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Title	Postnatal stress exposure infers depressive-like behavior and morphological changes in rats [an abstract of dissertation and a summary of dissertation review]
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Description	配架番号：2175
Degree Grantor	北海道大学
Degree Name	博士(医学)
Dissertation Number	甲第11693号
Issue Date	2015-03-25
Doc URL	https://hdl.handle.net/2115/58724
Rights(URL)	https://creativecommons.org/licenses/by-nc-sa/2.1/jp/
Type	doctoral thesis
File Information	Lyttle_Kerise_Alecia_abstract.pdf, 論文内容の要旨

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学位論文内容の要旨
（Summary of dissertation）

博士の専攻分野の名称　博士（医　学）　氏名：リトル　ケリス　アリシア
（Degree conferred: Doctor of Philosophy）　（Name of recipient: Lyttle Kerise Alecia ）

学位論文題名
（Title of dissertation）

Postnatal stress exposure infers depressive-like behavior and morphological changes in rats
（ラット幼若期ストレスはうつ様行動と形態学的変化を引き起こす）

Background and Objectives

The early postnatal period is characterized by increased sensitivity to adverse exposures. Studies have shown that exposure to early adverse experiences confer increased susceptibility to the development of depressive disorders. The modulatory effects of the early environment on adult psychiatry are thought to be mediated through enduring morphological changes in executive and limbic brain systems. The raphe nuclei serotonergic system is implicated in the regulation of emotional response. The executive brain system represented by the medial prefrontal cortex (mPFC) is of particular interest due to its role in the control of higher cognitive and executive functions including the regulation of emotional behavior. Notably, altered serotonergic parameters and prefrontal cortex abnormalities have been observed in depression. Although clinical studies have implied the negative effects of early life stress on brain development, causality and the detailed morphogenetic changes has not been clearly elucidated. The raphe nuclei serotonergic system is undergoing final arborisation while the prefrontal cortex is still undergoing significant maturation in the third postnatal week (Spear, 2000), during which rat pups are subjected to stress exposure. In particular, layer II/III of the prefrontal cortex is the last cortical layer to mature (Clancy et al., 2001) and is the site of processing of long-range inputs from subcortical limbic structures and may thus be susceptible to postnatal stress exposure. In the present study, I used an established model of early postnatal stress to investigate the presentation of depressive-like behavior and the neural mechanisms involved by focusing on raphe nuclei tryptophan hydroxylase 2 (TPH2) and glutamic acid decarboxylase (GAD) protein levels and mPFC layer II/III pyramidal spine densities using a rodent model. Additionally, I investigated the pharmacotherapeutic value of the selective serotonin reuptake inhibitor (SSRI), fluvoxamine on the behavioral deficits and altered neuronal morphology observed in rats subjected to 3 week footshock stress. Because it is well known that repeated treatment with SSRIs causes neurotrophic effects (Duman and Monteggia, 2006; Licznerski and Duman, 2013), I hypothesized that SSRI treatment could recover juvenile stress-induced behavioral and morphological impairments.

Methods

Male rats used for experiments were the offspring of timed-pregnant Wistar/ST rats. Pups were weaned at postnatal day 21 and group-housed (4 – 5 per cage). Food and water were provided *ad libitum.* At the post-adolescent stage behavioral characterization of locomotor activity and depressive-like behavior was carried out. The open field test was used for locomotor activity assessment in a square chamber (90 cm long × 90 cm wide × 40 cm high). Locomotor activity was assessed by measuring total distance travelled and the total number of crossings (crossings of the lines made by the division of the chamber into 9 cm × 9 cm squares) during a 15 min interval. LimeLight 2 software (Actimetrics USA) was used to score behavioral parameters.

Characterization of depressive-like behavior was assessed by the rat 2 day forced swim test with analysis of day 2 behavioral data. Forced swim test analysis was done by assessment of the occurring behavioral parameter at the end of each 5 sec interval. Behavioral parameters assessed included: (1)

immobility, swimming and climbing. Increased counts of immobile behavior is accepted as a depressive-like phenotype (Cryan et al., 2005).

Assessment of raphe nuclei TPH2 and GAD67 levels was performed by western blot. Dorsal and median raphe sections were assessed separately.

The Golgi-Cox staining technique was used for neuronal reconstruction of mPFC infralimbic and prelimbic cortex layer II/III pyramidal neuron using the FD Rapid GolgiStain™ Kit (FD Neurotechnologies, Inc.). Brains were extracted fresh and rinsed in purified water Millipore™. Brains were immediately transferred to the Golgi-Cox solution and stored in the dark for at least 2 weeks. Brains were further processed in Solution C for a minimum 48 hr at 4 °C. 100 μm mPFC coronal sections were used for photomicrographic analysis of pyramidal neuron spine densities.

Statistical data analysis was performed using IBM® SPSS® Statistics 21. Most parameters were analysed separately using a two-factor ANOVA with stress (no-FS or 3wFS) and the drug (vehicle or fluvoxamine) as between-subject factors. In cases in which there was a significant stress × drug interaction, it was followed by a one-factor ANOVA. $P < 0.05$ was considered statistically significant.

Results

Post-adolescent behavioral and morphological studies identified the presentation of increased depressive-like behaviors, elevated dorsal raphe TPH2 levels and reduced spine densities of layer II/III pyramidal neuron in the infralimbic cortex, but not in the prelimbic cortex of rats exposed to juvenile stress. Repeated fluvoxamine treatment recovered the increased depressive-like behavior and reduced spine densities observed in rats exposed to footshock stress.

Discussion

Juvenile stress exposure induced increased depressive-like behavior congruent with other studies which have demonstrated that adverse early-life stress results in behavioral deficits during adulthood. Investigation of raphe nuclei TPH2 identified a significant increase in dorsal raphe TPH2 levels following juvenile stress exposure which is consistent with previous studies of elevated dorsal raphe TPH2 following stress exposure. No significant changes in dorsal raphe GAD67 nor median raphe TPH2 and GAD67 levels may relate to the predominant role of dorsal raphe serotonergic mechanisms in regulation of stress-induced behavioral impairments.

Reduced apical and basal spine densities were observed in IL layer II/III pyramidal neurons of juvenile stressed rats. Layer II/III pyramidal neurons arborize throughout mPFC layers I and II/III enabling the sampling of diverse inputs from other brain regions (Spruston, 2008) including subcortical limbic structures involved in emotional arousal and it is expected that reduced spine densities would infer deficiencies in emotional regulation. It is likely juvenile stress-induced increased corticosterone levels mediate spine atrophy because Liu and Aghajanian (2008) demonstrated that glucocorticoid receptor antagonist reversed stress-induced reduction of spine density in the mPFC though they examined layer 5 in adult rats. 14 day oral administration of 10 mg/kg fluvoxamine recovered both increased forced swim test immobility and reduced spine densities associated with juvenile stress. It is likely that repeated fluvoxamine administration activates neurotrophic pathways responsible for behavioral and dendritic recovery.

Conclusion

The results demonstrate cortical and serotonergic sensitivities to stress exposures during the juvenile stage which mediate behavioral and morphological impairments, and provide a clue to find therapeutics for early life stress-induced emotional dysfunctions.