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Research Report

Environmental enrichment reverses reduction in glucocorticoid receptor expression in the hippocampus of and improves behavioral responses of anxiety in early malnourished rats

Roberto O. Soares[a,*], *Rodrigo C. Rorato*[b], *Diego Padovan*[c], *João-José Lachat*[a], *José Antunes-Rodrigues*[b], *Lucila L.K. Elias*[b], *Sebastião S. Almeida*[c]

[a]*Department of Surgery and Anatomy, FMRP, University of São Paulo, São Paulo, Brazil*
[b]*Department of Physiology, FMRP, University of São Paulo, São Paulo, Brazil*
[c]*Department of Psychology, FFCLRP, University of São Paulo, São Paulo, Brazil*

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ABSTRACT

We compared glucocorticoid receptor (GR) gene expression in the hippocampus of rats subjected to a low protein, "malnourished" diet (M; 6% protein) or a control, "well-nourished" diet (W; 16% protein), exposed to different environmental stimulation (environmental enrichment, E; no enrichment, N) between postnatal day 8 (P8) and P35. Rats were tested on the elevated plus maze (EPM) on P36. Male Wistar rats were split into two groups at birth according to diet (M or W) and subdivided according to environmental stimulation (E or N). GR expression was determined using real-time polymerase chain reaction and GR immunohistochemistry in the hippocampus. Our results showed that MN rats spent more time and made more entries into the open arms of the EPM compared to W rats. On the other hand, ME rats spent a similar percentage of time, and made a similar number of entries, in the open arms as WN rats. Following the EPM test, GR mRNA expression in the hippocampus was different in MN rats compared to W rats; expression was also different between M and ME rats; mRNA and expression of GR receptors in WN rats was similar to that observed in WE rats. These data also show that the effects of malnutrition on risk assessment in the EPM were reversed by E. Early malnutrition may alter GR expression in the hippocampus and environmental enrichment may exert a neuroprotective effect on malnutrition-induced brain injury.

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[*] Correspondence to: Laboratory of Neuroanatomy, Department of Surgery and Anatomy, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Avenida dos Bandeirantes 3900, Ribeirão Preto, São Paulo 14040-901, Brazil.
E-mail address: robertosoares@usp.br (R.O. Soares).

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1. Introduction

Studies in rats indicate that malnutrition during the prenatal period can alter development of the central nervous system (CNS), particularly hippocampal plasticity (Adlard and Smart, 1972; Smart et al., 1973; Morgane et al., 2002). Alterations in neurogenesis and CNS development, observed in early malnourished rats (Cintra et al., 1997; Kehoe et al., 2001) may result in deficits in development of the CA1 region of the hippocampus (Morgane et al., 2002) reductions in total dendritic ramification (Dobbing and Widdowson, 1965; Fishman et al., 1971; Krigman and Hogan, 1976) and number of synaptic spines (Durán et al., 2006). Early malnutrition may also lead to a reduction in glucocorticoid receptor (GR) expression in the brain, particularly in the hippocampus, and possibly alter regulation of corticosterone (CORT) negative feedback in the hypothalamic–pituitary–adrenal (HPA) axis (Adlard and Smart, 1972; Sampaio et al., 2008). Early malnutrition has been shown to increase adrenocorticotropic hormone release and free CORT levels (Kehoe et al., 2001; Sampaio et al., 2008) which are associated with low levels of brain-derived neurotrophic factor (Cintra et al., 1997; Mesquita et al., 2002). These results highlight the effects of early malnutrition on HPA axis activity.

Early malnourished rats show behavioral changes, including higher numbers of entry and time spent in the open arms in the elevated plus maze test (EPM) (Almeida et al., 1993; Hernandes and Almeida, 2003; Sampaio et al., 2008; Soares et al., 2013) as well as differences in head dipping and stretching behavior (De Oliveira and Almeida, 1985; Almeida et al., 1991; Santucci et al., 1994; Françolin-Silva and Almeida, 2004; Valadares et al., 2010; Soares et al., 2013), which indicate an altered anxiety response. This altered behavior is associated with failures in risk assessment, as animals demonstrate greater impulsivity when faced with a new situation, and impairments in anxiety response (Soares et al., 2013). Anxiety-related behavioral changes following protein malnutrition have also been observed in other animal tests of anxiety, such as the elevated T maze (Hernandes and Almeida, 2003; Silva Hernandes et al., 2005).

Although brain damage caused by malnutrition cannot be recovered through nutritional rehabilitation (Riul et al., 1999), studies have demonstrated that environmental stimulation may act as a neuroprotective factor against the effects of malnutrition (Santucci et al., 1994; Riul et al., 1999; Sampaio et al., 2008). Environmental stimulation or enrichment may alter GR expression in the hippocampus, directly interfering with the negative feedback mechanism of HPA axis (Dahlqvist et al., 1999; Gomes et al., 1999; Wigger and Neumann, 1999). In addition, rats exposed to an enriched environment had a thicker cortex when compared to rats living in standard conditions (Diamond et al., 1964; Rosenzweig et al., 1968; Rosenzweig and Bennett,1996). This increase in cortical thickness may be due to an increase in neuron size, dendritic number and length, and number of dendritic spines, induced by continued exposure to an enriched environment (Diamond et al., 1964; Will et al., 2004). Environmental stimulation also has effects on behavior, with rats living in an enriched environment showing increased exploratory behavior (Levitsky and Barnes, 1972), particularly in the open arms of the EPM (Fernández-Teruel et al., 1997, 2002; McIntosh et al., 1999).

We have previously demonstrated that rats subjected to postnatal malnutrition and exposed to an enriched environment for 1 h a day, during a critical period of CNS development, show similar numbers of entries and duration of time spent in the open arms of the EPM compared to well-nourished rats (Soares et al., 2013). These results demonstrate that environmental enrichment can protect against the deleterious effects of early malnutrition on CNS development. However, it is not clear how protein malnutrition may influence GR expression in the hippocampus, which can interfere with HPA axis activity. In addition, the effects of environmental enrichment on GR expression in the hippocampus are unknown. Therefore, the objective of the present study was to compare the effects of protein malnutrition and environmental enrichment on EPM performance and GR expression in the rat hippocampus.

2. Results

2.1. *Effects of malnutrition and enrichment on body weight*

There was a significant main effect of diet ($F_{(1,\ 19)}=7.37$, $p<0.05$) and postnatal day ($F_{(3,\ 57)}=118$, $p<0.001$) on dam

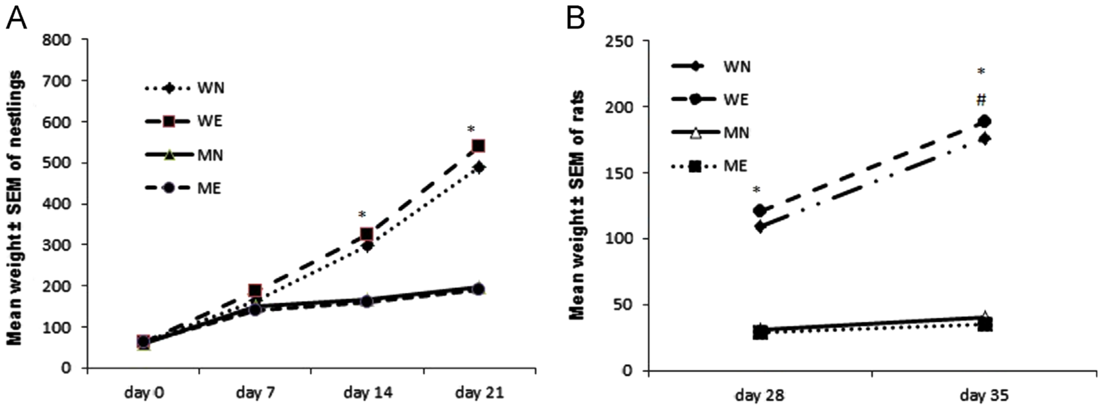

Fig. 1 – Body weight (g) of nestlings on postnatal day 0 (P0), P7, P14 and P21 (A) and body weight of post-weaning rats on P28 and P35 (B). $^{***}p<0.001$ compared to M groups and $^{\#\#\#}p<0.001$ compared to P35. WN=well-nourished, not enriched; WE=well-nourished, enriched environment; MN=malnourished not enriched; ME=malnourished, enriched environment. Data presented as mean±standard error of the mean.