Psychoneuroendocrinology 99 (2019) 8–19

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Postnatal impoverished housing impairs adolescent risk-assessment and increases risk-taking: A sex-specific effect associated with histone epigenetic regulation of *Crfr1* in the medial prefrontal cortex

Thiago Wendt Viola[a], Luis Eduardo Wearick-Silva[a], Kerstin C. Creutzberg[a], Érika Kestering-Ferreira[a], Rodrigo Orso[a], Anderson Centeno-Silva[a], Lucas Albrechet-Souza[b], Paul R. Marshall[c], Xiang Li[c], Timothy W. Bredy[c], Marco A. Riva[d], Rodrigo Grassi-Oliveira[a,*]

[a] Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brain Institute (InsCer), Developmental Cognitive Neuroscience Lab, Porto Alegre, RS, Brazil
[b] Louisiana State University, Health Sciences Center, Department of Physiology, New Orleans, United States
[c] University of Queensland, Queensland Brain Institute, Brisbane, Australia
[d] Università degli Studi di Milano, Department of Pharmacological and Biomolecular Sciences, Milan, Italy

ARTICLE INFO

Keywords:
Stress
Psychological
Prefrontal cortex
Decision making
Risk assessment
Epigenetics
Histones

ABSTRACT

While increasing evidence posits poor decision-making as a central feature of mental disorders, very few studies investigated the effects of early-life stress (ELS) on specific components of reward-related choice behaviors. Risk-taking (RT) involves the exposure to some danger, or negative consequences, in order to achieve a goal-directed behavior. Such behaviors are likely to be preceded by risk-assessment (RA), which is a dynamic cognitive process involving the acquisition of information in potentially dangerous situations. Here, we investigated the effects of being raised in impoverished housing conditions during early life (P2–P9) on RT, RA and dopaminergic and corticotrophinergic gene expression of adolescent male and female mice. Phenotypes were assessed by two protocols: the elevated plus-maze (EPM) and the predator-odor risk-taking (PORT). We found decreased RA in mice exposed to impoverished housing in the absence of a reward (EPM), with a more pronounced effect among females. Moreover, when exposed to a predatory olfactory cue, increased RT was observed in these females in a reward-related task (PORT), as well as decreased HPA axis responsivity. This sex-specific behavioral effect was associated with increased *Crfr1* mRNA expression in the medial prefrontal cortex (mPFC) and higher levels of the histone mark H3R2^{me2s}, a histone modification known to be involved in transcriptional activation, within the promoter of the *Crfr1* gene. These findings revealed that ELS exposure can impair the acquisition of environmental information in dangerous situations and increase RT in reward-related scenarios among females, with an important role regarding epigenetic regulation of the *Crfr1* gene.

1. Introduction

There is considerable evidence showing that early-life stress (ELS) exposure can negatively affect brain development, producing an array of clinically relevant behavioral and cognitive alterations (Blair and Raver, 2016; Harrison and Baune, 2014). These consequences may prime such vulnerable individuals toward the development of neuropsychiatric illnesses during adolescence as well as young adulthood (Grassi-Oliveira et al., 2008). While increasing evidence posits poor decision-making as a central feature of mental disorders (Kluwe-Schiavon et al., 2016b; Steward et al., 2016), very few studies investigated the effects of ELS on specific cognitive components of choice behaviors. Of particular interest, risk-assessment (RA) is a dynamic cognitive process that involves the acquisition of environmental information in potentially dangerous situations (Reis et al., 2012). Impairments in RA are often associated with risky choices and increased risk-taking (RT) particularly in reward-related situations, such as impulsive behavioral patterns towards reinforcing stimuli despite the negative consequences associated with such actions (Kusev et al., 2017; Reske et al., 2015). However, how ELS could potentially affect RA and RT later in life is presently unknown.

Substantial evidence suggests that the medial prefrontal cortex (mPFC) is a highly sensitive brain region to the effects of ELS (Chocyk et al., 2013), while the mPFC has a key contribution for behavioral

* Corresponding author at: Avenida Ipiranga, 6681, predio 11, sala 928 - Porto Alegre, RS - Brazil, 90619-900.
E-mail address: rodrigo.grassi@pucrs.br (R. Grassi-Oliveira).

https://doi.org/10.1016/j.psyneuen.2018.08.032
Received 10 May 2018; Received in revised form 21 July 2018; Accepted 24 August 2018

control, risk perception and reward processing (Crowley et al., 2017; Schall, 2001). In particular, dopaminergic neurotransmission through D1 and D2 receptors modulate neuronal inputs between the mPFC and the ventral striatum during reward processing (Jenni et al., 2017). Evidence indicates that pharmacological blockade of D1 or D2 receptors in the mPFC and striatum could hinder RA processing through alterations in risk or reward sensitivity (Jenni et al., 2017; Sonntag et al., 2014). In addition to dopaminergic neurotransmission, corticotrophin releasing factor (CRF) is also involved in RA and RT (Guillaume et al., 2013), particularly with respect to the influence of stress on reward sensitivity (Viola et al., 2016), as well as risk-seeking approach or avoidance (Georgiou et al., 2018). For example, CRF receptor type 1 (CRFR1) gene expression in the mPFC correlates with risk-avoidance behaviors following predator odor exposure in rodents (Schreiber et al., 2017), while the blockade of this receptor reverses stress-induced cognitive and executive impairments (Uribe-Marino et al., 2016). In this sense, stress can dynamically modulate the mesocorticolimbic dopamine system (*e.g.* mPFC and striatum) via actions of the neuropeptide CRF on its receptors (Holly et al., 2015).

Dopaminergic and corticotrophinergic signaling therefore represent good candidates for an investigation into the effects of ELS on RA and RT. Furthermore, emerging evidence supports the idea that the transcription of dopaminergic and corticotrophinergic genes is regulated through the activity of epigenetic mechanisms that can act as a rheostat, serving to turn up or turn down levels of gene expression in response to rapidly changing environmental demands (Baker-Andresen et al., 2013; Takase et al., 2013). More recently, it has been shown that epigenetic mechanisms can also prime genes for responsivity to future events (Stroud et al., 2017), and that the early postnatal environment has a crucial role on the corresponding changes in DNA or histones that is accompanied by gene expression and enduring behavioral phenotypes (Vialou et al., 2013). Therefore, the study of the epigenetic landscape is also of particular interest since it has been implicated in the neurobiology of a variety of cognitive and behavioral processes (Marshall and Bredy, 2016), but little is known regarding its role on gene expression regulation implicated on RA and RT processing.

On these bases, using a mice model of impoverished housing during early infancy, we investigated the effects of early life adversity on adolescent RA and RT behavior. We utilized the elevated plus-maze (EPM) for the investigation of anxiety and RA when a reward is not presented, and the predator-odor risk-taking (PORT) task for the investigation of RT behavior associated with a reward stimulus (Dent et al., 2014). The PORT task explores a conflict between two biologically relevant stimuli for rodents: the motivation to consume a sweet and highly palatable solution while being threat by predatory olfactory cues. Since previous studies highlighted sex differences in decision-making and RT behavior (Georgiou et al., 2018), as well as regarding vulnerability to the effects of ELS (Walker et al., 2017), we aimed to extend these findings with an investigation of the performance of both adolescent male and female mice in these behavioral tasks. In order to determine to what extent the dopaminergic and corticotrophinergic signaling may account for the effects of stress and sex in RA and RT processing, we measured gene expression levels of *Drd1*, *Drd2*, *Crf* and *Crfr1* in the mPFC and in the striatum.

Finally, histone acetylation/deacetylation and methylation of specific lysine residues on nucleosomal histone proteins (*i.e.*, H3-K9) within promoter regions are ways that chromatin remodeling can influence gene transcription. In particular, modifications of histone H3 in the mPFC have been associated with the effects of stress and fear on cognition (Bredy et al., 2007). Therefore, we also investigated the levels of $H3K9^{me3}$, a histone mark associated with transcriptional repression, and the levels of $H3R2^{me2s}$, a histone mark associated with gene expression, within the promoter region of behaviorally relevant candidate genes.

2. Methods

2.1. Animals

This study was performed with male and female C57BL/6 mice obtained from the colony of the Center for Experimental Biological Models (CeMBE), Pontifical Catholic University, Porto Alegre, RS, Brazil. The CeMBE is a facility devoted for the breeding of rodents in accordance with the sanitary standard SPF (Specific Pathogen Free). This building consists of breeding rooms, quarantine rooms, expedition services, material disinfection and sterilization, a warehouse, cold chambers, a diagnostic laboratory and operating rooms. In order to avoid any stress associated with animal transportation between different facilities, as well as to generate concomitant births, mice were bred in-house at the CeMBE, with technical support for the management of animals by a veterinarian staff team, duly registered at the Regional Veterinary Medicine Board. Two adult female C57BL/6 mice were bred with an adult male for 72 h. After this period, the male animal was moved to a new cage, while females remained together. Following two weeks and at the end of gestational period, females were separated in individual cages. Then, single-housed pregnant females were visually checked daily for the presence of pups. Following birth, 30 different mice litters were used, in which 15 were randomly assigned to standard reared group, and 15 were randomly assigned to impoverished housing condition (Rice et al., 2008). Each litter contributed with one to three pups per sex for each experimental group.

All animals were housed under a 12 h/12 h light–dark cycle in ventilated Plexiglas cages with temperature maintained at 21 ± 1 °C. Food and water were available *ad libitum*, with the exception when animals underwent water restriction for PORT behavioral training and testing. The experiments were conducted in accordance with the NIH laboratory animal care guidelines and approved by the Ethical Committee on the Use of Animals of the Pontifical Catholic University of Rio Grande do Sul. The experimental design and timeline of experiments are depicted in Fig. 1. All behavioral testing were conducted in the light phase of the light-dark cycle, with luminosity at the level of 75 lx. Mice were weighted at the postnatal day (P) 9, P30 and P40.

2.2. Model of impoverished housing during early infancy

We used the limited bedding and nesting (LBN) protocol (Rice et al.,

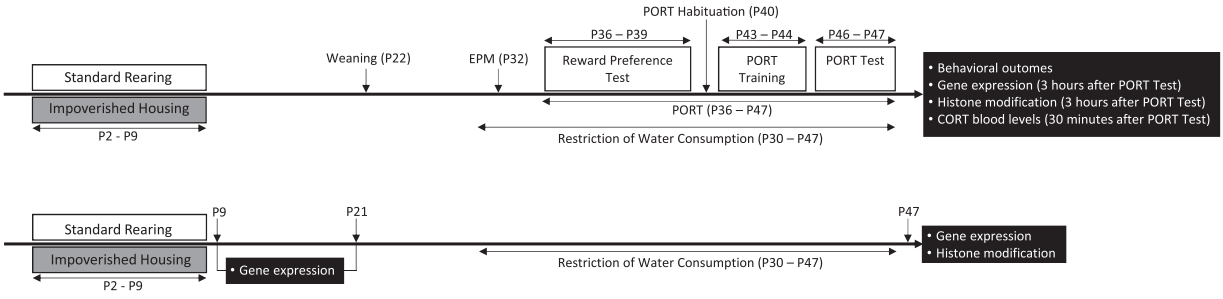

Fig. 1. Experimental design and study outcomes; EPM, elevated plus-maze; PORT, predator-odor risk-taking task.