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Behavioral and Cognitive Impact of Early Life Stress: Insights from An Animal Model

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Abstract

Background—Children subjected to traumatic events during childhood are reported to exhibit behavioral and cognitive deficits later in life, often leading to post-traumatic stress disorder (PTSD) and major depression. Interestingly, some children continue to remain normal despite being exposed to the same risk factors. These trauma-related behavioral and cognitive profiles across different stages of life are not well understood. Animal studies can offer useful insights.

Objective—The goal of this study was to determine the impact of early life exposure to traumatic events on behavioral and cognitive profile in rats by tracking the behavior of each rat at different ages.

Methods—We utilized the single prolonged stress (SPS), a rodent model of PTSD, to study the effects of early life stress. Male Sprague-Dawley rats were exposed to SPS on post-natal day (PND) 25. Tests to assess anxiety- and depression-like behavior, as well as learning and memory function were performed at PND32, 60 and 90.

Results—Rats exposed to SPS exhibited both anxiety- and depression-like behavior at PND32. And, short-term (STM) but not long-term memory (LTM) was impaired. Rats exposed to SPS at PND60 exhibited anxiety- but not depression-like behavior. STM but not LTM was impaired. Rats exposed to SPS at PND90 exhibited fearful (as indicated by elevated plus maze test) but not an overall anxiety-like behavior (in light and dark test). These rats also displayed significant depression-like behavior with no changes in STM or LTM. Interestingly, when data was further analyzed, two subsets of PND90 rats exposed to SPS were identified, “susceptible”: with depression-like behavior and “resilient”: without depression-like behavior. Importantly, while *resilient* group expressed early signs of anxiety- (at PND32 and PND60) and depression-like behavior (at PND32), these behavioral deficits were absent at PND90. On the other hand, *susceptible* PND90 rats exposed to SPS expressed later onset of anxiety-like behavior (at PND60), while depression-like phenotype was evident only later on at PND90.

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Conclusions—Our findings suggest that early life stress caused co-occurrence of anxiety and depression-like behavior at PND32 (mimics human early-adolescent period). This co-occurrence was lost at PND60 with demonstration of anxiety- but not depression-like behavior. Later, depression but not anxiety-like behavior was observed at PND90. It seems that behavioral adaptations occur at the critical PND60 stage (mimics human late-adolescent period), where behavioral and cognitive switching occurs, thereby, expressing susceptible and resilient phenotypes.

Keywords

Trauma; PTSD; Resilience; Early life stress; Psychological stress

1. Introduction

Adverse experiences during early life can contribute to development of psychiatric conditions later in life. In fact, adults with a history of experiencing traumatic experiences of either childhood abuse or other traumatic events are considered to be at increased risk of developing depression or post-traumatic stress disorder (PTSD) in their later life (Jurueña 2014). Relevant to this, in the United States alone, 1 in 58 children experience maltreatment (Fourth National Incidence Study of Child Abuse and Neglect). Approximately 15.5 million children witness physical or emotional abuse of a parent every year, thus becoming vulnerable to developing psychiatric conditions including PTSD and/or depression (McDonald, Jouriles et al. 2006).

Interestingly, not all children who experience traumatic events are at equal risk of developing later life psychiatric disorders. Some are resilient despite being exposed to the same risk factors, while others remain susceptible (Masten 2001, Silk, Vanderbilt-Adrianne et al. 2007, Miller, Chen et al. 2011). The relationship between early life trauma exposure and development of later life psychiatric symptoms is not clearly understood, and the mechanistic basis for resilience is also not known. While examining the link between early life stress and later life behavioral and cognitive well-being is important, conducting such studies in children with abuse or trauma history are difficult to carry out. Therefore, animal models are valuable in studying the behavioral consequences of early life stress across the developmental course and to distinguish occurrence of different developmental trajectories. Previous animal studies in rodents and monkeys have revealed that early life stress induced by maternal separation has negative effects on behavioral and neurobiological phenotype in adulthood (Lyons et al. 2010, Vetulani J. 2013). There is also a large body of literature suggesting that prenatal stress in rodents predisposes the animals towards anxiety- and depression-like behavioral phenotype in adulthood (Morley-Fletcher, Rea et al. 2003, Lee, Brady et al. 2007). However, role of early life stress remains unclear with lot of variations reported in different studies (Boersma, Bale et al. 2014) (Tamashiro 2015). Particularly, information on the role of early life stress in causing behavioral changes over the developmental course, is lacking.

Therefore, in the present study, using a well-established single prolonged stress (SPS) rodent model of PTSD, we examined the consequences of early life stress across different stages of

development in rats. The objective of this study was to determine the impact of early life stress by using SPS exposure at post-natal day (PND) 25 in rats, and to examine the behavioral and cognitive consequences at different developmental stages (early adolescent: PND32, late adolescent: PND60, and adult stage: PND90). Basically, male Sprague-Dawley rats were either exposed to SPS or control procedures on PND25, following which specific behavioral and cognitive parameters were examined at different time points of development. Examination of anxiety-like behavior, depression-like behavior, and learning and memory function were performed at PND32, 60 and 90; rodent life span corresponding to young, adolescent and adult stage. Our hypothesis is that early life SPS exposure leads to distinct age-specific behavioral phenotypes in rats.

2. Methods and Materials

2.1. Animals

Male Sprague-Dawley consolidated litters (PND12) were purchased from Charles River Laboratories (200 Charendon Street Boston, MA 02116-5092, USA.) The pups were separated from the female rats at PND21 and split into control and single prolonged stress (SPS) groups. Control group was subjected to control exposures while SPS group was subjected to SPS procedures at PND25 (Figure. 1). Rats were housed with a 12-hour light/dark cycle (lights on at 0600 h) in a climate-controlled room with food and water provided ad libitum. Experiments were conducted in accordance with the National Institutes of Health (NIH) guidelines, using protocols approved by the University of Houston Animal Care and Use Committee.

2.2. Single prolonged stress model

Single prolonged stress (SPS)—The SPS model comprises of three different types of stressors: 2 h restraint stress, 20 min forced swim stress and 2–3 min of ether anesthesia, which are expected to induce psychological, physical, and endocrinological stress respectively (Yamamoto, Morinobu et al. 2009). The rats at PND25 were subjected to a one time combined stress paradigm applied consecutively in one day: two-hour immobilization (compression with plastic Ziploc bag fastened with paper tapes with an opening at the nose of the rat) followed immediately by 20 min of forced swimming stress (in a tall cylindrical tank filled with water 50*20 cm), rest for 15 minutes, and a final 2–3 min exposure to ether anesthesia (with diethyl ether until loss of consciousness). The animals were then returned to their home cages and left undisturbed for 7 days. Control animals were not subjected to any stress except a gentle brief handling at PND25, and were kept in an undisturbed environment in the same room where SPS protocol and behavior experiments were conducted.

2.3. Behavior testing

All behavior tests were conducted and analyzed by the same person between 9 a.m. and 4 p.m. The experimenter was blinded to treatment.

2.3.1. Anxiety-like behavior tests—Two different anxiety-like behavior tests were carried out. First, light-dark (LD) test was conducted followed by elevated-plus maze (EPM) test in the next day, as previously published by us (Patki, Salvi et al. 2015).

2.3.1.1. Light-dark (LD) Test: The less time the rat spends in light is considered as an indication of anxiety-like behavior (Patki, Salvi et al. 2015). The LD box consists of two compartments, one light compartment, and one dark compartment. The barrier between the two compartments has a single opening for the rat to explore each compartment freely. Each session of LD test lasted 5 min, started by placing the rat into the light compartment. Total time spent in each compartment was recorded. The LD test was performed seven days (PND32), thirty-five days (PND60), and sixty-five days (PND90) after the SPS protocol.

2.3.1.2. Elevated plus maze (EPM) test: The less time the rat spends in the open arms of the EPM apparatus is considered as an indication of anxiety-like behavior (Patki, Salvi et al. 2015). The EPM consisted of four 43 cm long arms extending from a 10 cm² central area, with two arms open and two arms closed, placed 90 cm above the floor (Med Associates Inc.). The rat was placed in the center area facing the open arms of the maze and allowed to explore each arm freely for 5 min. Total time spent in both open and closed arms was recorded. The EPM test was performed eight days (PND33), thirty-six days (PND61), and sixty-six days (PND91) after SPS protocol.

2.3.2. Depression-like behavior test

2.3.2.1. Forced swim test (FST): More time spent by the rat in an immobile position is considered as an indication of depression-like behavior (Patki, Salvi et al. 2015). The apparatus of FST comprises of a water tank measuring 24 cm in diameter and 50 cm in height. Rats were individually placed into a water tank for 5 min at 25 °C. In response to the threat of drowning, the rat keeps swimming. When the rat stops struggling and floats motionlessly, it is a sign of “giving up,” which can be interpreted as despair-like behavior. The total time of immobility was recorded. The FST was performed nine days (PND34), thirty-seven days (PND62), and sixty-seven days (PND92) after the SPS protocol.

2.3.3. Memory function test

2.3.3.1. Radial arm water maze (RAWM) test: Short-term memory (STM) and long-term memory (LTM) tests were conducted using RAWM apparatus. The RAWM procedures were conducted as previously published (Patki, Solanki et al. 2014). The rats were subjected to 12 learning trials before STM and LTM tests, starting with the first set of six learning trials (trials # 1–6), followed by a 5 min rest period, then the second set of six learning trials (Trials # 7–12) were conducted. The STM was carried out 30 min after the completion of 12th learning trial, by releasing the rat from a random arm. The number of errors before the rat reached the goal arm was counted. The LTM was assessed 24 h after the end of the 12th learning trial. The RAWM was conducted ten days (PND35), thirty-eight days (PND63), and sixty-eight days (PND93) after the SPS protocol.

2.3.4. Cluster analysis—The forced swim test (immobility time) data at PND90 was used to identify two clusters using K-means cluster analysis (SPSS Statistics version 23, IBM, Armonk, North Castle, NY). ANOVA test followed by Tukey’s multiple comparison test were then performed on the control group and the two clusters using GraphPad Prism (GraphPad Software, La, Jolla, CA). The clusters were considered significantly different at $p < 0.05$.

3. Results

3.1. Behavior analysis

Behavior tests were conducted at different developmental stages following SPS procedure to determine the effects of early life stress on behavioral and cognitive functions at PND32, PND60, and PND90.

3.1.1. Analysis of anxiety- and depression-like behavior, as well as learning-memory functions

3.1.1.1. Post-natal day (PND) 32: First, behavior and cognitive analysis was conducted seven days after conclusion of SPS procedure at PND32 (rodent age mimicking human adolescent period). In the LD test, the rats exposed to SPS spent significantly less time in the light compartment as compared to control rats (CON: 42.60 ± 6.437 seconds, SPS: 24.27 ± 3.859 seconds; $p < 0.05$), indicative of increased anxiety-like behavior (Figure. 2A). Rats exposed to SPS at PND25 also spent significantly reduced time in the open arms in the EPM test as compared to control rats (CON: 25.00 ± 4.098 , SPS: 15.91 ± 2.263 ; $p < 0.05$), indicating increased anxiety-like behavior (Figure. 2B). In the FST, early life SPS caused a significant increase in the time of immobility in rats exposed to SPS compared to their matched controls during a 5-min session test (CON: 7.240 ± 2.510 , SPS: 39.24 ± 6.528 ; $p < 0.05$), indicative of depression-like behavior (Figure. 2C). The rats exposed to SPS made more errors when compared to their matched controls in the STM test (CON: 1.043 ± 0.4145 , SPS: 1.527 ± 0.3102 ; $p < 0.05$), (Figure. 2D). In the LTM test (24 h after the STM test), there was no significant difference between rats exposed to SPS and their matched controls (Figure. 2E). In summary, after SPS procedure conducted at PND25, rats exposed to SPS exhibited heightened anxiety- and depression-like behavior, and short-term memory impairment at PND32 (Figure. 2A–D).

3.1.1.2. Post-natal day (PND) 60: After conclusion of SPS procedure at PND25 and behavioral tests at PND32, behavior tests were carried out at PND60 to assess if the effects of SPS lasted until PND60. In the LD test, rats exposed to SPS continued to exhibit significant anxiety-like behavior when compared to respective controls (CON: 65.88 ± 12.22 , SPS: 26.44 ± 3.068 ; $p < 0.05$) (Figure. 3A). In the EPM test, rats exposed to SPS also exhibited anxiety-like behavior compared to their matching controls, indicated by significantly less time spent in the open arms (CON: 64.96 ± 9.415 , SPS: 34.29 ± 3.895 ; $p < 0.05$) (Figure. 3B). However, in the FST test, rats exposed to SPS spent equal time staying immobile when compared to their matched controls (CON: 20.56 ± 5.161 , SPS: 24.76 ± 2.36 ; $p < 0.05$) (Figure. 3C), indicative of no depression-like behavior at PND60. In learning-memory function tests, the rats exposed to SPS showed impairments in STM but not LTM, similar to the results obtained at PND32. Rats exposed to SPS made more errors in STM when compared to their matched controls (CON: 0.1200 ± 0.06633 , SPS: 0.6852 ± 0.1710 ; $p < 0.05$) (Figure. 3D), but they made comparable number of errors compared to controls in LTM test (Figure. 3E). In summary, following SPS procedure at PND25, anxiety-like behavior and short-term memory impairment persisted until PND60 (Figure. 3A, B, D, and E). However, depression-like behavior was not evident at PND60 (Figure. 3C).

3.1.1.3. Post-natal day (PND) 90: At PND90, i.e. 68 days after first exposure to SPS, the same set of behavior tests were carried out on the same set of rats to assess whether behavioral impairments lasted into this stage. In LD and EPM test, heightened anxiety-like behavior that we observed at PND32 and 60 were not observed at this age. At PND90, rats exposed to SPS spent comparable time in the illuminated area in LD test compared to their age matched controls (CON: 55.44 ± 7.820 , SPS: 44.98 ± 4.619 ; $p = 0.2297$) (Figure. 4A). The time that rats exposed to SPS spent in the open arms was significantly different from that of the control rats (CON: 42.28 ± 7.524 , SPS: 24.95 ± 3.203 ; $p < 0.05$) (Figure. 4B). We do not interpret decreased time in open arms of EPM as an indication of anxiety-like behavior but interpret it as fearful behavior. Unlike LD apparatus, EPM has open, exposed and elevated apparatus, which makes this test more fearful and high-risk for the rats. At PND90, the rats did not show changes in LD test, but exhibited difference between SPS and control groups in EPM test. We interpret EPM results as indicative of fearful behavior instead of overall anxiety-like behavior. Interestingly, in the FST, rats exposed to SPS exhibited significantly increased immobility time during the 5-min test when compared to control rats (CON: 22.80 ± 3.126 , SPS: 43.82 ± 3.762 ; $p < 0.05$) (Figure. 4C). Short-term memory impairment observed at PND32 and 60 did not last at PND90. Rats exposed to SPS made comparable numbers of errors in both STM (CON: 0.8182 ± 0.2244 , SPS: 0.7818 ± 0.1735 ; $p = 0.9067$) (Figure. 4D) and LTM (CON: 1.909 ± 0.4648 , SPS: 2.073 ± 0.3112 ; $p = 0.7761$) (Figure. 4E) tests when compared to their matched controls. In summary, following SPS procedure at PND25, while anxiety-like behavior and short-term memory impairment did not persist until PND90 (Figure. 4A, B, and D), depression-like behavior was observed at PND90 (Figure. 4C).

3.1.1.4. Cluster analysis of PND90 FST data: We further examined PND90 FST data using K-mean cluster analysis utilizing IBM SPSS (IBM, Armonk, NY) to identify subgroups in rats exposed to SPS. Based on the time of immobility in FST, the rats exposed to SPS appeared to occur in two clusters. From a total of 55 rats exposed to SPS, 28 rats exhibited less immobility time, and belonged to the same cluster as controls. We considered these rats to be the “*resilient type*”. The other 27 rats belonged to the second cluster and exhibited longer immobility time in the FST. These rats were considered as the “*susceptible type*”, (Figure. 5 panel I). We then performed ANOVA test on the control group and the two clusters (resilient and susceptible), followed by Tukey’s multiple comparison test using GraphPad Prism (GraphPad Software, La, Jolla, CA). ANOVA test indicated the two clusters were significantly different ($p < 0.05$). Tukey’s multiple comparison test indicated that the 27 rats in the susceptible group had significantly longer time of immobility when compared to their matched controls, or to the 28 rats in the resilient group (Figure. 5 panel II). However, the 28 rats within the resilient group were not significantly different from their matched controls (Figure. 5).

3.1.1.5. Correlation between emergence of behavioral phenotype and different stages of development: After identification of susceptible (with depression-like behavior) and resilient (without depression-like behavior) phenotypes of PND90 rats in the FST test, we back-traced behavior performance of these rats at PND32 and PND60. Both depression- and

anxietylike behaviors were assessed within each group at different stages of development *i.e* susceptible rats at PND32, 60 and 90 and resilient groups at PND32, 60 and 90.

PND90 susceptible rats (depressive phenotype) with significantly longer FST immobility time than control or resilient rats (Control: 42.60 ± 6.437 , Resilient: 21.83 ± 4.682 , Susceptible: 30.05 ± 6.991) (Figure. 6 panel IC), did not exhibit greater FST immobility at PND32 (Control: 7.240 ± 2.510 , Susceptible: 14.00 ± 1.984 ; $p = 0.0820$) (Figure. 6 panel IA), or at PND60 (Control: 20.56 ± 5.161 , Susceptible: 29.42 ± 2.879 ; $p = 0.1762$) (Figure. 6 panel IB). On the other hand, PND90 resilient rats (no depressive phenotype) with no demonstration of higher FST immobility time (Control: 22.80 ± 3.126 , Susceptible: 27.52 ± 2.084 ; $p = 0.1988$) (Figure. 6 panel IC), exhibited significant immobility time at PND32 (Control: 7.240 ± 2.510 , Resilient: 37.31 ± 6.248 ; $p < 0.05$) (Figure. 6 panel IA), but not at PND60 (Control: 20.56 ± 5.161 , Resilient: 22.31 ± 3.233 ; $p = 0.7640$) (Figure. 6 panel IB).

PND90 susceptible rats (no anxiety phenotype) spent comparable time in light at PND32 when compared to controls (Control: 42.60 ± 6.437 , Susceptible: 30.05 ± 6.991 ; $p = 0.1972$) (Figure. 6 panel IID) and significantly less time in the light compartment at PND60 (Control: 65.88 ± 12.22 , Susceptible: 32.42 ± 4.732 ; $p < 0.05$) (Figure. 6 panel IIE). On the other hand, PND90 resilient rats (no anxiety-like behavior) spent less time in light compartment at both PND32 (Control: 42.60 ± 6.437 , Resilient: 21.83 ± 4.682 ; $p < 0.05$) and 60 (Control: 65.88 ± 12.22 , Resilient: 23.28 ± 3.910 ; $p < 0.05$) (Figure. 6 panel IID, F). Upon mapping the presence/absence of behavioral impairments over time in susceptible and resilient rats, a period of behavioral switching clearly emerged at PND60 (Figure. 6 panel IIIG).

In summary, rats that did not show depression-like behavior (the resilient type) at PND90 showed heightened anxiety- and depression-like behavior early on at PND32, while the susceptible rats displayed late onset of anxiety-like behavior at PND60, but not at PND32 (Figure. 6 panel IIIG).

4. Discussion

A growing body of research focusing on childhood trauma has established that early life traumatic experiences, including physical, psychological, or combined, alter susceptibility to mood disorders in later life, especially major depression (Scheller-Gilkey, Moynes et al. 2004, Brunson, Kramar et al. 2005, Pietrek, Elbert et al. 2013). Although negative consequences of early life trauma are well recognized (Kessler, Davis et al. 1997), yet, many aspects still remain unknown. For example, what is the most vulnerable age when early life trauma exerts most serious and long lasting effects? Whether early life trauma causes one psychiatric condition such as anxiety or depression and whether one or both persist over time, or does one disorder transform into another? Whether early life trauma causes learning and memory impairments? Are these deficits permanent? Answers to these questions cannot be easily sought, as conducting these studies in humans are quite challenging. Although animal models cannot accurately reveal the impact of early life traumatic events on psychiatric symptoms occurring in later life, rodent models are excellent tools that can provide useful insights. In this study, using a modified version of our published model of

PTSD (SPS) (Patki, Li et al. 2014), we examined the role of early life stress in regulation of behavioral and cognitive function and also examined the phenomenon of resilience. After exposing the rats to SPS at PND25 (equivalent to human childhood), behavioral tests were conducted at PND32 (equivalent to early human adolescent or late childhood), PND60 (equivalent to early human adulthood or late adolescent), and PND90 (equivalent to human adulthood) (Sengupta 2013). Learning and memory function also were analyzed at each stage.

We observed that early life SPS induced anxiety-like behavior and short-term memory impairment at initial stages of development (PND32 and 60). When SPS rats reached adult stage, anxiety-like behavior previously evident at PND32 and 60 transformed into depression-like behavior at PND90. Previous evidence has suggested that SPS exposure during adulthood leads to significant anxiety- and depression-like behavior, and these negative effects discontinue about two weeks after the initial exposure (Wu, Tian et al. 2016). On the other hand, early life stress, including prenatal and postnatal stress, is reported to cause long-lasting behavioral as well as morphological changes in brain regions that are the centers of emotional regulation, like hippocampus, amygdala, and prefrontal cortex (Wang and Schmidt 2016). Our study assessed the long-term effects of early life SPS exposure by tracking the behavioral performance of each rat across different ages and also examined the extent and severity of these changes over the developmental course (PND32, 60, and 90). The short-term memory impairment observed at PND32 and 60 was not evident at PND90. Early life stress is well known to affect memory (Raine, Park et al. 2001, Morris, Le et al. 2016). Memory function deficit observed in our study during PND32-60 period may be due to stress-induced elevation of glucocorticoids, which can accelerate hippocampal cell loss because of stress-induced acceleration of neurotoxicity mechanisms (Finsterwald and Alberini 2014). Evidence in the literature suggests that chronic exposure to glucocorticoids lead to neuronal cell damage and morphological changes, suggesting neurotoxic effects of glucocorticoids (Suri and Vaidya 2013). It is believed that glucocorticoids by activating glucocorticoid receptor enable nuclear translocation of certain transcription factors that encode N-methyl-D-aspartate (NMDA) receptor. An increase in NMDA receptor triggers excessive influx of Ca^{2+} , which leads to excitotoxicity and cell death. Thus, these events might be relevant in SPS-mediated learning-memory function effects (Xiao, Feng et al. 2010). Restorative processes (Hoffman, Krigbaum et al. 2011) occurring later in the developmental course most likely dominate over toxicity mechanisms and hence restore learning and memory mechanisms at PND90. Therefore, STM and LTM were no longer evident at PND90. Previous studies have reported that stress induced increase in glucocorticoid causes reduction in brain derived neurotrophic factor (BDNF), which impairs neurogenesis (Suri and Vaidya 2013). It seems reasonable that after PND60, normalized glucocorticoid levels lead to replenishment of BDNF stores, which enhances the restorative processes causing recovery of memory function at PND90. Interestingly, there appears to be a critical period between PND32-PND60 where anxiety-like behavior switches to depression-like behavior. This also seems to be a critical window when cognitive features become permanent, beyond which learning cannot be compromised and memories are solidified. This data has important clinical relevance as it informs us of a behaviorally

sensitive period during which potentially critical biological changes occur, which are responsible for triggering the onset of depression in the later course of life.

Furthermore, at PND90, one subgroup of rats exposed to SPS was more susceptible to depression-like behavior. Using our FST data collected at PND90, we tracked behavioral performance of each rat in each behavioral test. The idea was to gain information about the developmental stage at which behavioral impairments become evident prior to the occurrence of susceptible and resilient phenotypes. We observed that resilient rats showed heightened anxietylike behavior at both PND32 and 60, and depression-like behavior at PND60. However, susceptible rats did not show anxiety-and depression-like behavior at PND32, but exhibited anxiety-like behavior at PND60. Our data indicates that after early life exposure to SPS, rats at earlier stages (PND32 and 60) showed an onset of anxiety-like behavior but did not continue to have behavioral impairments at PND90. However, those rats that remained normal at earlier stages (PND32) showed anxiety-like behavior at PND60, and later continued to have depressionlike behavior at PND90. Involvement of epigenetic modifications in regulation of this behavioral phenotype seems an attractive possibility. Perhaps, epigenetic modifications occur in specific brain areas at particular developmental period of life, positively regulating anxio-depressive circuits (Stankiewicz, Swiergiel et al. 2013) to cause recovery of anxiety-like behavior. Reports of an interaction between individual genetic vulnerabilities and environmental risk factors are already available (Wu, Feder et al. 2013, Zannas and West 2014, Nestler 2016). Thus, stress in early life can potentially affect neurodevelopmental processes by epigenetically modifying gene expression during specific developmental stages, permanently changing brain structure and function, either in a positive or a negative manner. Relevant to this, the relationship between early life stress and oxidative stress seems interesting (Schiavone, Colaianna et al. 2015), and how oxidative stress might affect later behavioral performance is another interesting question. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is the key transcription factor that regulates the expression of critical antioxidant proteins, which can protect against oxidative stress. Therefore, one interesting possibility is that early life stress causes epigenetic modifications in the promoter region of Nrf2, resulting in altered expression of Nrf2, while altering antioxidant capacity. Brain region selectivity in terms of stress response and differential susceptibility to oxidative stress responsiveness is beginning to be revealed (Bremner 1999, McEwen 2016). Role of hippocampus, amygdala and the PFC is emerging to be quite important. Exactly how these brain regions would modulate neuronal circuits remains to be seen.

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Abbreviations

PTSD	post-traumatic stress disorder
SPS	single prolonged stress

PND	postnatal day
LD	light and dark test
EPM	elevated plus maze test
STM	short term memory
LTM	long term memory
FST	forced swim test
NMDA	N-methyl-D-aspartate
BDNF	brain-derived neurotrophic factor
Nrf2	nuclear factor (erythroid-derived)-like 2

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Figure 1. Schematic representation of the experimental regimen

Male Sprague-Dawley consolidated litters were acclimatized for one week and separated from mother at PND21. The rats were then subjected to single prolonged stress (SPS: 2 h restraint stress, 20 min forced swim stress and 2–3 min anesthesia) at PND25 as previously published (Patki, Li et al. 2014) with some modifications. Behavior tests including light and dark (LD), elevated plus maze (EPM) and forced swim test (FST) were conducted using our published protocols (Patki, Salvi et al. 2015). Short-term (STM) and long-term memory (LTM) was examined using radial arm water maze (RAWM) test according to our previously published protocols (Patki, Salvi et al. 2015). The same set of behavior tests were carried out at PND32 (LD at PND32, EPM at PND33, FST at PND34, STM at PND35, and LTM at PND36), PND60 (LD at PND60, EPM at PND61, FST at PND62, STM at PND63, and LTM at PND64), and PND90 (LD at PND90, EPM at PND91, FST at PND92, STM at PND93, and LTM at PND94). Rats were sacrificed after the conclusion of all behavior tests at PND90 and at PND95.

Figure 2. Examination of anxiety- and depression-like behavior and short and long term memory function tests conducted at PND32

Male Sprague-Dawley rats were subjected to control or SPS exposures at PND25. One week later, behavior and cognitive tests were conducted at PND32. Total time spent in the light compartment in light-dark (LD) test (A) and in the open arms in the elevated plus maze (EPM) test (B) measured anxiety-like behavior respectively. Total immobility time in the Forced swim test (FST) was used to examine depression-like behavior (C). Number of errors made in the short-term memory (STM) test (D) and long-term memory (LTM) test (E) examined learning and memory function in radial arm water maze apparatus (RAWM) comprising of six swim paths. Group designations: Control exposures (CON: open bars, $n = 25$ rats); early life single prolonged stress (SPS: black bars, $n = 55$ rats). (*) indicates significantly different from control at $p < 0.05$. Bars represent means $\pm$ SEM.

Figure 3. Examination of anxiety- and depression-like behavior and short and long term memory function tests conducted at PND60

Male Sprague-Dawley rats were subjected to control or SPS exposures at PND25. Five weeks later, behavior and cognitive tests were conducted at PND60. Total time spent in the light compartment in light-dark (LD) test (A) and in the open arms in the elevated plus maze (EPM) test (B) measured anxiety-like behavior. Total immobility time in the Forced swim test (FST) was used to examine depression-like behavior (C). Number of errors made in the short-term memory (STM) test (D) and long-term memory (LTM) test (E) examined learning and memory function in the radial arm water maze apparatus (RAWM) comprising of six swim paths. Group designations: Control exposures (CON: open bars, $n = 25$ rats); early life single prolonged stress (SPS: black bars, $n = 55$ rats). (*) indicates significantly different from control at $p < 0.05$. Bars represent means $\pm$ SEM.

Figure 4. Examination of anxiety- and depression-like behavior and short and long term memory function tests conducted at PND90

Male Sprague-Dawley rats were subjected to control or SPS exposures at PND25, and behavioral tests at PND32 and 60. Nine weeks after SPS exposure, behavior and cognitive tests were conducted at PND90. Total time spent in the light compartment in light-dark (LD) test (A) and in the open arms in the elevated plus maze (EPM) test (B) measured anxiety-like behavior. Total immobility time in the Forced swim test (FST) was used to examine depression-like behavior (C). Number of errors made in the short-term memory (STM) test (D) and long-term memory (LTM) test (E) examined learning and memory function in radial arm water maze apparatus (RAWM) comprising of six swim paths. Group designations: Control exposures (CON: open bars, $n = 25$ rats); early life single prolonged stress (SPS: black bars, $n = 55$ rats). (*) indicates significantly different from control at $p < 0.05$. Bars represent means $\pm$ SEM.

Figure 5. Cluster analysis of depression-like behavior at PND90

PND90 FST data was examined using K-mean cluster analysis with IBM SPSS (IBM, Armonk, NY) (**panel I**). Two clusters were identified in cluster test at PND90 as a function of immobility time in FST (**panel II**). Control group $n = 25$; Resilient group $n = 28$; Susceptible group $n = 27$. (*) indicates significantly different from control as well as resilient groups at $p < 0.05$.

Figure 6. Retrospective data analysis of susceptible and resilient rats

Performance of susceptible and resilient PND90 rats was traced back in FST (**panel I**) and LD (**panel II**) tests at PND32 (**A** and **D**), 60 (**B** and **E**) and 90 (**C** and **F**). A diagrammatical representation of occurrence of either anxiety- and depression-like behavior or their cooccurrence is provided in different colors (**panel III G**). Presence of anxiety-like behavior is indicated in green and its absence in black. Presence of depression-like behavior is indicated in maroon and its absence in black, while cooccurrence of the two behaviors is indicated in yellow. A black colored dotted box in the center indicates a potential behavior

switch period (**panel IIIG**). Group designations: Control exposures (CON: open bars, n = 25 rats); Resilient (Grey bars, n = 28 rats); Susceptible (Black bars, n = 27 rats). (*) Significantly different at $p < 0.05$. Bars represent means $\pm$ SEM.