Brain Research 1657 (2017) 130–139

Contents lists available at ScienceDirect

Brain Research

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

Research report

Tactile stimulation partially prevents neurodevelopmental changes in visual tract caused by early iron deficiency

Everton Horiquini-Barbosa [a,b,*], Robbin Gibb [c], Bryan Kolb [c], Douglas Bray [c], Joao-Jose Lachat [b]

[a] Department of Psychology, Faculty of Philosophy, Sciences and Letters of Ribeirao Preto, University of Sao Paulo, Sao Paulo, Brazil
[b] Laboratory of Neuroanatomy, Department of Surgery and Anatomy, Ribeirao Preto Medical School, University of Sao Paulo, Sao Paulo, Brazil
[c] Canadian Centre for Behavioural Neuroscience, University of Lethbridge, Alberta, Canada

ARTICLE INFO

Article history:
Received 20 August 2016
Received in revised form 29 November 2016
Accepted 2 December 2016
Available online 9 December 2016

Keywords:
Tactile stimulation
Neonatal handling
Neurodevelopment
Optic nerve
Astrocyte
Oligodendrocyte
Myelinated fibers

ABSTRACT

Iron deficiency has a critical impact on maturational mechanisms of the brain and the damage related to neuroanatomical parameters is not satisfactorily reversed after iron replacement. However, emerging evidence suggest that enriched early experience may offer great therapeutic efficacy in cases of nutritional disorders postnatally, since the brain is remarkably responsive to its interaction with the environment. Given the fact that tactile stimulation (TS) treatment has been previously shown to be an effective therapeutic approach and with potential application to humans, here we ask whether exposure to TS treatment, from postnatal day (P) 1 to P32 for 3 min/day, could also be employed to prevent neuroanatomical changes in the optic nerve of rats maintained on an iron-deficient diet during brain development. We found that iron deficiency changed astrocyte, oligodendrocyte, damaged fiber, and myelinated fiber density, however, TS reversed the iron-deficiency-induced alteration in oligodendrocyte, damaged fiber and myelinated fiber density, but failed to reverse astrocyte density. Our results suggest that early iron deficiency may act by disrupting the timing of key steps in visual system development thereby modifying the normal progression of optic nerve maturation. However, optic nerve development is sensitive to enriching experiences, and in the current study we show that this sensitivity can be used to prevent damage from postnatal iron deficiency during the critical period.

1. Introduction

According to the World Health Organization, iron deficiency is the most common nutritional disorder, affecting approximately two billion people globally in both developing and developed countries (WHO, 2002, 2005). Iron is an essential micronutrient required for normal neurodevelopment and cell function as it participates in a variety of critical processes including DNA, RNA and protein synthesis, cell proliferation and cell differentiation and myelination (Beard et al., 1993; Beard and Connor, 2003; Beard et al., 2003; Lozoff and Georgieff, 2006; Greminger et al., 2014). Iron demand increases during the period of rapid brain growth, and its absence has been directly related to disrupted central nervous system (CNS) development. There is an accumulating body of evidence indicating that iron deficiency leads to irreversible structural (DeMaman et al., 2008), ultrastructural (DeMaman et al., 2010), electrophysiological (Rocinholi et al., 2008), neurochemical (Beard, 2003), behavioral (Lozoff et al., 2006) and cognitive abnormalities (Lozoff et al., 2006; Murray-Kolb and Beard, 2007).

Previous studies from our group have reported that rats maintained on an iron-deficient diet during neurodevelopment experience robust morphologic and morphometric impairments in the optic nerve. These include vacuolization and nuclear invagination, increases in both astrocyte and damaged fiber density, and decreases in oligodendrocyte density; all possibly attributed to alteration in maturational mechanisms (Demaman et al., 2008). We have also shown that damage related to neuroanatomical parameters was not satisfactorily reversed after iron replacement, indicating that iron deficient nutrition during the first postnatal month results in irreversible consequences to the normal development of the CNS (DeMaman et al., 2008, 2010), which might interfere with the normal functions of the visual system and cause visual dysfunction.

Some effects of iron deficiency are permanent, but a degree of protection can be obtained through an enriched early experience because the brain is remarkably responsive to its interaction with the environment and this can shape the developing brain. Previous studies revealed that malnourished rats achieved some prophy-

* Corresponding author at: Department of Psychology, University of Sao Paulo, Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, São Paulo, Brazil.
E-mail address: horiquini@usp.br (E. Horiquini-Barbosa).

http://dx.doi.org/10.1016/j.brainres.2016.12.003
0006-8993/

laxis by exposure to an enriched environment (Soares et al., 2013; De Oliveira Soares et al., 2014; Barbosa et al., 2016; Soares et al., 2015). There is now an abundance of literature reinforcing the fact that tactile stimulation (TS) treatment is a positive enriching experience (Kolb and Gibb, 2007; Imanaka et al., 2008; Gibb et al., 2010; Kolb and Gibb, 2010; Richards et al., 2012; Freitas et al., 2015; Horiquini-Barbosa and Lachat, 2016). This work includes studies showing that tactile stimulation accelerates visual development both in rat pups and in human infants (Guzzetta et al., 2009), as well as our initial findings showing that optic nerve development is accelerated in rats that were exposed to neonatal TS (Horiquini-Barbosa and Lachat, 2016). It is plausible that such an experience may offer preventative intervention in animals maintained on iron deficient diet early in life. Although the focus on TS treatment-driven plasticity in the brain has traditionally been on the altered morphology of neurons and in particular the synapse, we have shown that oligodendrocyte density and cerebrovasculature distribution also demonstrate robust alterations in response to TS.

Since iron deficiency is the most common form of malnutrition in the world, being most prevalent and severe in young children and TS is totally non-invasive, low-cost and effective procedure and with potential application to humans, the proposal of the current work was to explore whether exposure to TS treatment could also be employed to prevent neuroanatomical changes in iron-deficient rats. To elucidate the therapeutic effects of TS on the neuroanatomy of optic nerve from rats maintained on an iron-deficient diet, a qualitative fine structure analysis was performed to evaluate the developmental patterns in the cytoarchitecture. In addition, a quantitative assessment was carried out to determine whether the observed changes in optic nerve tissue were significantly different among the groups or at different ages.

2. Results

2.1. Body weight and hematology

On the day of birth, we found no significant difference among newborns, confirming that all rats were in the same nutritional condition at the beginning of the experimental treatment. A significant effect of diet condition ($F_{(1,20)} = 469.63$; $P < 0.001$) and days ($F_{(5,100)} = 1539.75$; $P < 0.001$) was observed, but no significant effect of stimulation condition variable ($F_{(1,20)} = 0.25$; $P = 0.620$ [n.s]) nor an interaction effect ($F_{(5,100)} = 1.65$; $P = 0.154$ [n.s]). These data suggest that rats maintained on an iron-deficient diet presented a lower body weight when compared to age-matched rats maintained on iron-adequate diet, and this significant difference was age-dependent, indicating that animals in iron-deficient groups have a lower growth rate according to the body weight gain. TS treatment did not change the body weight in the iron deficient rats (Table 1; Fig. 1A and B).

Table 1
Body weight of the pups in lactation and post-lactation period.

Age	Body weight (g)[1]			
	ANTS	ATS	DNTS	DTS
P0[2]	6,67 ± 0,16	6,66 ± 0,32	6,89 ± 0,69	6,67 ± 0,49
P7	17,78 ± 1,49	18,04 ± 1,22	17,06 ± 1,90	16,90 ± 1,84
P14	32,48 ± 3,22	32,88 ± 3,77	30,76 ± 4,35	30,45 ± 3,93
P21	53,56 ± 3,56	53,17 ± 4,14	39,11 ± 6,59*	39,53 ± 3,76*
P28	87,67 ± 4,11	87,25 ± 4,26	63,17 ± 4,17*	63,33 ± 6,99*
P32	112,17 ± 10,20	112,67 ± 3,82	68,83 ± 6,49*	68,67 ± 5,53*

[1] Values are means ± SEM.
[2] Based on body weight, all rats were in the same nutritional condition at the beginning of the experimental treatment.
* Compared to age-matched pups maintained on iron-adequate diet ($p \leqslant 0.05$).

Rats maintained on an iron-deficient diet showed a significant decrease in Hb and Hct levels whereas statistical analyses revealed no effect of TS treatment. A three-way ANOVA on each of Hb and Hct revealed a main effect of diet ($F_{(1,20)} = 208.333$; $P < 0.001$) and ($F_{(1,20)} = 298.496$; $P < 0.001$), respectively; and effect of days ($F_{(2,40)} = 18.885$; $P < 0.001$) and ($F_{(2,40)} = 33.780$; $P < 0.001$), respectively; but no significant effect of stimulation ($F_{(1,20)} = 0.030$; $P = 0.864$ [n.s]) and ($F_{(1,20)} = 0.002$; $P = 0.966$ [n.s]), respectively; nor the interaction effects ($F_{(2,40)} = 0.018$; $P = 0.982$ [n.s]) and ($F_{(2,40)} = 0.028$; $P = 0.972$ [n.s]), respectively. These results confirm that rats maintained on an iron-deficient diet experienced a severe iron deficiency anemia (Fig. 1C and D).

2.2. Qualitative analysis

2.2.1. Overview of optic nerve ultrastructure

To interpret the iron-deficiency related structural and ultrastructural alterations or any protective effect by TS that affected the optic nerve fibers, it is necessary to understand the normal appearance of astrocytes, oligodendrocytes and blood vessel in light micrographs (Fig. 2; A, B), and also characteristic features of myelinated and unmyelinated fiber profiles in electron micrographs (Fig. 3; A, B).

Many glial cells and blood vessels were present in both the peripheral and central portions of the nerve. At high magnification (1000×), glial cells could be identified as astrocytes or oligodendrocytes, and myelinated nerve fibers could be clearly identified. Astrocytes presented a large pale and oval nucleus with apparent dark nucleoli, a thin rim of condensed chromatin beneath the nuclear envelope and the nucleus is surrounded by a large amount of pale cytoplasm. Oligodendrocytes were stained more strongly with toluidine blue compared with the astrocyte, and presented a dark and irregular shaped nucleus, in which is possible to note that the chromatin is clumped throughout the nucleoplasm (Fig. 2).

The normal myelinated fibers of the optic nerve are composed of an axon with pale axoplasm in which there are cytoskeletal structures, *i.e.,* neurofilaments and microtubules, and oval-shaped mitochondrial profiles. The myelin sheath is a greatly extended and modified plasma membrane wrapped concentrically around the axon, and it is produced by oligodendrocytes, the most common type of neuroglial cells in white matter (Figs. 2 and 3).

The unmyelinated fibers are frequently in a compacted bundle and they present an oval and smaller profile. The unmyelinated axon has a pale axoplasm in which there are neurofilaments, microtubules, and mitochondrial profiles (Fig. 3).

2.2.2. Effect of tactile stimulation treatment in the optic nerve of iron deficient rats

The iron deficiency led the optic nerve fibers to undergo a variety of alterations that were mostly initiated by the degeneration of the myelin sheath. The delaminated or loosened myelin was the most common form of alteration caused by iron deficiency and at P18, the extent of these early myelin ultrastructure changes varied from mild cases where myelin decompaction divided only few myelin lamellae, to extreme cases where myelin decompaction divided several lamellae. At this early stage of degeneration, the interstitial space between fibers increased and areas with vacuolation were visible (Fig. 2 – C1). The axons of retinal ganglion cell were still intact, and presented healthy characteristics even with myelin alteration (Fig. 3 – C1). The optic nerves from rats maintained on an iron-deficient diet, but treated with TS, presented several characteristic iron deficiency-related alterations, such as delaminated myelin. However, the tissue was better organized with less damaged fibers when compared to age-matched non-treated iron-deficient rats, indicating a possible protective effect (Fig. 2 – D1 and Fig. 3 – D1).