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RESEARCH ARTICLE

The impact of maternal separation and isolation stress during stress hyporesponsive period on fear retention and extinction recall memory from 5-week- to 1-year-old rats

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Abstract

The purpose of the present study was to determine whether age would disrupt fear retention and extinction memory in rats pre-exposed to maternal separation and isolation stress; these rats are called MS rats. MS stress was induced by exposing rat pups into maternal separation followed by isolation stress from peer groups (MS) daily/6 h during stress hyporesponsive period, while controls rats that were undisturbed during this period are called NMS rats. 5, 8, 15 and 52 weeks later, these animals were exposed to classical fear conditioning test by pairing auditory stimulus (conditioned stimulus, CS+) with electric footshock. 24 h later, conditioned freezing response to CS+ was measured during fear retention, extinction and extinction recall trials. The normal ageing per se did not affect the formation of fear memory, retention and fear extinction memory. MS stress, on the other hand, disrupted fear memory at young adulthood age exhibiting increased freezing response to CS+ during retention test and reduced during fear extinction memory test when compared to NMS groups. On the other hand, rats at adolescence age exhibited reduced freezing during fear retention and enhanced freezing response to CS+ during extinction recall test. However, MS-induced changes in freezing response during fear retention and extinction tests were not seen in adulthood and 1-year-old age groups. These data demonstrate the young adulthood age is highly vulnerable to fear memory and extinction processes. The differences in freezing response to CS+ during fear conditioning from adolescence to old age, thus, appear to be related to the maturation of the limbic circuit.

Keywords Maternal separation stress and isolation stress · Stress hyporesponsive period · 5 week · 8 week · 15 week · 1 year · Fear retention · Fear extinction memory

Introduction

The maternal separation and isolation stress during stress hyporesponsive period (SHRP) from postnatal days 4–14 involves activation of hypothalamus-pituitary adrenal (HPA) axis leading to hypercortisolism in the rodent models (both mouse and rat) (de Kloet et al. 2005; Levine 2001; Schmidt et al. 2003). The increased cortisol level can impair the stress coping mechanisms in rat adulthood (8 weeks) (Lajud et al. 2012) and sensitize the limbic circuit in young children which may further increase the risk for psychiatric disorders (Gunnar and Quevedo 2008) and cardiovascular problems later in life (Miller et al. 2011). These individuals when challenged with corticotropin releasing hormone (CRH) during adulthood can have diminished or blunted HPA axis response in rodent models (McEwen et al. 2015). This was supported by clinical studies indicating that severity of the psychiatric disorders like anxiety disorders, schizophrenia and panic attacks increases as intensity of the adverse experiences is more in childhood (Quintino-dos-Santos et al. 2014; Bale et al. 2010; Yamauchi et al. 2005) and like seen with the children of mothers who had a history of infection during pregnancy (Brown 2006; Knuesel et al. 2014; Sotowright et al. 1995). The studies have also shown that panic reactions, depression, anxiety and hallucinations increase with increasing adverse experiences during early childhood (Anda et al. 2006).

In addition to clinical evidences, the studies also suggest that an exposure to stress during the 'critical time window'

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can cause increased anxiety with increased fear retention memory in the adult male Sprague–Dawley and Wistar rats (Kosten et al. 2006; Madruga et al. 2006; Sampath et al. 2014), increased vulnerability to ethanol consumption (Nylander and Roman 2013), facilitated extinction of social fear (Zoicas and Neumann 2016), increased fear memory (Toda et al. 2014) as well as several emotional and social behaviours in rats (Beery and Kaufer 2015; Gonzalez et al. 2001) and mice (Reiss et al. 2007).

Based on the above studies, it is evident that disrupted maternal care during the 'critical time window' of the development impairs the HPA axis response to stressors during adulthood (8 weeks). However, it is unclear whether MS stress during SHRP impairs fear memory and extinction across all the age groups from adolescence (5 weeks) to older age (< 1 year). The classical fear conditioning paradigm was used to induce fear, which involves presentation of neutral stimuli with mild electric footshock as unconditioned stimulus in mice (Laxmi et al. 2003). Repeated presentation of conditioned stimuli leads to a gradual decline in the fear response, a process termed as fear extinction. The present study has examined the long-term effects of early maternal separation followed by isolation stress on fear memory and its extinction from 5-week- to 1-year-old age group.

Materials and methods

Male Wistar rats weighing 140–330 g (5 week-, 8 week-, 15 week-, > 1-year-old) were used to study the effect of ageing and early life stress on fear retention and extinction memory. All the experiments were conducted according to the ethical guidelines and approved by the Institutional Animal Ethics Committee (IAEC, NIMHANS) (AEC/46/286/NP), Bengaluru, India. Maximum care was taken to reduce the discomfort to the animals during the maternal separation protocols and experimental procedures. Total $n = 26$ pregnant rats, procured from CARF, NIMHANS, Bengaluru, India, were used in the study Food and water were provided ad libitum. The rats were kept in well-ventilated polypropylene cages in a climate-controlled room having adequate exposure to the light–dark cycle (12 h:12 h).

Maternal separation stress procedure

Pregnant rats were randomly divided into MS and NMS groups. Rats that were exposed to stress during P4–P14 were considered as early maternal separation and isolation stress group (MS) and a group of rats that were not exposed to stress were assigned to animals with no MS stress (NMS) group. The rat litters from MS group were housed with the dam in a cage for the entire period of pre-weaning except at the time of maternal separation procedure, while controls (NMS) were not disturbed throughout the pre-weaning period (P0–P21).

Maternal separation and isolation stress (MS) was conducted for a period of 6 h (10.00–16.00 h) daily and this stress protocol was carried out from P4 to P14 (10 days). At the end of the separation period, rat pups were returned to the home cage and reunited with dams. These MS rat litters were undisturbed for rest of the period until weaning. On P21, rat pups were weaned from the mother (Fig. 1). The total number of rats used in the present study are NMS, ($n = 56$), MS ($n = 61$) and these rats were randomly distributed in different age groups (5 weeks, 8 weeks, 15 weeks and 1 year).

Fear conditioning apparatus

Differential auditory fear conditioning and extinction protocols were carried out in two different contexts placed inside the sound isolation box (23″W × 20″D × 24″H, Coulbourn Instruments, USA). The conditioning chamber was a rectangular box (12″W × 10″D × 12″ H, Coulbourn Instruments, USA) with metal grids on the floor for delivering electric foot shock. In order to deliver auditory stimulus, one speaker (4Ω, Coulbourn Instruments, USA) was kept on the wall of the chamber. The video camera was mounted on the rooftop of the isolation box for monitoring the behaviour of rats. Programmable tone generator and shocker (Habitest system, Coulbourn Instruments, USA) were used to deliver tones and foot-shock during the experiment.

Total time: day 1 habituation: 6.6 min/session—two sessions per day, total: 13.2 min. Day 2 fear conditioning: 5.16 min. Day 3 retention of fear memory: 6.6 min. Days 4–6 extinction training: 13.6 min/session—two session per day, total: 27.2 min. Day 16 recall of extinction memory: 13.6 min.

Differential fear conditioning

A total of 117 rats were trained in an auditory-cued differential fear conditioning paradigm. The rats from both the groups were at first exposed to two different contexts (Coulbourn Instruments, USA). The differential fear conditioning was carried out in one context (context A), while extinction training was carried out in another context (context B). Context A: rectangular box having floor grids, a video camera mounted on the roof-top of the isolation cubicle, house light-on, conditioning chamber cleaned with 70% alcohol before placing rat into the chamber. Context B: same as that of context A. Here, the conditioning chamber was refreshed with peppermint soap.

Fear conditioning and extinction protocol were based on the previous study in the laboratory (Sampath et al. 2014). The rats were handled at least for 3–4 days before the

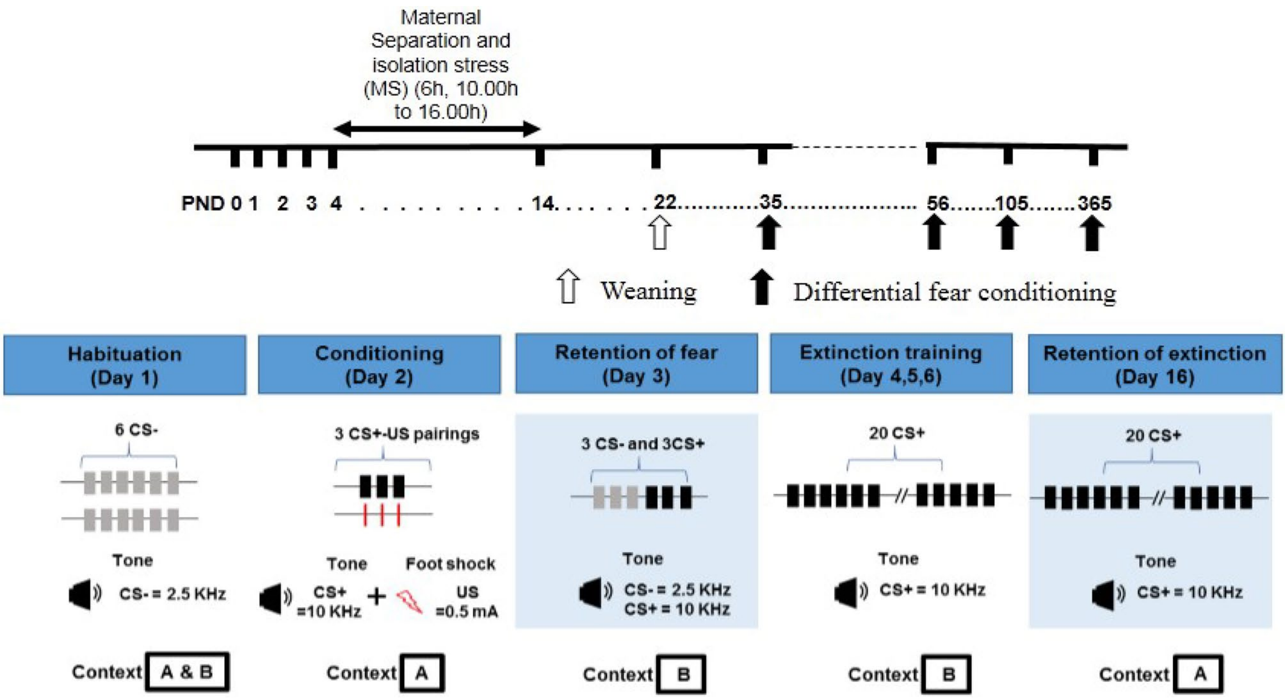

Fig. 1 Outline of the study: experimental design: rats were subjected to the tone habituation with 6CS− (2.5 kHz) in both context A and context B on day 1. Followed by auditory fear conditioning (3CS+ and 3US pairing during conditioning) using a US (0.5 mA) combined with CS (10 kHz) in context A. 24 h later, rats were subjected to fear retention test using 3CS− and 3CS+. On day 3, rats did not receive foot shock, but only tones CS− (2.5 kHz) and CS+ (10 kHz) were given to test the retention of acquired fear memory. Extinction training sessions were given in context B from days 4 to 6, giving 20 CS+ with 2 sessions per day. 10 days after last session of extinction training, recall of extinction memory was performed in context A using 20 CS+

beginning of an experiment. Briefly (Fig. 1), rats were first habituated to two contexts—context A and context B with the presentation of six auditory tones (CS−) (2.5 kHz, 70 dB) lasting for 10 s with inter-tone interval of 20 s. Rats were allowed to explore freely in the conditioning chamber before and after the termination of auditory tone. The inter-trial interval between two habituation sessions was 6 h. On day 2, auditory-cued differential fear conditioning was carried out in context A. Rats were allowed to explore the conditioning chamber for 120 s before the presentation of three conditioned stimuli (CS+) (10 kHz auditory tone for 10 s duration, 90 dB)/foot-shock (US) (co-terminated with 0.5 mA of foot shock for a period of 1 s) pairings. CS+/US pairings were separated by 20 s. The fear conditioning protocol was repeated after 6 h in the same context to reinforce the conditioned fear memory. On day 3, rats were placed in context B and were introduced to both CS− (3 times) and CS+ tones (3 times) without pairing with the US. The inter-trial interval was 20 s. The rats were left in the conditioning chamber for 120 s before and after termination of last CS+. On day 4, the extinction sessions were carried out in context B with repeated presentation of 20 CS+ tones and inter-tone interval of 20 s. This trial was carried out for two sessions per day with an interval of 6 h. On day 16, i.e., 10 days after the last session of extinction training (day 16), rats were once again placed in the conditioning chamber in context A to test recall of extinction memory. In this extinction recall test session, rats were once again exposed repeatedly to 20 CS+ tones lasting 10 s with an inter-tone interval of 20 s. The fear memory was based on the time spent in freezing (lack of movements except for respiratory movements) to CS which were analysed (Sampath et al. 2014; Seidenbecher et al. 2003) using FreezeFrame software (Coulbourn Instruments Co., USA). We describe the percentage freezing of the first session of extinction training on fourth day and last session of extinction training on sixth day. We intently chose two extremes of the extinction training because most of the relevant outcomes of the study were found in these extremes only.

Further fear extinction index was calculated using the following formula:

$$\mathrm{FE}_{\mathrm{index}} = \frac{F_{\mathrm{recall}}}{F_{\mathrm{extinction\ recall}}},$$

where $\mathrm{FE}_{\mathrm{index}}$ is the fear extinction recall index, F_{recall} is the freezing response in the first fear extinction session and

$F_{\text{extinction recall}}$ is the freezing response in the fear extinction recall session. The value of FE_{index} is 1 when F_{recall} is equal to $F_{\text{extinction recall}}$. The value of FE_{index} is more than 1 when $F_{\text{extinction recall}}$ is less than F_{recall}. This indicates the presence of an extinction memory.

Data analysis

Fear conditioning-induced changes in freezing to CS− and CS+ were analysed by repeated measure with two-way analysis of variance (ANOVA). Further, Bonferroni post-hoc test was applied to calculate significant difference within and between the experimental groups, to compare changes in behavioural responses for different animals as the ratio of average freezing to CS− over CS+ during recall of fear memory.

Results

MS stress animals exhibit enhanced fear memory during retention test to CS+ than controls in PND56 (8 weeks) but not in PND105 (15 weeks), PND35 (5 weeks) and PND365 (1 year) age groups

Here, we investigated the impact of MS stress for 10 days of SHRP on fear acquisition, retention of conditioned fear, extinction and extinction recall of fear memory from 5-week-old age groups to 1-year-old rats. We found that MS stress for 10 days exhibited increased fear memory retention in both 8 weeks and 15 weeks after the termination of MS stress, whereas similar effect on fear memory retention was not observed in 5-weeks- and 1-year-old as compared to controls (Fig. 2a–d).

Further, between-groups analysis using two-way ANOVA test revealed, on the other hand, that MS group of 8-week-old rats showed significant increase in freezing behaviour to CS+ ($p < 0.01$) as compared to that of NMS groups. The significance differences were based on groups ($F_{1,204} = 42.41$, $p < 0.0001$) and stimulus ($F_{5,204} = 18.04$, $p < 0.0001$). The differences in freezing behaviour to CS+ between-group was not evident in other age groups—5 weeks, 15 weeks and 1-year-old (Fig. 2a–d). Together, these results indicate that young adulthood rats pre-exposed to MS stress are highly vulnerable to fear responses showing increased fear memory retention.

Acquisition of fear extinction is age-dependent from young to old

It was assumed that the acquisition of fear extinction may depend upon the age at which they are exposed to extinction training sessions (Jovanovic et al. 2014) and on the 'new learning' after exposure to classical fear conditioning. In the present study we found that acquisition of fear extinction did not depend upon the age. The rats at adolescence showed 'new learning' ability when compared to that at young adulthood. Both NMS and MS rats were exposed to fear extinction training in context B after 24 h of fear conditioning. At 5 weeks, adolescence age, both NMS and MS group of rats exhibited progressive decline in conditioned fear response across extinction training session 1–6 (block of 20 trials). The MS rats acquired fear extinction learning faster than that of controls showing progressive reduction in the fear response to CS+ at the first extinction session

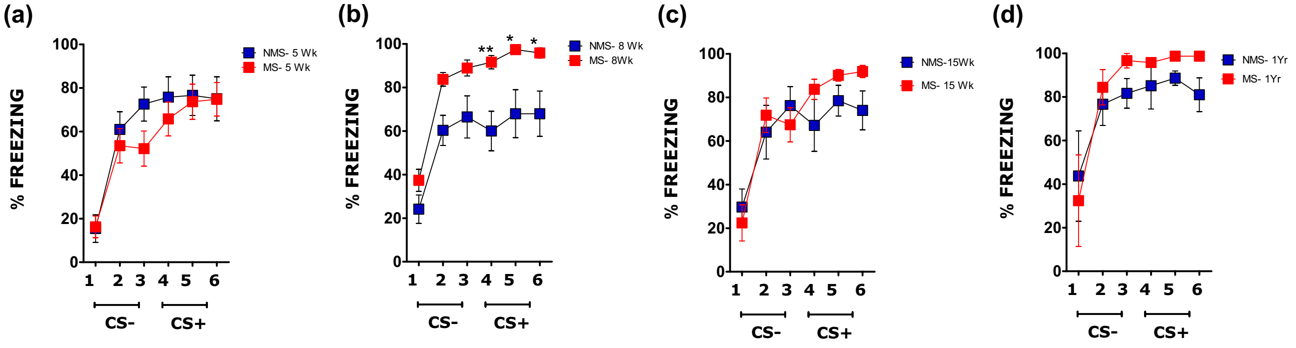

Fig. 2 The percent freezing response to CS− and CS+ by NMS and MS groups from **a** 5 weeks, **b** 8 weeks, **c** 15 weeks and **d** 1-year-old rats. Both groups of rats exhibited robust increase in freezing response to CS+ and less freezing to the CS− during fear retention period. Two-way ANOVA followed by Bonferroni post-hoc test was applied to find the difference between and within group. $**p < 0.01$, $*p < 0.05$ within-group comparison between CS+ and CS−; NMS (control group) (5 weeks, $n = 14$; 8 weeks, $n = 17$; 15 weeks, $n = 10$; 1 year, $n = 15$) and MS (maternal separation and isolation group) (5 weeks, $n = 16$; 8 weeks, $n = 19$; 15 weeks, $n = 12$; 1 year, $n = 15$); CS− unpaired stimulus, CS+ paired stimulus. *X*-axis shows response of rats towards tones, CS− (stimulus, 1–3) and CS+ (stimulus, 4–6) during retention of fear memory. *Y*-axis shows percentage freezing calculated during CS− and CS+ presentation in both MS and NMS rats

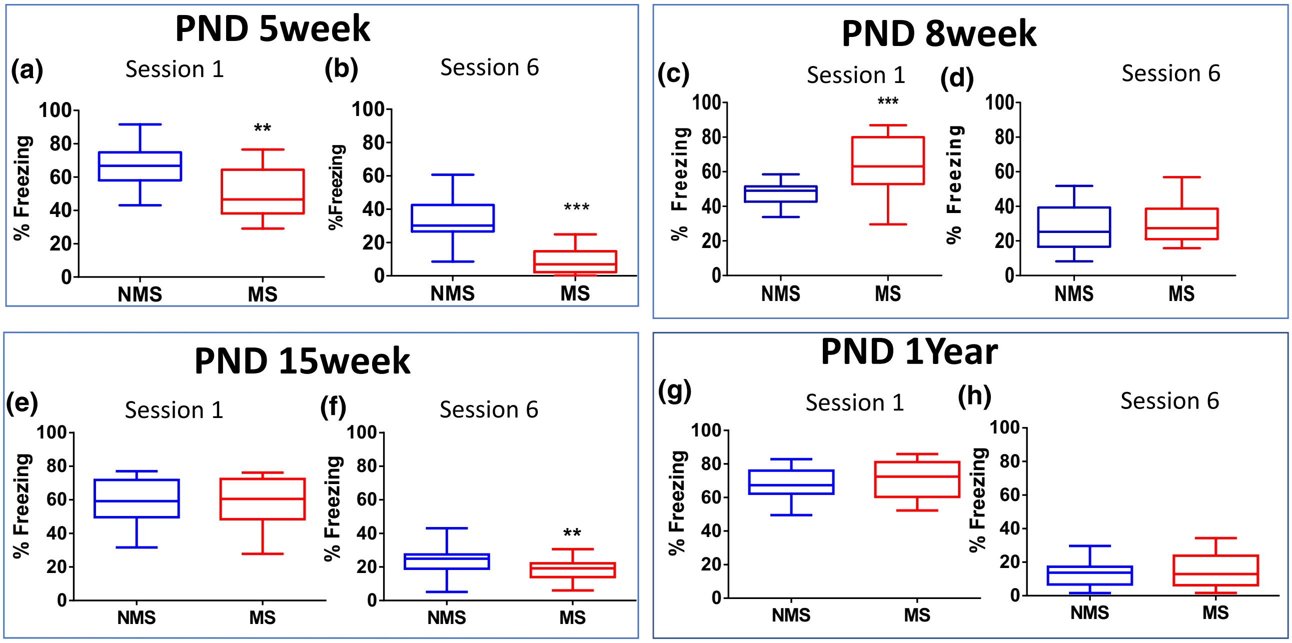

Fig. 3 The per cent freezing response to CS+ during acquisition of fear extinction trials in NMS and MS group of rats. **a, c, e, g** First extinction session of 5 weeks, 8 weeks, 15 weeks and 1-year-old group; **b, d, f, h** last extinction session of 5 weeks, 8 weeks, 15 weeks and 1-year-old rats, respectively. Unpaired *t* test, $***p<0.0001$, $**p<0.01$ as compared to NMS groups. *NMS* control group, *MS* stressed group. Two-way ANOVA followed by Bonferroni post-hoc test was also analysed, ($****p<0.0001$ stimulus, $****p<0.0001$ group, **a**), ($p=0.0008$ stimulus, $****p<0.0001$ group, **b**), ($****p<0.0001$ stimulus, $****p<0.0001$ group, **c**), ($****p<0.0001$ stimulus, **d**), ($****p<0.0001$ stimulus, **e**), ($*p=0.0126$ group, **f**), ($****p<0.0001$ stimulus, **g**), two factors comparable, stimulus and group. *X*-axis shows comparison of NMS (blue) and MS (red) group during extinction training sessions. *Y* axis shows percentage freezing showed by MS and NMS rats

is significantly different between-groups ($t_{1,38}=3.787$, $p<0.0005$) (Fig. 3a). The difference in freezing level is also different between groups in the last fear extinction session ($t_{1,38}=6.997$, $p<0.0001$) (Fig. 3b) when compared to NMS rats. These results indicate that early MS stress has ameliorated the stress coping mechanisms showing increased acquisition rate of fear extinction for rats at adolescence age (PND 5 weeks).

The acquisition of fear extinction was different for young adulthood age groups when compared to adolescence and 15-week- and 1-year-old age groups. The freezing behaviour to CS+ during acquisition phase, MS rats at young adulthood initially showed significant increase in freezing behaviour (session 1) ($t_{1,38}=4.333$, $p<0.0001$) (Fig. 3c) and gradually reduced at the end of sixth extinction session (Fig. 3d). The rats at young adulthood (8 weeks) as well as 1-year-old MS rats did not show significant differences in freezing behaviour following extinction training (session 6) as compared to age-matched control (Fig. 3d, h) even though initially (session 1) 8-week-old group showed significant increase in freezing response ($t_{1,38}=4.333$, $p<0.0001$) (Fig. 3c).

Similar to adolescence group rats at adulthood (15 weeks) age MS rats did not show differences in the freezing response during the initial phases of extinction training when compared to NMS groups (Fig. 3e). However, following five sessions of extinction training, MS exhibited significant decrease in freezing response to CS+ ($t_{1,38}=2.597$, $p<0.01$) when compared to age-matched controls (Fig. 3f). Together, these results indicate that the magnitude of 'new learning' is high in 5- and 15-week-old MS rats (Fig. 3b, f) and not in 8-week- and 1-year-old MS groups when compared to age-matched controls (Fig. 3d, h).

Recall of fear extinction

When extinguished fear reappears in a context different from the fear conditioning environment, it is called fear extinction recall. It is evident from the (Fig. 4a) that there was differential fear extinction recall memory across age groups such that 5 week old MS rats had stronger fear extinction memory, while, 8 week old MS rats had poor extinction memory (Fig. 4b). This difference in the freezing behaviour during recall session was not seen in 15-week- and 1-year-old groups (Fig. 4c, d). The fear extinction recall in 8-week-old MS rats did not appear to be mitigated by extensive extinction training. (Fig. 4b) represents increased freezing response to CS+ by MS group of rats during extinction

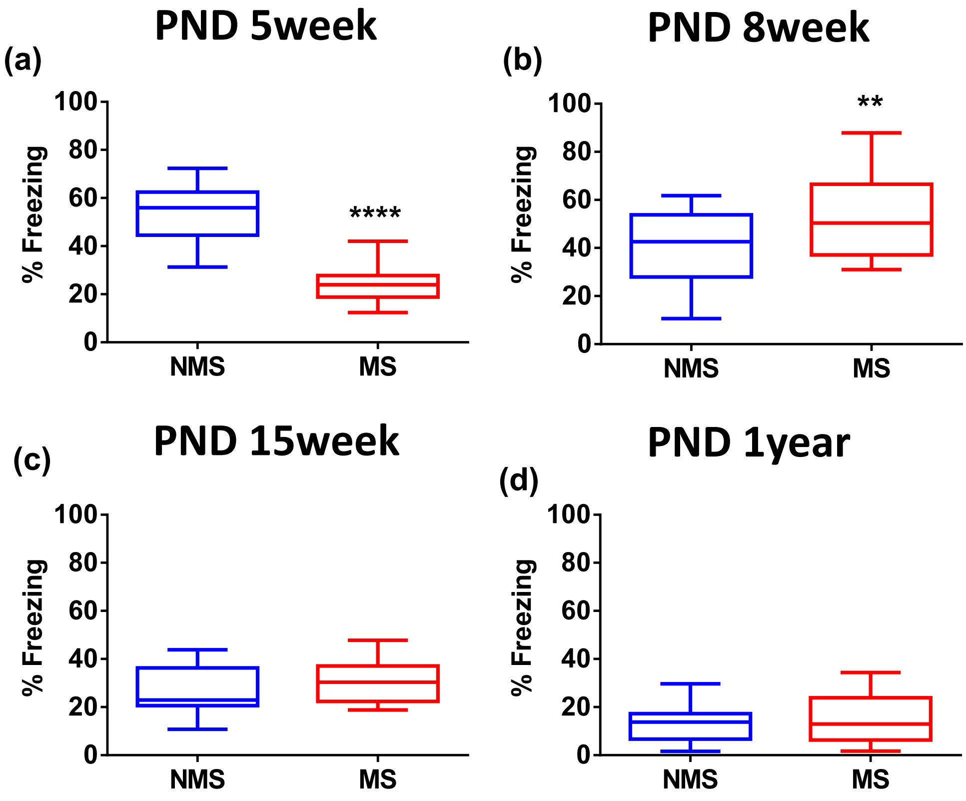

Fig. 4 The percent freezing response to CS+ during fear extinction recall test. **a** 5 weeks, **b** 8 weeks, **c** 15 weeks and **d** 1-year-old group. Unpaired *t* test, $^{****}p<0.0001$; $^{**}p<0.02$ when compared to NMS group. *X*-axis shows comparison of NMS (blue) and MS (red) group during extinction training sessions. *Y* axis shows percentage freezing showed by MS and NMS rats

recall phase when compared to NMS rats 8 weeks old ($t_{1,38}=2.439$, $p<0.02$). On the contrary, 5-week-old MS rats exhibited reduced freezing response during fear extinction recall test when compared to NMS rats ($t_{1,38}=9.376$, $p<0.0001$) indicating stronger extinction memory following extensive extinction training in adolescence age (Fig. 4a).

A two-way ANOVA test showed there was significant effect of groups ($F_{1,170}=12.66$, $p<0.0001$) as well as interactions between stimuli (blocks of 4 stimuli) ($F_{4,170}=15.37$, $p<0.0005$). The multiple comparisons with the Bonferroni post hoc test (Fig. 5b) indicated that the MS group showed more freezing during the first block ($p<0.05$) and the last block ($p<0.01$) of CS+ presentation which suggest that MS rats in young adulthood age group showed an increase in the fear response at the immediate and the late extinction phase.

It was interesting to note that that 5-week-old MS group showed progressive decrease in freezing response to CS+ indicating stronger fear extinction memory when compared to the age-matched controls. A two-way ANOVA has further revealed significant effect of group ($F_{1,96}=9.282$, $p<0.005$) and stimuli across blocks of stimuli ($F_{4,96}=3.685$, $p<0.007$). The decrease in freezing response was stronger to first block (blocks of 4 stimuli) ($p<0.01$) and to third block ($p<0.05$) based on Bonferroni post hoc test (Fig. 5a).

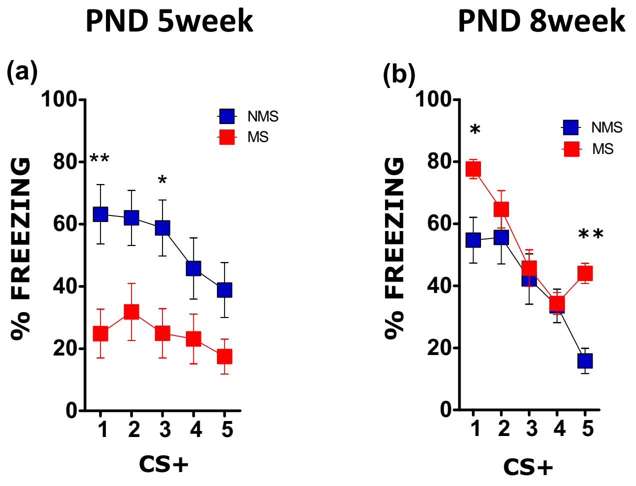

Fig. 5 Fear extinction recall test in NMS and MS group. **a** 5 weeks after MS stress, when exposed to fear extinction test showed significant reduction in freezing response to CS+. **b** 8-week-old rats exhibit impaired fear extinction recall memory. $^{**}p<0.01$, $^{*}p<0.05$ when compared to NMS group. *X*-axis shows response of rats towards CS+ presentation (stimulus, 1–5) during recall of extinction. Since 20 CS+, each CS+ is a block of 4 CS+ tones during fear extinction recall test in both NMS and MS group of rats

Finally, the study has evaluated the extent of fear memory and extinction process by calculating FE_{index} (Fear extinction index) and $FE_{recall\ index}$ (Fear extinction recall index). FE_{index} was calculated based on the ratio between 1st and 6th extinction training sessions. As shown in (Fig. 6a), irrespective of the age groups, both NMS and MS rats showed

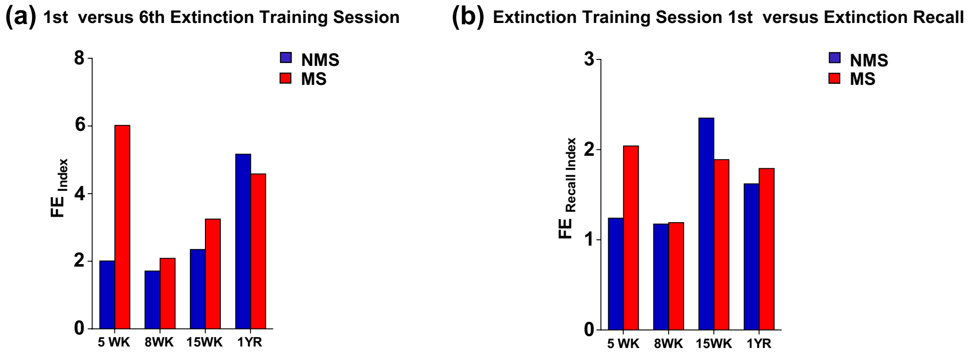

Fig. 6 Fear extinction recall index ($\mathrm{FE}_{\mathrm{index}}$) and Fear extinction recall index ($\mathrm{FE}_{\mathrm{recall\ index}}$). Please note that $\mathrm{FE}_{\mathrm{index}}$ is more than 5 (**a**) and $\mathrm{FE}_{\mathrm{recall\ index}}$ is more than 2 (**b**) in MS group of 5 weeks when compared to NMS group. Rats from all other groups showed more than 1 with regard to $\mathrm{FE}_{\mathrm{index}}$ and $\mathrm{FE}_{\mathrm{recall\ index}}$

extinction learning process showing FE Index more than 2. The strength of extinction learning was significantly different within 5-week-old age groups showing stronger extinction learning in MS group than NMS group.

Since strength of the extinction learning itself was different in 5-week-old MS than NMS rats, it is possible to have significant difference in extinction recall index also. As shown in (Fig. 6b), MS rats of 5 weeks once again showed stronger extinction recall Index when compared to NMS group of rats. These results indicate magnitude of fear extinction memory was retained in the 5-week-old even after 10 days of last extinction training session.

Discussion

Important observations from the present study show that fear retention memory is stronger in 8-week-old MS rats, compared to controls. The increase in fear memory was not evident in 5-week-, 15-week- and 1-year-old rats. Second, MS-induced elevations in fear extinction learning was evident in 5-week- and 15-week-old animals and not in 8-week- and 1-year-old animals. On the other hand, recall of fear extinction memory was stronger in 5-week-old and weaker in 8 week-old MS animals. Finally, we provide evidence that normal ageing per se did not have any impact on either fear retention or fear extinction processes.

The stronger fear memory in young adulthood, but not in adolescent age, indicates that the impact of early MS stress surfaces mainly at young adulthood when challenged with the task that triggers limbic circuit. The formation and retention of fear memory generally depends upon the established synaptic connectivity of amygdala with other limbic brain regions. The faulty connectivity in the limbic circuit may cause abnormal changes in fear acquisition and retention. Under normal circumstances, adolescent period per se, the prefrontal cortex and limbic system undergo reorganization in terms of establishing synaptic connections between brain regions (van Eden et al. 1990; Sowell et al. 1999, 2001; Zuo et al. 2005) due to which risk taking, high exploratory behaviour, social interaction and playful behaviour are prominently high at adolescent age (Spear 2000), while at young adulthood, the number of synapses appears relatively constant until the late stages of life (Masliah et al. 1993; Peters et al. 1998; Rakic et al. 1986). Even though synaptic connectivity is established at young adulthood, several lines of evidence indicate that any disturbances at the critical time window of the development could lead to impaired physiological functions later in life. In line of these arguments, animals exposed to MS stress exhibited increased fear during retention stage suggesting an impact of stress on establishing stronger connectivity in the limbic circuitry. These behavioural changes also could be attributed to developmental reorganization of limbic circuitry at SHRP due to stress (Huttenlocher 1990; Markus and Petit 1987). In contrast to young adulthood age, rats at adolescence show high levels of risk-taking behaviour which is associated with increased spine dynamics suggesting that cortical neurons are highly plastic (Grutzendler et al. 2002) at this period of age. Hence, animals having pre-exposure to stress, subjected to classical fear conditioning may not show different fear retention behaviour from animals without stress. In addition to 5-week-old rats, the early MS stress did not impair fear retention memory at 15-week- and 1-year-old age groups. This can be correlated with the synapse maturation seen in the diverse regions of cerebral cortex at adulthood (Zuo et al. 2005) which may not be sensitive to fear acquisition.

The MS animals, unlike fear retention memory, showed that adolescence (5 weeks) and adult (15 weeks) animals were able to acquire new extinction learning after the termination of aversive stimuli. This is different in young adulthood and at old age group as new extinction learning was impaired in 8 week- and 1-year-old animals. The fear extinction memory, on the other hand, showed contrasting pattern between adolescence (5 weeks) and young adulthood (8 weeks) age group animals. The animals that showed increased extinction learning in adolescence age were able to form a stronger extinction memory, while rats that showed weaker extinction learning in young adulthood showed weaker extinction memory. These results suggest that young adulthood is highly sensitive period and showed increased susceptibility to fear memory tasks. Interestingly, younger rats once acquired were able to exhibit fear extinction and retain the fear extinction memory for a longer period of time, while 15-week-old rats, even though able to extinct faster than control, did not show any differences in fear memory as compared to controls. In addition, the study found MS did not affect the acquisition of fear conditioning as well as fear extinction, but impaired mainly the retention of memory at young adulthood age. The differences in fear memory formation between age groups in these MS animals may be attributed to stronger activation of HPA axis, which is much severe at young adulthood and not at peri-adolescent and adulthood or not even at older age groups (Lupien et al. 2009).

The age-associated changes in fear memory may be explained based on the degree of activation of HPA axis. The increased fear extinction learning in adolescence and older animals could be due to increased sensitivity of HPA axis to stress leading to increased corticosterone levels, as well as prolonged hyperthermic and inflammatory responses than the younger ones (Koenig et al. 2018). This suggests that dysregulated pituitary cytokine interactions and brain cell activation in the HPA axis may result in behavioural alterations. The indifferences in the fear memory processes between MS and control groups in 1 year could be due to the reduced locomotor activity even though increased corticosterone is found in older animals after exposure to novel environmental stressors (Koenig et al. 2018).

Recently, studies have shown age associated changes in fear extinction processes such that 17-day-old rats failed to show fear extinction when compared to 24-day-old rats (Langton et al. 2007; Kim and Richardson 2007a, b; Yap and Richardson 2007). Similarly, in another study they show that younger animals perform much better than the older animals in contextual associative learning (Luu et al. 2008) suggesting brain development is a continuous process from young to old age. In task-based functional resonance imaging study, it is suggested that the developmental changes in amygdala-prefrontal cortex connectivity is age-dependent across 4–24 age groups in humans. As a result, the functional connectivity having positive impact in children less than 10 years changes to negative in children of 10 years and more (Gee et al. 2013). These results, together, suggest that acquisition and extinction of fear memory may depend upon the neural development of the fear circuits (Fareri and Tottenham 2016; Malter Cohen et al. 2013).

The underlying neurophysiological mechanisms of fear retention memory formation involves the activation of the amygdala (LaBar et al. 1998; LeDoux 2003; Schafe and LeDoux 2000) as well as synchronized connectivity between the amygdala and dorsal hippocampus (Seidenbecher et al. 2003). The amygdala activation in response to such fear induced arousal results in the activation of HPA axis (Flandreau et al. 2012; van Stegeren et al. 2007; de Kloet et al. 2005) leading to increased corticosterone. The hypercortisolism in rodents are generally associated with increased fear memory formation (Roozendaal 2002, 2003). The increased fear memory formation in MS animals in the present study thus could be attributed to accentual increase in corticosterone (Rees et al. 2006) which results in increased fear memory retention. This hypothesis is supported by human studies wherein they show that orphanage children maintain higher level of cortisol throughout the day even after inoculation (Gunnar et al. 1996; Jacobson et al. 1999; Lewis and Ramsay 1995). The age-associated differences in freezing behaviour in MS rats could be related to HPA axis maturation (Koenig et al. 2018) wherein adolescent and older rats do not exhibit more freezing behaviour than controls. Even though basal corticosterone is high in MS animals (Aisa et al. 2009), activation of HPA axis through classical fear conditioning (McEwen 2003) did not alleviate fear retention and extinction processes in older animals. This could be due to blunted HPA axis activation to stress and elevated resistance to negative feedback mechanisms in the aged (Gaffey et al. 2016).

In summary, the present study demonstrated that young adulthood age has a profound impact on fear retention and extinction memory following MS stress unlike adolescence (5 weeks) and old (1 year) age groups. The increased fear retention memory with subsequent impaired retention of extinction memory indicates that MS stress during SHRP impairs only the retention and not the acquisition at two different stages of fear conditioning—fear acquisition and fear extinction learning. This suggests that hormonal changes that occur at young adulthood (8 weeks) appear to be significant in impairing memory retention.

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Compliance with ethical standards

Conflict of interest The author declares that no competing interests exist.

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