Hormones and Behavior 70 (2015) 30–37

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Regular article

# Exposure to social defeat stress in adolescence improves the working memory and anxiety-like behavior of adult female rats with intrauterine growth restriction, independently of hippocampal neurogenesis

Miyako Furuta [a,b,*], Midori Ninomiya-Baba [b], Shuichi Chiba [b], Toshiya Funabashi [a], Tatsuo Akema [a], Hiroshi Kunugi [b]

[a] Department of Physiology, St. Marianna University School of Medicine, Kawasaki, Japan
[b] Department of Mental Disorder, National Center of Psychiatry and Neuroscience, Tokyo, Japan

ARTICLE INFO

*Article history:*
Received 23 July 2014
Revised 19 January 2015
Accepted 23 January 2015
Available online 25 February 2015

*Keywords:*
Anxiety-like behavior
Working memory
Female rat
Hippocampal cellular proliferation
Hippocampal neurogenesis
Intrauterine growth restriction
Social defeat stress

ABSTRACT

Intrauterine growth restriction (IUGR) is a risk factor for memory impairment and emotional disturbance during growth and adulthood. However, this risk might be modulated by environmental factors during development. Here we examined whether exposing adolescent male and female rats with thromboxane A2-induced IUGR to social defeat stress (SDS) affected their working memory and anxiety-like behavior in adulthood. We also used BrdU staining to investigate hippocampal cellular proliferation and BrdU and NeuN double staining to investigate neural differentiation in female IUGR rats. In the absence of adolescent stress, IUGR female rats, but not male rats, scored significantly lower in the T-maze test of working memory and exhibited higher anxiety-like behavior in the elevated-plus maze test compared with controls. Adolescent exposure to SDS abolished these behavioral impairments in IUGR females. In the absence of adolescent stress, hippocampal cellular proliferation was significantly higher in IUGR females than in non-IUGR female controls and was not influenced by adolescent exposure to SDS. Hippocampal neural differentiation was equivalent in non-stressed control and IUGR females. Neural differentiation was significantly increased by adolescent exposure to SDS in controls but not in IUGR females. There was no significant difference in the serum corticosterone concentrations between non-stressed control and IUGR females; however, adolescent exposure to SDS significantly increased serum corticosterone concentration in control females but not in IUGR females. These results demonstrate that adolescent exposure to SDS improves behavioral impairment independent of hippocampal neurogenesis in adult rats with IUGR.


## Introduction

Intrauterine growth retardation (IUGR) in humans and other mammals can be caused by maternal undernutrition or by a variety of prenatal stresses. There is a potential link between IUGR and impaired behavioral responses for learning and memory (Geva et al., 2006b; Huang et al., 2008; Illa et al., 2013; Saito et al., 2009; Xuelan et al., 2008), despair (Drago et al., 1999), and anxiety (Geva et al., 2006b; Illa et al., 2013; Vasiliadis et al., 2010). The social impact of IUGR-related learning and emotional difficulties manifests at school age or in adolescence (Alati et al., 2009; Geva et al., 2006a,b; Leitner et al., 2007; O'Keeffe et al., 2003; Scherjon et al., 2000; Vasiliadis et al., 2010), and these difficulties are undoubtedly precursors of adult psychopathology. In normal rats, the adolescent period in particular is considered to be one that is sensitive to stressors (Bingham et al., 2011), and stressors are generally thought to increase the risk of developing behavioral disorders in adulthood (Kovalenko et al., 2014). This is because of the relatively late maturation of brain areas that are targeted by stress, such as the prefrontal cortex and the hippocampus. However, a recent report showed that normal adolescent rats have a remarkable ability to recover from social defeat stress (SDS) (Buwalda et al., 2013). It remains unknown whether being subjected to SDS during adolescence results in persistent behavioral differences between adult IUGR and non-IUGR rats.

IUGR individuals may be vulnerable to negative social interactions, given that rats with IUGR that is induced by systemic exposure to thromboxane A2 (TXA2) during pregnancy exhibit brain disturbances that can include delayed neural migration (Sasaki et al., 2000), reduced cortical plate density (Hayakawa et al., 1999; Saito et al., 2009), reduced levels of brain-derived neurotrophic factor (BDNF) mRNA (Fukami et al., 2000), and suppressed BDNF cellular signaling (Ninomiya et al., 2010). However, the adaptability of IUGR rats to stress is more complex than it seemed at first. Specifically, when corticosteroid release in response to acute noise stress was compared in control rats versus rats with protein deprivation-induced IUGR, the responses of the

* Corresponding author at: Department of Physiology, St. Marianna University School of Medicine, Sugao 2-16-1, Miyamae, Kawasaki, Kanagawa 216-8511, Japan. Fax: +81 44 977 0172.
*E-mail address:* mfuruta@marianna-u.ac.jp (M. Furuta).

http://dx.doi.org/10.1016/j.yhbeh.2015.01.010
0018-506X/

hypothalamic–pituitary–adrenal (HPA) axis in the two groups of rats were similar (Nolan et al., 2001). In addition, adult rats with IUGR induced by prenatal dietary restriction exhibit increased neurogenesis in the hippocampus, and acute restraint stress does not affect this change in neurogenesis (Uban et al., 2010). The correlation between the stress-induced reduction of hippocampal neurogenesis and impaired behavioral responses is well-documented in normal subjects (McCormick et al., 2010; Ming and Song, 2011; Samuels and Hen, 2011), raising the question of whether stress causes deterioration in the behavioral responses of IUGR individuals.

It is thought that adolescents with IUGR may be more prone to experiencing socially adverse conditions, such as bullying. Understanding the prognosis of adolescent social stress in adulthood could help determine an appropriate social environment for IUGR adolescents. The objective of the present study was to investigate whether exposure to SDS, which is considered the best way to model bullying in a laboratory setting (Koolhaas et al., 1997b), during the adolescent period affects hippocampal-dependent working memory and anxiety-like behavior with regard to circulating corticosterone levels in adult IUGR rats. To understand the neural mechanisms underlying behavioral changes, hippocampal cellular proliferation and survival to neural differentiation were also determined.

## Methods

### *Animals and study design*

Female Long–Evans rats (Institute for Animal Reproduction, Ibaraki, Japan) that were purchased at 8 days of pregnancy were kept in individual cages (276 × 445 × 204 mm) in conditions of controlled light (12L:12D, light on at 16:00) and temperature (21–24 °C). Food (standard chow) and water were available ad libitum. Rats were allowed to deliver spontaneously, and all pups were fed by their own mothers. Five days after birth, the rat pups were sexed, and the litters were culled to 4 male and 4 female pups per litter. The present study consisted of behavioral, immunohistochemical, and endocrine assessments. Only female rats were used for immunohistochemical assessments because we observed no effect of IUGR on behavioral and endocrine assessments in males. At 10 weeks of age, all animals were assigned to one of the three experimental cohorts (Fig. 1). To prevent litter effects, no more than two pups per litter were assigned to each cohort (Holson and Pearce, 1992). On postnatal day 1.5 and at 10 weeks of age, the body weight of randomly chosen rats was measured ($N = 8$ per group). All animal use procedures were in accordance with the guidelines of the Ethics Review Committee for Animal Experimentation of the National Institute of Neuroscience, Japan.

### *Induction of IUGR*

To induce IUGR, a synthetic TXA2 analogue (9,11-dideoxy-9α, 11a-methanoepoxy-prosta-5Z, 13E-dien-1-oic acid; Cayman Chemical, Michigan, USA) was administered to the pregnant female rats as described previously (Hayakawa et al., 1999). Briefly, rats were assigned to either a control group or a TXA2 group on gestational day 13. An osmotic pump (model 2ML1; Alza Corp., California, USA) containing 2 ml of TXA2 solution (12.5 μg/ml) was implanted into the lower portion of the peritoneal cavity with aseptic precautions under sodium pentobarbital anesthesia (31.5 mg/kg body weight) on the same day. TXA2 was delivered continuously for 10 days, from gestational day 13 to 22, at a rate of 20 ng/h. Control pregnant rats were treated with phosphate-buffered saline (PBS).

### *Social defeat stress*

The pups in each experiment ($N = 8$ per gender per group) were subjected to SDS from postnatal day 45 to 54. The SDS model we used was based on the resident-intruder paradigm (Koolhaas et al., 1997a; Miczek, 1991) and was implemented using a method similar to that reported recently (Rygula et al., 2005). The recipient pups were exposed to an aggressor of the same strain and gender that was 10 weeks older for 10 min each day for a total of 10 days. During the brief exposure period, all test rats exhibited signs of stress and subordination, including vocalization, flight response, and submissive posture. After 10 min of contact, the test (recipient) rats were separated from the aggressor by placement of the test rats in an adjacent compartment of the same cage, separated by a plastic divider with holes for the next 24 h; they then were housed individually until the start of the experiments. Non-stress controls were also housed individually starting on postnatal day 45.

### *Behavioral testing*

The elevated plus maze, which consists of two opposing open arms (50-cm length × 10-cm width) with 3-mm-high ledges and two opposing equal-sized closed arms with 30-cm-high transparent walls, joined by a central platform (10 × 10 cm) (O'Hara & Co., Tokyo, Japan), was conducted as described previously (Furuta et al., 2013; Komada et al., 2008). At the beginning of the test, the rats were placed at the junction of the open and closed arms, facing one of the closed arms. The total number of arms entries, the number of open arm entries, the percent of open arm entries, and the time spent in open arms on video recording were calculated using Y-maze software (O'Hara & Co.) Rodents generally avoided the open arms of the maze and preferred to remain in the closed arms (Osborn et al., 1998). Thus, an increase in time spent in the open arms indicated a reduction in anxiety-like behavior.

The same individual rat that experienced the elevated plus maze test was subjected to the T-maze test 3 days later. The forced alternation task was conducted using an automatic T-maze (O'Hara & Co.) as described previously (de Brabander et al., 1991; Stam et al., 1989; Takao et al., 2008). For 2 days before the beginning of the first trial, rats were subjected to 15-min adaptation sessions during which they were allowed to freely explore the T-maze, including both arms, with all doors open. Each rat was first placed in the start arm of the T-maze. Upon leaving the start arm, the rat had to choose between entering the left or the right goal arm. With repeated trials, the animals should display less of a tendency to enter a previously visited arm. The percentage of alternations (number of turns in each goal arm) was recorded. This T-maze forced alternation task was used to quantify working memory.

### *Immunohistochemistry*

Each rat was injected intraperitoneally with BrdU (100 mg/kg in EDTA) for 5 days. To detect cellular proliferation and neural differentiation (Encinas and Enikolopov, 2008; Miller and Nowakowski, 1988), the animals were given an overdose of pentobarbital sodium (Kyoritsu Seiyaku Corp., Tokyo, Japan) and perfused with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer at 24 h or 3 weeks after the final injection, respectively. Brains were post-fixed in the perfusion solution for 24 h at 4 °C, cryoprotected for at least 24 h in 30% sucrose in 0.1 M phosphate buffer, and then frozen at −80 °C. Free-floating 30-mm hippocampal sections were subsequently collected at −20 °C.

For the determination of BrdU labeling, sections were incubated for 30 min in 6 N HCl to denature the DNA. After being washed in PBS, sections were blocked with 1.5% goat serum for 1 h at room temperature and incubated for 24 h at 4 °C with primary rat monoclonal anti-BrdU antibody (diluted 1:50; AbD Serotec, Oxford, UK). They were then incubated for 1 h at room temperature with Alexa Fluor 488-conjugated secondary antibody (diluted 1:200; Molecular Probes, Oregon, USA). For double staining with BrdU and NeuN, DNA was denatured as described above, and the sections were blocked with 1.5% goat serum for 1 h at room temperature, and then incubated for 24 h at 4 °C with