 cm) and maintained under standard laboratory conditions (12 h light/ dark cycle; 22–25 °C) with *ad libitum* access to feed (chow pellets) and water. Plentiful amounts of paddy husk were provided as bedding and the cage was cleaned once in 2 days. Animals were treated according to the regulation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India) and all the protocols are approved (BDU/IAEC/2016/NE/33/dated 17.03.2016) by Institutional Animal Ethical Committee (IAEC), Bharathidasan University, Tiruchirappalli. During the experiment, utmost care was taken to minimize the animal suffering and reduce the number of animal use.

Early-Life Stressful Social Experience (SSE) Paradigm

Experimental group pups were allowed to experience the social stress by introducing the stranger (ST) into their cage in the presence (mother with pups; M+P) or absence of mother (maternally separated pups; MSP) as shown in experimental time line (Fig. 1). For each group, four dam and their pups were used. Upto postnatal day (PND)—23,

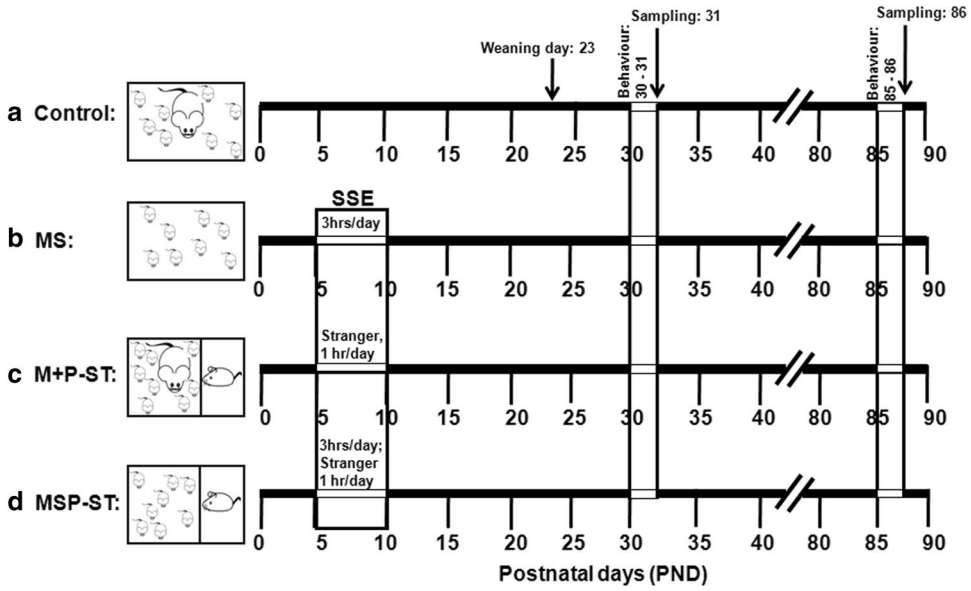

Fig. 1 Schematic representation showing the timeline based on experimental procedures of the study. (**a**) Control group (CON), (**b**) maternal separation (MS), (**c**) mother and their pups exposed to stranger (M+P-ST), (**d**) maternally separated pups alone exposed to stranger (MSP-ST)

control group pups and their mother (CON) were undisturbed, except during cleaning and general handling. For MS group, first the mother was transferred to another cage with home cage bedding and then the pups were transferred. Immediately, mother was transferred back to the home cage. MS paradigm was carried out for 3 h [09:00–12:00 h] from PND-5 to 10. To provide stressful social experience to M+P and MSP, specially designed cage standard laboratory cage: 43 cm × 27 cm × 15 cm—divided by wire mesh into two chambers, to prevent the physical contact of stranger with the mother/ pups and it is kept in the same room at animal house. The home cage bedding was used during exposure to stranger in order to avoid additional stress and modification of maternal behaviour (Ershov et al. 2018). For M+P-ST group, first mother and then their pups were transferred (from PND 5–10; 09:00–12:00 h) to one-half of the specially designed cage with home cage bedding and stranger (Senescence male; 18 months old) was placed (10:00–11:00 h) in another half of the cage. For MSP-ST group, pups from their mother were separated to the specially designed cage with home cage bedding from PND-5 to 10; (09:00–12:00 h) and then stranger was placed (10:00–11:00 h) in another half of the specially designed cage.

Social Interaction Test

The three-chambered apparatus was constructed based on the specification (Ferland and Schrader 2011) and social interaction test (SIT) was carried out to analyze sociability. SIT was conducted for all experimental groups during the adolescent age (PND-30, 31; Con: $n = 20$; MS: $n = 21$; M+P-ST: $n = 18$; MSP-ST: $n = 19$) and adult (PND-85, 86; only for males; Con: $n = 9$; MS: $n = 13$; M+P-ST: $n = 9$; MSP-ST: $n = 12$). SIT was conducted for two sessions—training session (Day 1) and testing session (Day 2). At first, subject animals were transferred to the experimental room one hour before testing/ training sessions to acclimatize. On day one, during the training session, subject animal was placed in the centre chamber (CC) for 5 min to habituate by closing the two doorways to Chamber 1 (C1) and Chamber 2 (C2). Subject animal was trained to interact with a conspecifics-1 (referred as stranger-1: ST-1) was placed in the C1 and empty cylindrical chrome wire cage (EC) was placed in C2. After 5 min, the two doorways were opened and the subject was allowed to explore the apparatus and interact with ST-1 for 10 min. On day two, ST-1 was placed in C1 and another new conspecifics-2 (referred as stranger-2: ST-2) was placed in C2. The subject was placed in CC, and allowed to enter the chambers to interact with strangers for the period of 10 min. To avoid the olfactory cues, the apparatus was wiped clean with 75% ethanol immediately after every training/ testing. The person performing the experiment was not aware of the groups' details. During training and testing, the subject's interaction with the ST-1/ ST-2 and time spent in each chamber was video recorded and analyzed.

Sample Preparation

Samples were collected immediately after the behavioural test on PND-31 in adolescent rats and on PND-86 in adult rats (male only). Animals representing each group (Con; MS; M+P-ST; MSP-ST) to be dissected were euthanized ($n = 6$ from each group), and the amygdala region was dissected out as described elsewhere (Morse et al. 2015), and total RNA, total protein and histone protein were isolated.

Total RNA

Total RNA was isolated from amygdala using TRI Reagent (Cat # T9424; Sigma-Aldrich) according to the manufacturer's instruction and RNase inhibitor (Cat # 61110110001A; Genei Laboratories) was added finally before storing at − 80 °C. Total RNA (2 µg/sample) was reverse-transcribed using random/ oligo-dT primers (Cat # 170–8891; iScript™ cDNA synthesis kit; Bio-Rad Laboratories) and stored at 4 °C.

Total Protein

Total protein was isolated from amygdala by homogenizing with ice-cold lysis buffer [Tris-Hydrochloric acid (Tris-HCl) pH 7.5, Sodium chloride (NaCl), Ethylenediaminetetraacetic acid (EDTA), Dithiothreitol (DTT), Tergitol (NP-40), Sodium orthovanadate (Na_3VO_4), Phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail (Cat # P8340; Sigma-Aldrich)]. The homogenates were incubated on ice for 30 min and then centrifuged at 12,000 rpm for 30 min at 4 °C. The clear supernatant were collected in fresh tube and centrifuged again at 12,000 rpm for 30 min at 4 °C. The final supernatants were collected and stored in aliquots at − 80 °C.

Histone Proteins

Histone protein was isolated from amygdala by homogenizing with TX buffer (Tris-HCl, NaCl, EDTA, Triton 100, protease inhibitor cocktail). The homogenates were incubated on ice for 15 min and then centrifuged for 10 min at 4 °C. Pellet was then dissolved in 0.2M HCl-TX buffer and isolated in ice for 30 min which was followed by centrifugation for 10 min at 4 °C. The supernatant was collected and stored at -80 °C.

Quantitative Real-Time PCR (qRT-PCR)

The qRT-PCR was performed using real-time reaction mixture (Cat # 170-8880AP; iQ™ SYBR® green supermix, Bio-Rad Laboratories) with specific primers and cDNA in CFX-96 Touch™ Real-time PCR Detection system (Bio-Rad Laboratories). The expression level of *Dnmt3a, Tet3 and Hsp-70* were estimated using specific primers (Online Resource 1). The amplification was confirmed by monitoring the dissociation curve followed by melting curve analysis and visualized in Native polyacrylamide gel electrophoresis (12%) staining with Ethidium bromide. The level of expression were normalized to the internal control (*Hsp70)* and presented as mean fold change CFX Manager™ version 2 software, CFX-96 Touch™ Real-time PCR Detection system (Bio-Rad Laboratories).

Western Blot Analysis

The concentration of total/ histone protein samples were estimated using Bradford method by measuring the absorbance at 595 nm using Biophotometer plus (Eppendorf). Equal concentration of total/ histone proteins (60 µg) was resolved on 10% polyacrylamide gel. The separated proteins were transferred electrophoretically to polyvinylidene difluoride (PVDF) membrane (Cat #: IPVH00010; Millipore) using a semi-dry western apparatus (SD 20; Cleaver Scientific Ltd). The membranes were blocked in Tris-buffered saline (TBS) containing 0.1% Tween-20; 5% non-fat milk for 2 h at room temperature. The membrane was then incubated at 4 °C for 8 h with one of the specific primary antibodies (Online Resource 1). Membrane-bound antibodies were detected by incubating for 3 h with secondary antibody conjugated with alkaline phosphatase. Subsequently, alkaline phosphatase activity was detected with 5-bromo-4chloro-3-indolyl phosphate disodium salt (BCIP)/ nitroblue tetrazolium chloride (NBT) (Cat # S3771; Promega Biotech Ltd) according to the manufacturer's instructions. Images were acquired with a Molecular Imager ChemiDoc XRS System (Bio-Rad Laboratories) and trace quantity of each band was measured using Image Lab 2 software (Bio-Rad Laboratories). The data were presented as fold relative to control group.

Statistical Analysis

Data were presented as a mean ± standard error of the mean (SEM) and plotted with KyPlot (ver 1.0) for graphical representation. Univariate ANOVA (SPSS, ver. 24) was performed to test the behavioural analysis between groups and among groups for testing and training. One-way analysis of variance (ANOVA; Sigma Stat, ver 3.1) was used to examine the significant difference between groups in molecular data. Data were shown as mean ± SEM and asterisk indicates significant difference between groups (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). Differences were considered significant if $P < 0.05$.

Results

Behavioural Analysis

Early-Life Stressful Social Experience (SSE) Affects Social Interaction in Adolescent Rats

Social interaction test revealed that during training irrespective of group, all individuals had active interaction with ST-1 than EC. There was no significant interaction between groups × subject and between groups (Fig. 2a). Similarly, significant difference was detected in time spent in the different (ST-1, CC, EC) chamber. Notably, they spent time in chamber ST-1 than CC/ EC. However, there was no significant interaction was detected between groups × chamber and between groups (Fig. 2c; Online Resource 2).

During testing, experimental group individuals' actively contact with either ST-1 or ST-2 and their interaction with the subject were significantly. However, control and M+P-ST groups preferred to interact with ST-2 than ST-1, in contrast MS and MSP-ST groups preferred to interact with ST-1 than ST-2 (Fig. 2b). In addition, significant difference was detected in the preference to subjects between groups and interaction between groups × subjects. When we analyzed the time spent by individuals at different chambers, we found significant difference in their preference to different chambers. Interestingly, control and M+P-ST spent more time in ST-2 chamber but MS and MSP-ST group preferred to stay in ST-1 chamber. A significant interaction was detected between groups × chamber preference, however, there was no difference between groups (Fig. 2d; Online Resource 2).

Early-Life Stressful Social Experience (SSE) Affects Social Interaction in Adult Rats

Interestingly, adults during training did not show preference to subjects, except MSP-ST group. MSP-ST group actively interact with ST-1 than EC. Observed preference to subjects between groups was not significantly different and interaction between groups × subject (Fig. 3a). Similarly, there was no significant difference in the time spent by the individuals in different chamber. The analysis revealed that control group spent equal time with ST-2 and ST-1 chamber while MSP-ST group with ST-1 and CC. Whereas, MS and M+P-ST groups preferred to spend equal time in all three chambers and no significant difference were detected. Detected interaction between groups × chamber preference

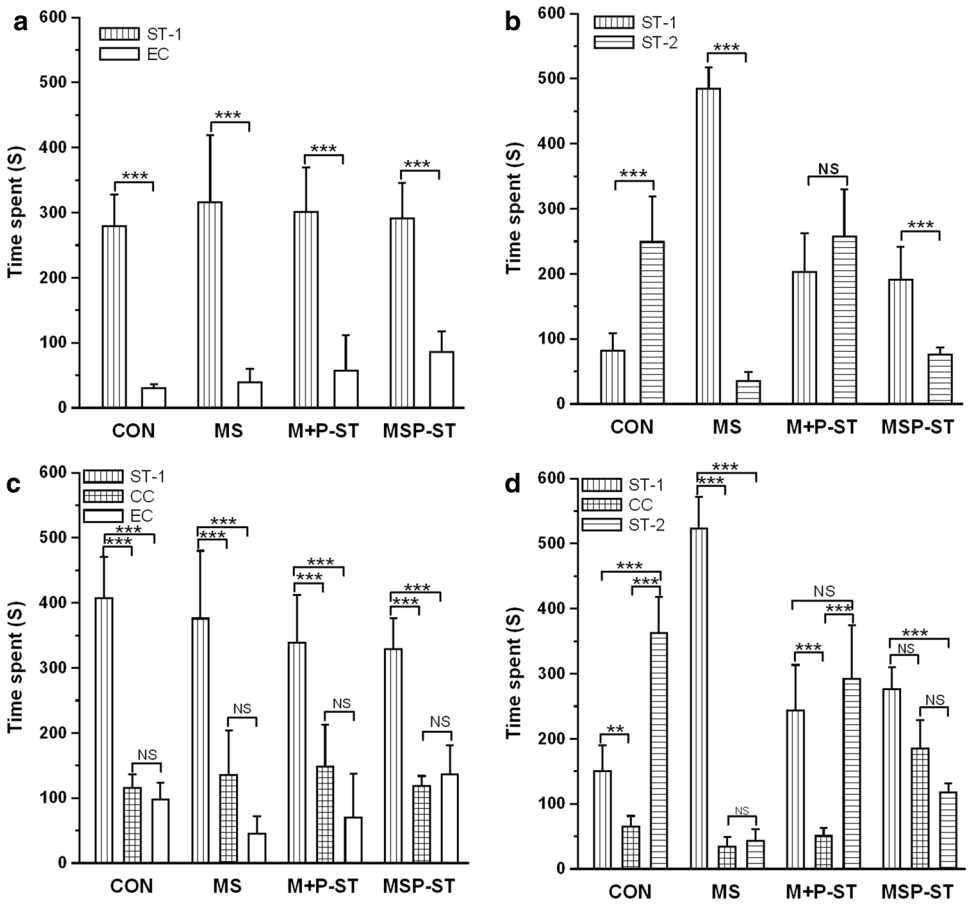

Fig. 2 Effect of early-life SSE on social interaction in adolescent rats. Different experimental group's individual's active contact during training (**a**) and testing (**b**). Experimental group individuals spent more time with ST-1 than CC or EC during training (**c**) and time spent with ST-1/ CC/ ST-2 during testing (**d**)

and between groups was not significantly different (Fig. 3c; Online Resource 2).

In the testing session, we detected significant difference in active contact with subjects, control preferred to interact with ST-2 and MS, MSP-ST preferred with ST-1. Their preference to the subjects [$P < 0.001$] and interaction between groups × subjects was significantly different (Fig. 3b). Similarly, the time spent by individuals represent each group in different chambers was significantly different. Control group spent more time in ST-2 chamber than CC/ST-1, whereas MS and MSP-ST preferred to stay in ST-1 chamber than CC/ST-2. Interestingly, M+P-ST showed equal preference in ST-1 and ST-2 chamber than CC. Significant differences were found in interaction between groups × chamber preference but not between groups (Fig. 3d; Online Resource 2).

Early-Life SSE Alters the Expression of Dnmt3a and Tet3 in Amygdala of Adolescent Rats

Exposure to the early social stress resulted in alternation of *Dnmt3a* and *Tet3* expression in amygdala of adolescent rats. The analysis revealed similar pattern of *Dnmt3a* and *Tet3* expression (Fig. 4a, c). We found that level of *Dnmt3a* and *Tet3* in MS, M+P-ST and MSP-ST was elevated than control and significantly different. However, the level of *Tet3* in M+P-ST group did not reach significant level compared to control. In fact, early SSE-induced expression of *Dnmt3a* and *Tet3* were significantly higher in MS and MSP-ST groups than M+P-ST. In comparison, the level of *Dnmt3a* and *Tet3* was significantly higher in MSP-ST than MS (Fig. 4b, d; Online Resource 3). This results suggest that early SSE significantly alters the expression of enzymes involve in methylation/ demethylation process in adolescent rats of amygdala region.

Early-Life SSE Alters the Expression of Dnmt3a and Tet3 in Amygdala of Adult Rats

The estimated the level of *Dnmt3a* and *Tet3* in amygdala of adult rats showed that SSE alters the expression. The level of *Dnmt3a* and *Tet3* altered in experimental groups (Fig. 5a, c). Estimated level of *Dnmt3a* and *Tet3* in MS was not significantly different from control but significantly reduced in M+P-ST group. However, the level of *Dnmt3a* and *Tet3* were significantly higher in MSP-ST than control. In comparison, the level in M+P-ST was significantly lower than MS. Whereas the estimated level of *Dnmt3a* and *Tet3* in MSP-ST was significantly higher than MS (Fig. 5b, d; Online

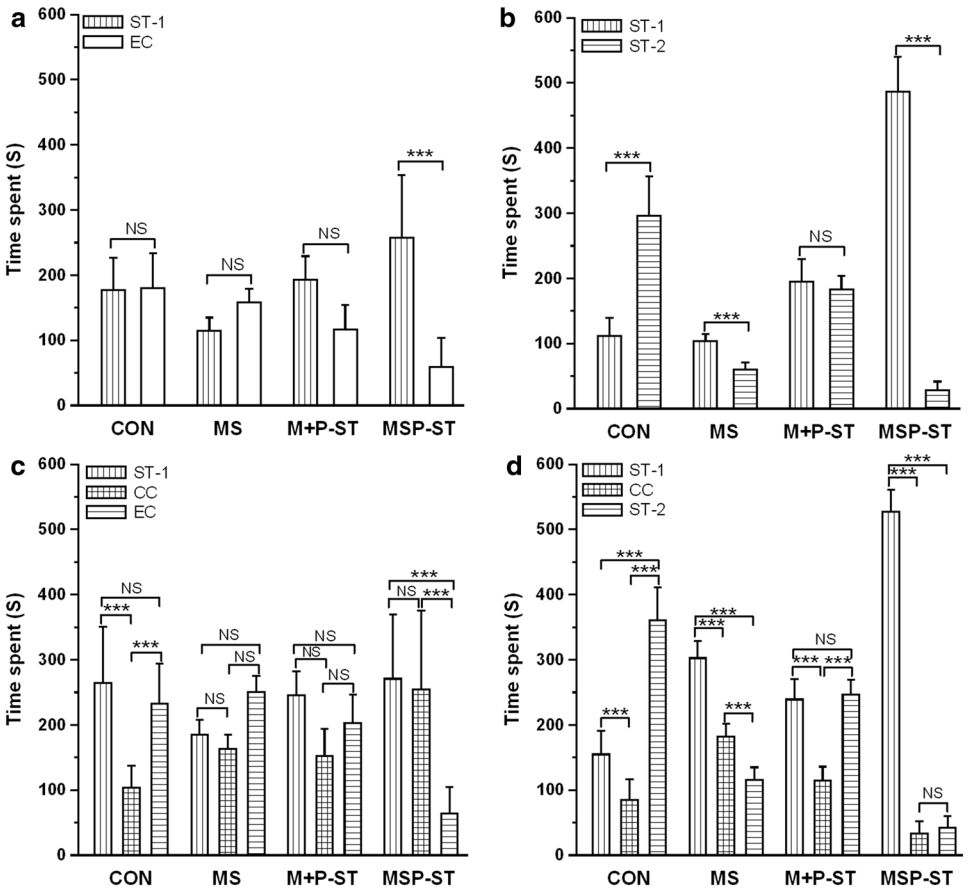

Fig. 3 Impact of early-life SSE on social interaction of adult rats. Active contacts of adult rats during training (**a**), and testing (**b**) in social interaction test. Individuals representing different groups spent time differently with ST-1/ CC /EC during training (**c**) and time spent with ST-1/ CC/ ST-2 during testing (**d**)

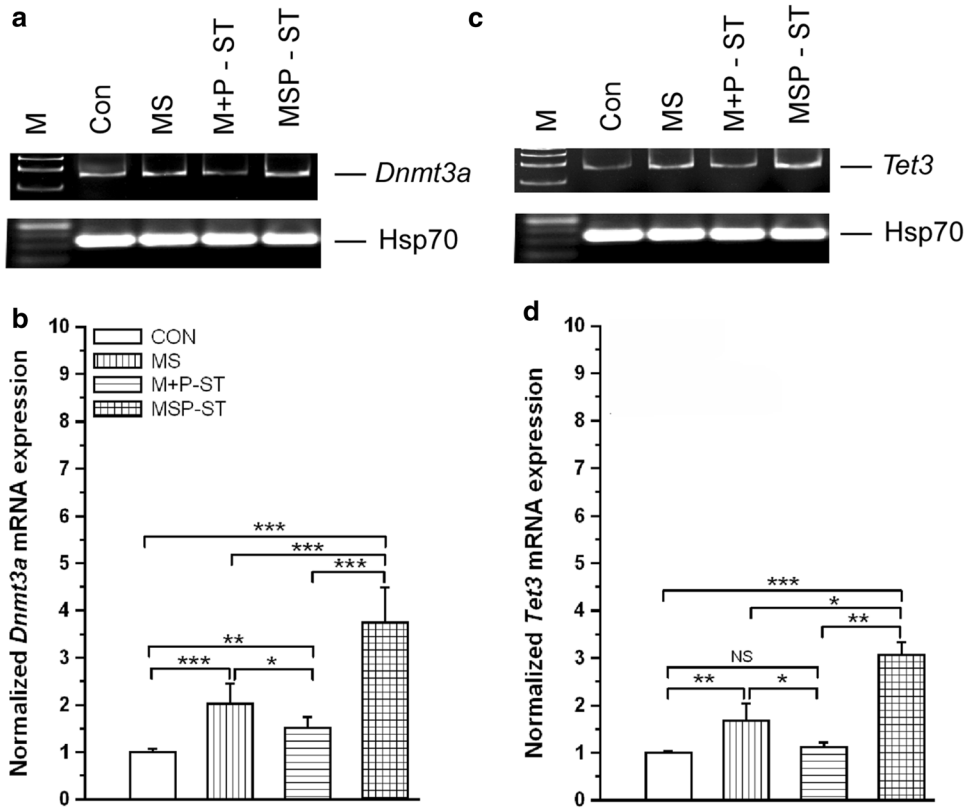

Fig. 4 Quantitative RT-PCR analysis showing the effect of early-life SSE on the level of expression in adolescents' amygdala. Gel image (**a, c**) showing the expression pattern of DNA methyltransferases (*Dnmt3a)*, Ten-eleven translocation (*Tet3)* and Heat shock protein (*Hsp70)*. Estimated normalized expression level of (**b**) *Dnmt3a* and (**d**) *Tet3* in experimental groups

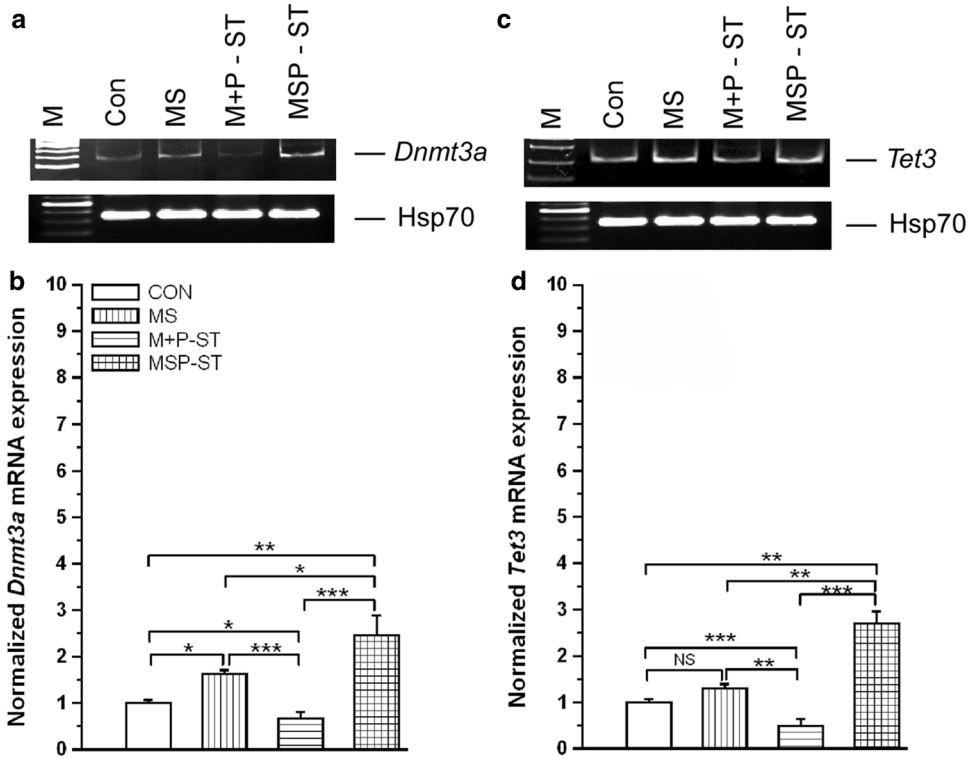

Fig. 5 Early-life SSE alters the mRNA expression of DNA methyltransferases (*Dnmt3a)* and Ten-eleven translocation (*Tet3)* in adults' amygdala. Quantitative RT-PCR analysis showing (**a, c**) *Dnmt3a, Tet3* and *Hsp70*. Estimated normalized expression level of (**b**) *Dnmt3a* and (**d**) *Tet3* in experimental groups

Resource 3). Observed results suggest that early social stress induced changes in methylation/ demethylation enzymes in amygdala of adolescent age possibly.

Early-Life SSE Alter MeCP2 and REST Level in Amygdala of Adolescent Rats

Since we observed notable change in *Dnmts* and *Tets*, following SSE, we sought to examine MeCP2 and REST in amygdala of adolescent rats. Western blot analysis showed that MeCP2 and REST level (Fig. 6a) was significantly elevated in MS, MSP-ST groups but reduced in M+P-ST group when compared with control. As seen in Fig. 6b,c, the estimated MeCP2 and REST was significantly higher in MS and MSP-ST than M+P-ST but we did not detect significant difference between MS and MSP-ST in MeCP2 while there was significant difference in REST (Online Resource 4). Observed variation in MeCP2 and REST shows that the early-life SSE alters the expression in adolescent age.

Early-Life SSE Alter MeCP2 and REST Level in Amygdala of Adult Rats

Following the SSE in early life, we examined the level of MeCP2 and REST in amygdala of adult rats. Similar to the changes observed in adolescent rats, level of MeCP2 and REST in amygdala of adult rats was altered by early life SSE (Fig. 7a). The estimated level of MeCP2 in MS and MSP-ST

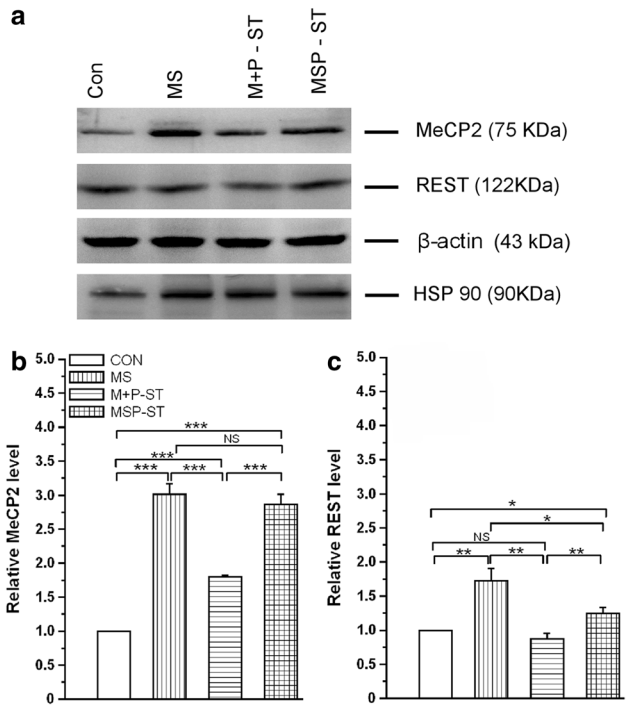

Fig. 6 Methyl CpG binding protein-2 (MeCP2), Repressor Element 1 Silencing Transcription Factor (REST) and Heat shock protein-90 (HSP-90) expression was altered in adolescent rats' amygdala by early-life SSE. (**a**) Representative western blots showing the level of MeCP2, REST, β-actin and HSP 90 expression. Estimated relative level of MeCP2 (**b**) and REST (**c**) in experimental groups

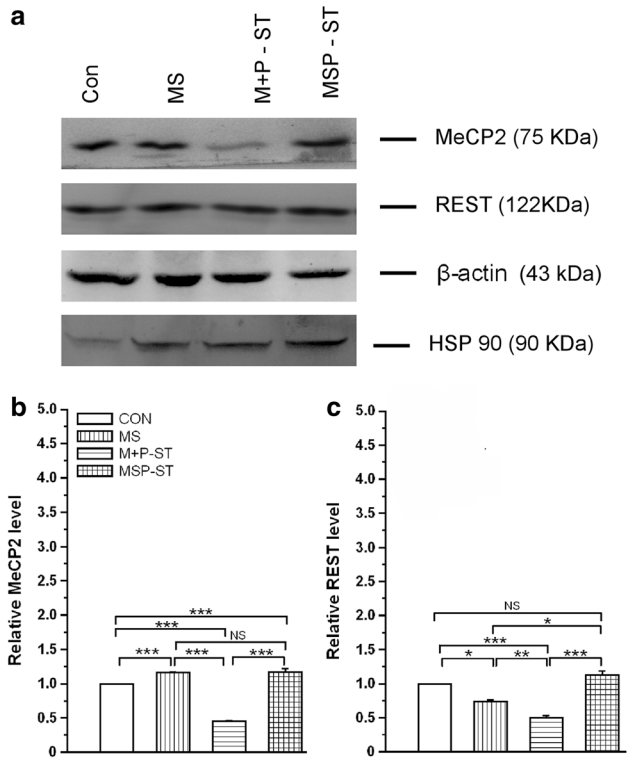

Fig. 7 Early-life SSE alters methyl CpG binding protein-2 (MeCP2), Repressor Element 1 Silencing Transcription Factor (REST) and Heat shock protein-90 (HSP-90) level in adult rats' amygdala. (**a**) Representative western blots showing expression level of MeCP2, REST, β-actin and HSP 90. Estimated relative level of MeCP2 (**b**) and REST (**c**) in experimental groups

was significantly higher than control but significantly lowered in M+P-ST group. Comparative analysis showed that the level of MeCP2, REST in MS and MSP-ST significantly elevated than M+P-ST. However, there is a significant difference between MS and MSP-ST only in the level of REST but not in MeCP2 (Fig. 7b, c; Online Resource 4).

Early-Life SSE Alter the Level of Acetylation and Specific Methylation at Histone Residue in Amygdala of Adolescent Rats

We found that level of H3K14ac was altered in adolescent rats, which experienced SSE stress in early (Fig. 8a). The estimated level of H3K14ac in MS and MSP-ST was significantly lower than control but no significant difference detected between M+ P-ST and control. Estimated level in MS and MSP-ST significantly lower than M+P-ST. In comparison, H3K14ac level in MS was significantly lower than and MSP-ST (Fig. 8b; Online Resource 5). To further understand the effect of early SSE on methylation, we examined the level of H3K4me2/me3 in amygdala. The estimated level of H3K4me2 in MS and MSP-ST were significantly higher than control but lowered in M+P-ST. Whereas, detected levels in MS and MSP-ST were significantly higher than M+P-ST group. In addition, we found that the level of H3K4me2 in MSP-ST was significantly higher than MS (Fig. 8c). In contrast, the detected level of H3K4me3 in M+P-ST was significantly higher than MS, MSP-ST and control. Whereas, H3K4me3 level in MS was significantly lower than MSP-ST and control. In comparison, higher level of H3K4me3 was found in MSP-ST than control (Fig. 8d; Online Resource 5).

Similarly, we examined the level of H3K9ac, me2, me3 (Fig. 9a). We found that level of H3K9ac was significantly lower in MS, M+P-ST and MSP-than control. Whereas, H3K9ac level was significantly higher in M+P-ST than MSP-ST and MS. In comparison, the level of acetylation did not differ between MS and MSP-ST (Fig. 9b). When we examined the level of H3K9me2, level of H3K9me2 was significantly higher in M+P-ST than MS and MSP-ST and control. Whereas, estimated level in MS and MSP-ST significantly lower than M+P-ST, however, MSP-ST showed significantly higher than MS (Fig. 9c). Contrast to H3K9me2, the detected level of H3K9me3 in MS, M+P-ST and MSP-ST was significantly higher than control. Estimated level in MS and MSP-ST was significantly higher than M+PM+P-ST group. However, MSP-ST was significantly higher than MS in H3K9me3 level (Fig. 9d; Online Resource 5). These results suggest that acetylation (H3K14; H3K9) and specific methylation (H3K4; H3K9) possibly contribute to the transcriptional regulation of stress responsive genes in amygdala.

Early-Life SSE Alter the Level of Acetylation and Specific Methylation at Histone Residue in Amygdala of Adult Rats

To further understand the effect of early SSE in adult rats, we examined the level of acetylation, di- and tri-methylation of the specific histone residue (H3K4; H3K9) in amygdala of adult rats (Fig. 10a). The level of H3K14ac was significantly higher in MS, M+P-ST and MSP-ST groups than control. However, there was no detectable significant difference among experimental groups [MS; M+P-ST; MSP-ST] in any comparison (Fig. 10b). The H3K4me2 level was significantly elevated in the rats' experienced social stress in their early life. The analysis showed that level of H3K4me2 was significantly higher in MS, M+P-ST and MSP-ST than control group. The estimated level in MS and MSP-ST were significantly higher than M+P-ST. In comparison, no significant difference detected between MS and MSP-ST (Fig. 10c). The detected H3K4me3 level was significantly higher in MS and MSP-ST than control group but there was no significant difference between control and M+P-ST. In comparison, MSP-ST level of H3K4me3 was significantly higher than MS (Fig. 10d; Online Resource 5).

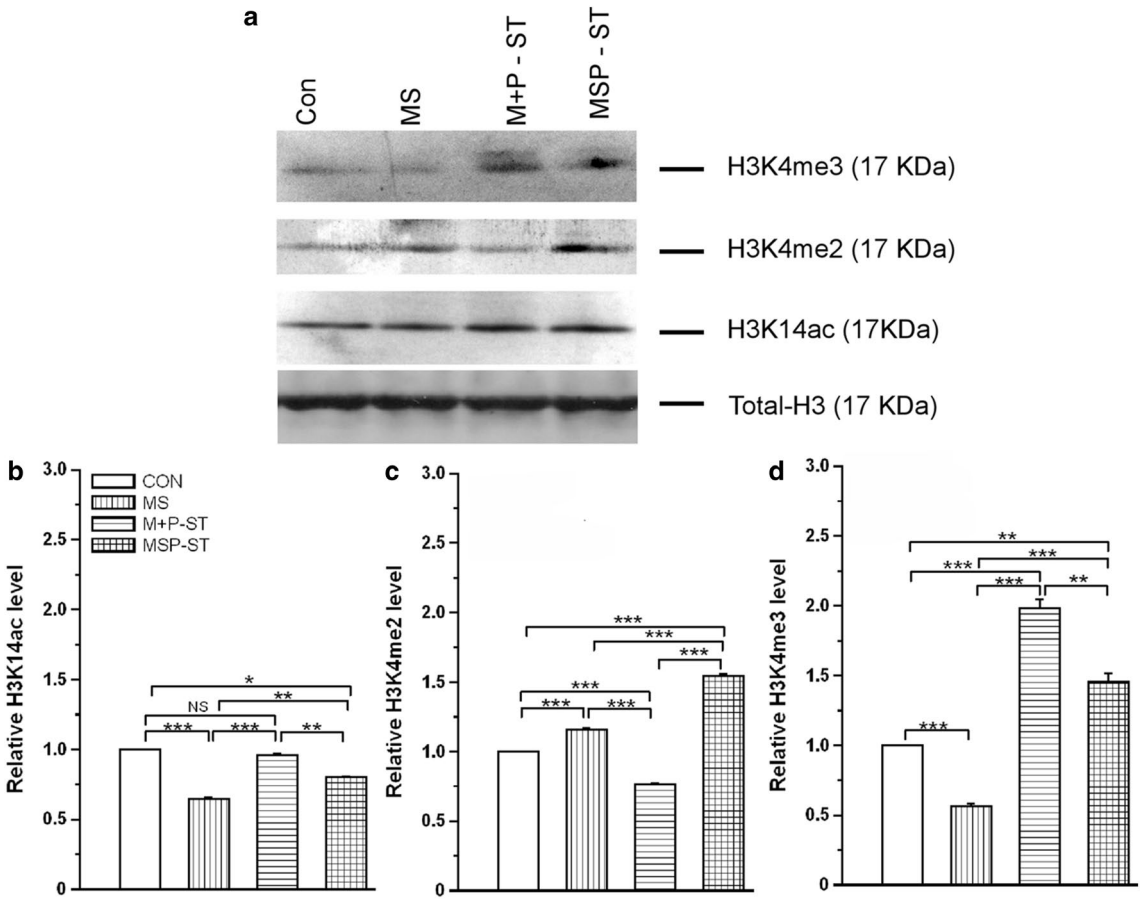

Fig. 8 The level of H3K14ac, H3K4me2/me3 in adolescent rats' amygdala altered by early-life SSE. (**a**) Representative western blots showing the variation in H3K14ac, H3K4me2 and H3K4me3 in experimental groups. (**b**) H3K14ac, (**c**) H3K4me2 and (**d**) H3K4me3 showing the detected relative level in experimental groups

Similarly, the level of H3K9ac, me2, me3 was altered in the amygdala of adult rats by early-life SSE (Fig. 11a). We found that the level of H3K9ac was significantly elevated in MS, M+P-ST and MSP-ST than control group. Among them, MSP-ST and MS group were significantly higher than M+P-ST. In comparison, MSP-ST was significantly higher than MS group (Fig. 11b). When we examine the H3K9me2 and me3, we observed similar pattern. The estimated level in MS, M+P-ST and MSP-ST groups showed significantly higher level than control group. Whereas, MS group showed significantly higher level than M+P-ST and MSP-ST but there was no significant difference between M+P-ST and MSP-ST group (Fig. 11c). We detected the level of H3K9me3 significantly higher in MS, M+P-ST but MSP-ST level did not vary than control. Among them, H3K9me3 level was significantly higher in MS than M+P-ST and MSP-ST. Whereas the detected level of H3K9me3 in MSP-ST was significantly lower than M+P-ST (Fig. 11d; Online Resource 5).

Early-Life SSE Alter the Expression of BDNF in Adolescent and Adult Amygdala

To establish the promoter, methylation changes of adolescent and adult rats were associated with alternation in BDNF protein level. Western blot analysis showed that BDNF level was altered by early SSE in amygdala of adolescent and adult rats (Fig. 12a). In adolescent and adults, the level of BDNF in MS and MSP-ST was significantly lower than control group. In comparison, the level of BDNF in MS was significantly lower in group. Note to mention that the detected BDNF level did not reach significant difference between control and M+P-ST (Fig. 12b, c; Online Resource 6). Observed results showed that early-life SSE may affect the BDNF expression in later life.

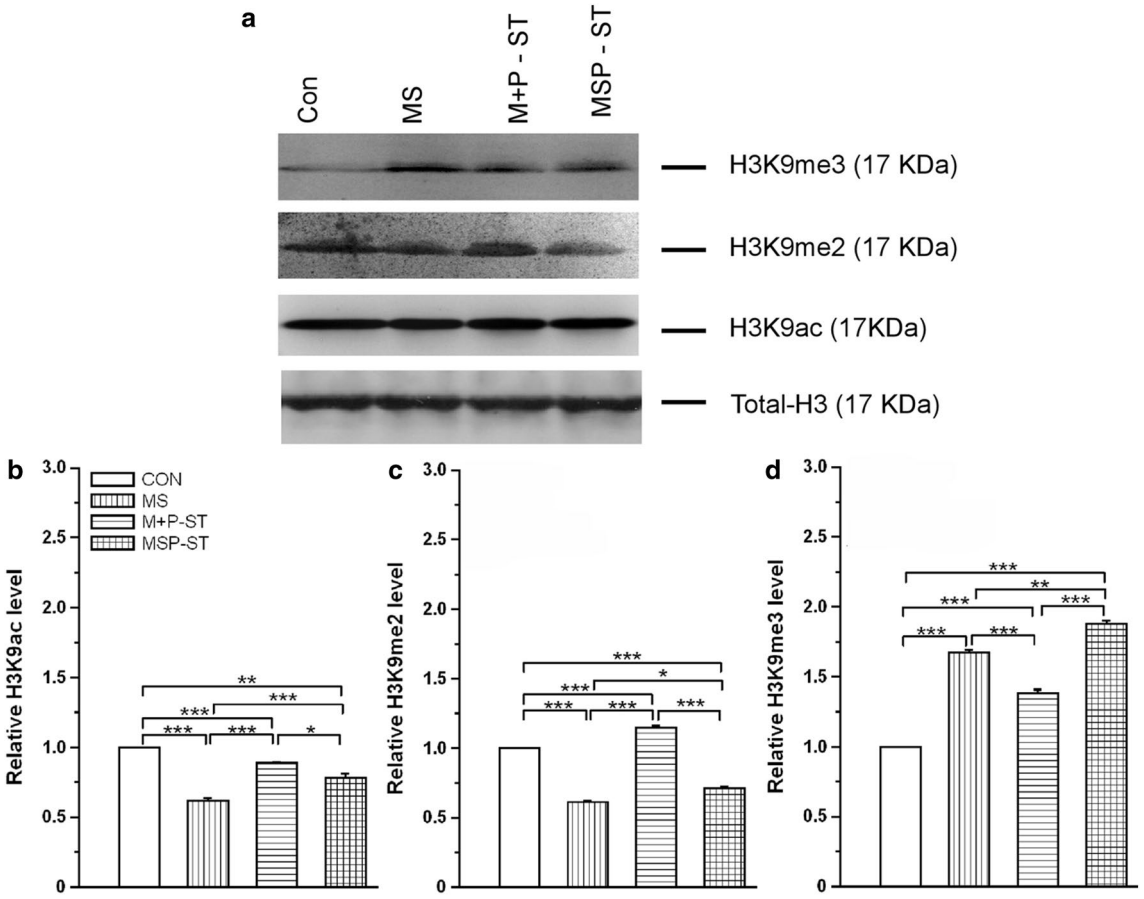

Fig. 9 Early-life SSE alters the level of H3K9ac, H3K9me2/ me3 in adolescent rats' amygdala. (**a**) Variation in the level of H3K9ac, H3K9me2 and H3K9me3 shown in representative western blots. (**b**) H3K9ac, (**c**) H3K9me2 and (**d**) H3K9me3 showing the differential levels in experimental groups

Discussion

Recent studies in early-life stress model have shown different behavioural effects, *i.e*. increased anxiety or decreased anxiety (D′Amato and Cabib 1987; Leite et al. 2016). Thus, in this study, we tested whether the presence/ absence of mother during early-life stressful social experience (SSE) contribute to the experience-dependent behavioural changes in adolescent and adult. We found that individuals' experienced social stress (SS) in the absence of their mother (MSP-ST) showed less social interaction with the conspecifics than the control, and the group experienced SS in the presence of their mother (M+P-ST). The SSE of MSP-ST group in the absence of their mother may suppress the exploratory or sociability behaviour. Mothers' physical contact such as grooming, licking, warmth and maternal odor may suppress the stranger-induced stress in M+P-ST group (Kalin et al. 1994; Moriceau et al. 2009; Nugent et al. 2015). Indeed, earlier studies demonstrate that separating the pups from their mother during early post-natal period has a pronounced effect on their pups in later life (Anier et al. 2014; Bose et al. 2015; Wang et al. 2017). Similarly, we have also observed lower levels of exploration and sociability with the unknown conspecifics in the MS group. When we tested their social behaviour on second day with known and unknown conspecifics, the MS group actively contact with the ST-1 than ST-2. Individuals from MSP-ST group interacted with ST-1 and spend more time at the center chamber during adolescent period but contrast in adulthood. One can expect that animals in a familiar context may have less anxiety/ stress (Uchida et al. 2010; Luchetti et al. 2015). MS has been known to elicit the stress (Anier et al. 2014; Bose et al. 2015), the presence of a stranger can create additional stress and may alter the feedback mechanism, and impair the stress response (Moriceau et al. 2004; Luchetti et al. 2015). In this study, observed behavioural profile demonstrate that the presence of mother possibly suppresses the SSE-induced stress and the associated social interaction later in life.

Recent studies have demonstrated that ELS-induced behavioural disorders are possibly governed by epigenetic mechanisms, specific histone modifications, DNA methylation and alternations in small-RNA molecules (Kao et al.

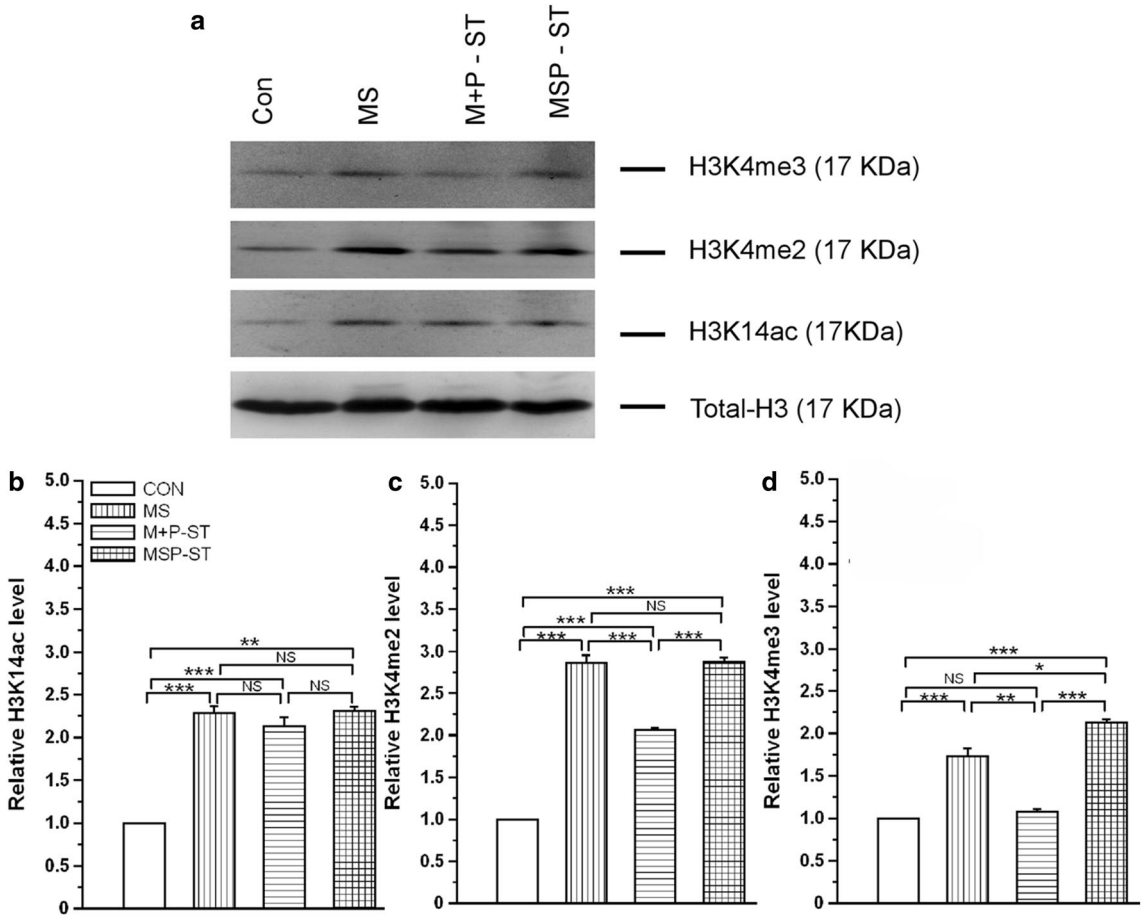

Fig. 10 SSE in early-life alters the level of H3K14ac, H3K4me2/ me3 in adult rats' amygdala. (**a**) Western blots showing the alternations in H3K14ac, H3K4me2, and H3K4me3. In different experimental groups estimated relative level of H3K14ac (**b**), H3K4me2 (**c**) and (**d**) H3K4me3

2012; Wang et al. 2017), which are known to activate or repress the transcriptional process based on specific residue. One can expect that the SSE can alter the methylation level through the induction of DNMT3a (Murgatroyd et al. 2009; Anier et al. 2014). The elevated level of DNMT3a in the amygdala of adolescent and adult MS, MSP-ST group increased accordingly than M+P-ST group. On the other hand, TET-3 has been known to respond to the stress (Moriceau and Sullivan 2006; Murgatroyd et al. 2009), the level of TET-3 was elevated in the adolescent and adult rats MS and MSP-ST group but not in the M+P-ST group. Therefore, it is possible that early-life SSE could alter the gene expression, and lead to behavioural impairment (D′Amato and Cabib 1987; Roth et al. 2009; Pusalkar et al. 2016). Indeed, MeCP2 and REST are the other class of enzymes that governs the process of methylation (File and Hyde 1978; Moriceau and Sullivan 2006; Bondar et al. 2018), and contribute to the activation / repression of specific gene or genome level according to the individuals' experience (Veenema et al. 2007). Elevated level of MeCP2 and REST was detected in the adolescent, adult MSP-ST group but not in the M+P-ST group. The observed reduction of *Dnmt3a, Tet3*, MeCP2 and REST in M+P-ST group than control possibly associated with maternal overprotection during SSE (Murgatroyd and Spengler 2014; Perera et al. 2015).

Different laboratories have shown that stress alter methylation at the specific residues based on context/ experience (McGann et al. 2014; Singh-Taylor et al. 2018). Particularly, methylation of histones at specific sites (H3K4) can facilitate the active transcription and on the other hand (H3K9) possibly silence the transcription process (Zimmermann et al. 2015). We noted elevated levels of H3K4me2 in amygdala of both adolescent and adult MS and MSP-ST group compared to other groups. Together with previous reports (McLeod et al. 2007), H3K4me2 data suggest that SSE alter the methylation status and contribute to the development of behavioural disorders. In part, the level of H3K4me3 was decreased in adolescent rats, but in contradiction increased in adult rats. However, earlier studies posted differential pattern of H3K4me3 level according to the type of stress and specific brain region (McLeod et al. 2007; Hunter et al. 2009; Gupta et al. 2011; Wang et al.

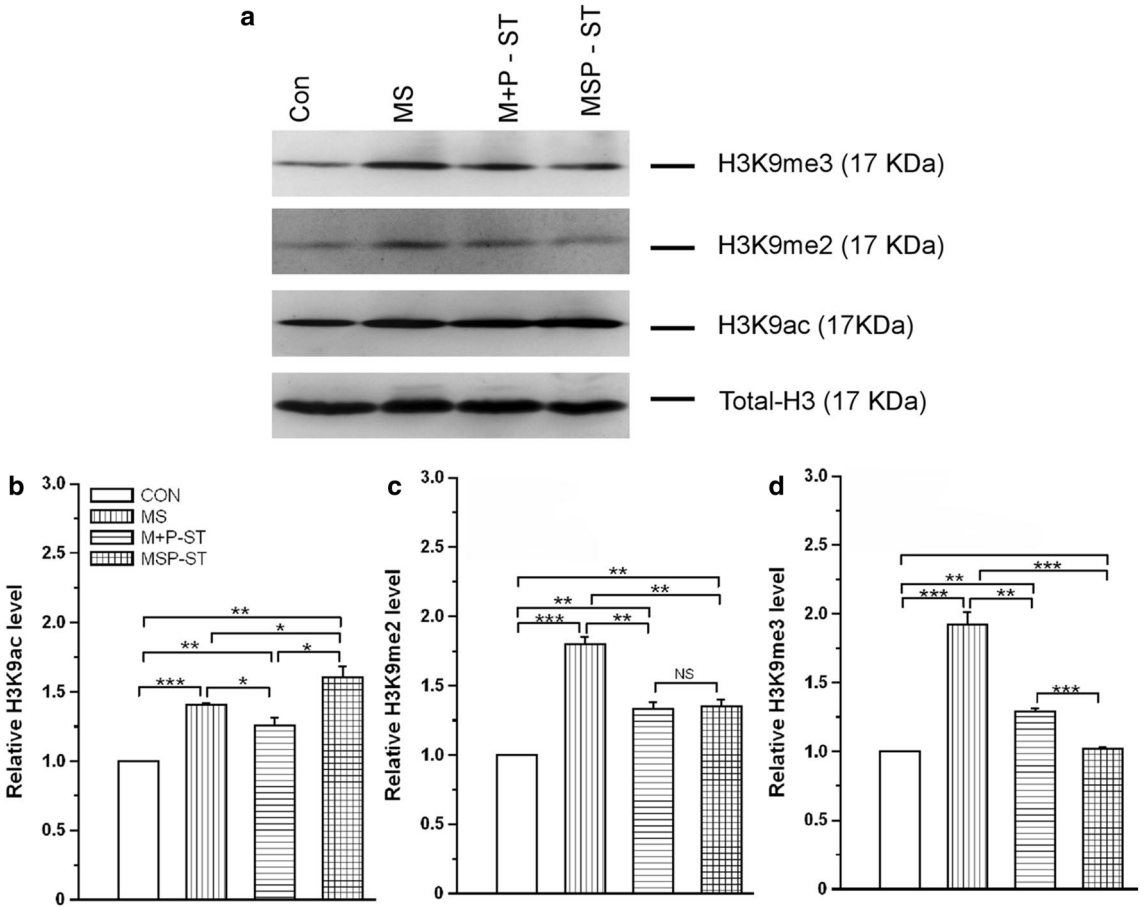

Fig. 11 Early-life SSE alters the level of H3K9ac, H3K9me2 and H3K9me3 in adult rats' amygdala. (**a**) Western blots showing variation in the expression of H3K9ac, H3K9me2 and H3K9me3. H3K9ac (**b**), H3K9me2 (**c**) and H3K9me3 (**d**) estimated relative level in different experimental groups

2017). The developmental regulations possibly contribute to the contradiction in the level of H3K4me3 in adolescent and adult rats which experienced the same type/ level of stress. In case of H3K9 methylation, we found contrasting pattern of H3K9me2 and H3K9me3 in adolescent rats. Note to mention that H3K9me2 is known to repress the gene expression by actively recruit the DNA methylation complex and in contrast H3K9me3 mediates the active transcription (McGann et al. 2014). The down regulated level of H3K9me2 and elevated level of H3K9me3 in the MS, MSP-ST groups than the other groups. Supporting to our data, earlier studies in other stress models reported that stress reduce the levels of H3K9me2 (Kao et al. 2012; Wang et al. 2017) and elevated level of H3K9me3 (Sailaja et al. 2012; Gere et al. 2012). Furthermore, earlier studies suggest that a reduction in the level of H3K9me2 may reduce the synaptic plasticity, neurotransmission and then leads to the development of depressive-like behaviour (Want et al. 2017). Note to mention that, we have not observed significant changes in the level of both H3K9me2 and H3K9me3 in adults. It is possible that neuronal development could alter the stress-induced modifications (Ruthenburg et al. 2007; Bose et al. 2015).

Subsequently, we examined the levels of acetylation of histones at lysine residues (H3K9ac; H3K14ac), and our analysis showed that SSE altered the acetylation at H3K9 and H3K14. We found that early-life SSE reduced the level of acetylation in H3K9, H3K14 in the MSP-ST group and the MS group than other groups in adolescent rats. The level of H3K9ac and H3K14ac were increased in the early-life SSE groups, specifically in MSP-ST group during their adolescent period. Similar to our observations, earlier studies (Covington et al. 2011; Gere et al. 2012; Sailaja et al. 2012) reported the declining level of H3K9ac and H3K14ac in different stress model. In fact, the level of H3K9ac and H3K14ac has been increased in adults, possibly due to the accumulation of HDAC (Berger 2007) in brain region. The reason may be based on the fact that following the stressful experience occurred during the early stages; chances are more for hypoacetylation to occur in the histone thus

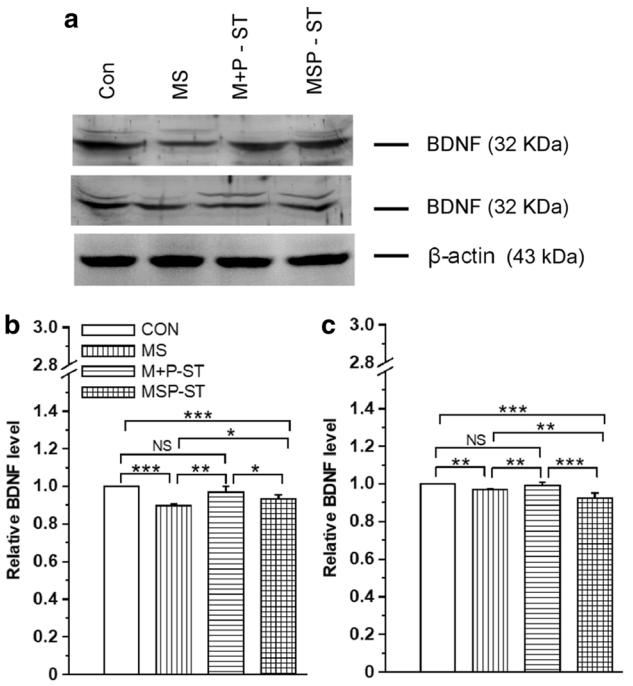

Fig. 12 Early-life SSE alters the expression of BDNF in the amygdala of adolescent and adult. (**a**) Western blots showing the expression pattern of BDNF in amygdala of adolescent (first) and adult (second), and their estimated relative level of BDNF in adolescent (**b**) and (**c**) adults

changing their open chromatin conformation and transcriptional activation (Lachner and Jenuwein 2002; Hunter et al. 2012).

Earlier studies have been reported that the interaction between DNA and histone modification can alter the DNA methylation (Bose et al. 2015). Notably, several studies linked the social stress and methylation status of BDNF (Boersma et al. 2014; Doherty et al. 2016) with depressive-like behaviour/ sociability. This suggests that methylation/ demethylation of *bdnf* gene is linked to the development and functioning of the brain; thus, we analyzed level of BDNF in adolescent and adult rats. We observed that there was a significant decline in the MS and MSP-ST groups when compared to control and M+P-ST groups. The result suggests that stress can suppress BDNF expression, thus showing impaired social interaction (Roth et al. 2009; Mitchelmore and Gede 2014; Boersma et al. 2014) in MS and MSP-ST groups but not in others.

Taken together, our study demonstrates that early-life SSE can significantly impair social behaviour in later life. The observed changes in core enzymes (DNMT3a; TET3; MeCP2; REST) balancing the methylation and its associated epigenetic changes in acetylation (H3K9ac/14ac), methylation (H3K4me2/me3/K9me2/me3), and level of BDNF in amygdala could be linked with early-life SSE. Finally, the observed results suggest that presence of mother possibly counteracts with the stressful stimuli and suppress the SSE-induced changes in epigenetic status and social behaviour in later life.

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Compliance with Ethical Standards

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

Ethical Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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