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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.

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.

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 (Juruena, 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 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 et al., 2007; Miller et al., 2011). The relationship between early life trauma exposure and development

Abbreviations: PTSD, post-traumatic stress disorder; SPS, single prolonged stress; PND, post-natal 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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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, 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 et al., 2003; Lee et al., 2007). However, role of early life stress remains unclear with lot of variations reported in different studies (Boersma 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 (Fig. 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

2.2.1. 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 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 min, 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.

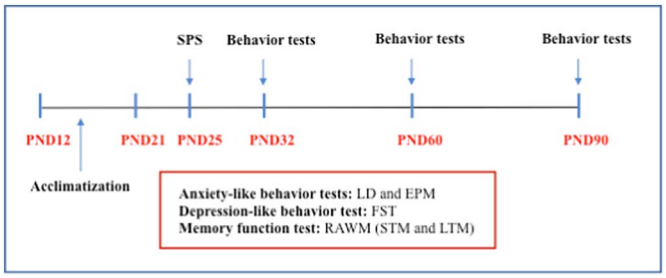

Fig. 1. Schematic representation of the experimental regimen. Male Sprague-Dawley consolidated litters were acclimatized for one week and separated from mother at PND21. The rats were then subjected to single prolonged stress (SPS: 2 h restraint stress, 20 min forced swim stress and 2–3 min anesthesia) at PND25 as previously published (Patki et al., 2014a, 2014b) 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 et al., 2015a, 2015b). Short-term (STM) and long-term memory (LTM) was examined using radial arm water maze (RAWM) test according to our previously published protocols (Patki et al., 2015a, 2015b). 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.

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 et al., 2015a, 2015b).

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 et al., 2015a, 2015b). 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 et al., 2015a, 2015b). The EPM consisted of four 43 cm long arms extending from a 10 cm^2 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 et al., 2015a, 2015b). The apparatus of FST comprises of a water tank measuring 24 cm in diameter and 50 cm in height. Rats