Please cite this article in press as: Antonioli-Santos R et al. The fine tuning of retinocollicular topography depends on reelin signaling during early postnatal development of the rat visual system. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.06.001

Neuroscience xxx (2017) xxx–xxx

THE FINE TUNING OF RETINOCOLLICULAR TOPOGRAPHY DEPENDS ON REELIN SIGNALING DURING EARLY POSTNATAL DEVELOPMENT OF THE RAT VISUAL SYSTEM

RACHEL ANTONIOLI-SANTOS, [a,c]
BRUNA LANZILLOTTA-MATTOS, [a]
CECÍLIA HEDIN-PEREIRA [b,d] **AND**
CLAUDIO ALBERTO SERFATY [a*]

[a] *Federal Fluminense University, Biology Institute, Neurobiology Department, Laboratory of Neuroplasticity – Niteroi, PO Box: 100180, Brazil*

[b] *Federal University of Rio de Janeiro, Institute of Biomedical Sciences, Laboratory of Cellular Neuroanatomy – Rio de Janeiro, Brazil*

[c] *Institute of Biomedical Research, Marcílio Dias Navy Hospital, Rio de Janeiro, Brazil*

[d] *Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil*

Abstract—During postnatal development, neural circuits are extremely dynamic and develop precise connection patterns that emerge as a result of the elimination of synaptic terminals, a process instructed by molecular cues and patterns of electrical activity. In the rodent visual system, this process begins during the first postnatal week and proceeds during the second and third postnatal weeks as spontaneous retinal activity and finally use-dependent fine tuning takes place. Reelin is a large extracellular matrix glycoprotein able to affect several steps of brain development, from neuronal migration to the maturation of dendritic spines and use-dependent synaptic development. In the present study, we investigated the role of reelin on the topographical refinement of primary sensory connections studying the development of retinal ganglion cell axon terminals in the rat superior colliculus. We found that reelin levels in the visual layers of the superior colliculus are the highest between the second and third postnatal weeks. Blocking reelin signaling with a neutralizing antibody (CR-50) from PND 7 to PND 14 induced a non-specific sprouting of ipsilateral retinocollicular axons outside their typical distribution of discrete patches of axon terminals. Also we found that reelin blockade resulted in reduced levels of phospho-GAP43, increased GluN1 and GluN2B-NMDA subunits and decreased levels of GAD65 content in the visual layers of the superior colliculus. The results suggest that reelin signaling is associated with the maturation of excitatory and inhibitory synaptic machinery influencing the development and fine tuning of topographically organized neural circuits during postnatal development.

Key words: brain development, retinotectal, axonal fine tuning, plasticity, visual system, topographical maps, reelin, extracellular matrix molecules, NMDA receptors.

INTRODUCTION

One of the most important features of brain development is the formation of precise neuronal connections, which ensures an adequate processing of sensory, motor and cognitive functions. In the rat visual system, retinal ganglion cells (RGCs) axons project to subcortical nuclei, mainly the superior colliculus (SC) (Linden and Perry, 1983). Retinocollicular pathways have been extensively used to address the development of topographical maps (Huberman et al., 2008), (for a review), as a model of neuronal circuitry maturation. The ipsilateral visual pathway of rodents is a particularly useful tool, since it forms spatially defined clusters of axon terminals during postnatal development, as revealed by bulk intravitreal injections of neuronal tracers (Serfaty et al., 2005). Therefore, this neuronal pathway has been used as a biological model to easily access the emergence of topographical maps driven by synapse elimination during postnatal development (Land and Lund, 1979; Campello-Costa et al., 2006; de Velasco et al., 2012).

The refinement of topographical patterns of axon terminals emerges as a result of the elimination of excess axon arbors driven by molecular gradients together with electrical activity (Huberman et al., 2008) during a time window spanning the first and second postnatal weeks, in rodents. Thus, an adult-like visual topography emerges during an early postnatal period through a rapid, large-scale removal of axons and axon arbors from topographically inappropriate positions simultaneously with the increase in axonal branching and arborization at topographically correct locations (Simon and O'Leary, 1992). This has been demonstrated for the ipsilateral retinocollicular pathway that undergoes a similar process resulting in a convergence into discrete clusters at the ventral border of the collicular visual layers (Serfaty et al., 2005). Several conditions appear to contribute to this stage of neural circuit development. For instance, it has been shown that pharmacological or nutritional alterations in serotonin content, caffeine

*Corresponding author. Adrees: Instituto de Biologia, Universidade Federal Fluminense, P.O. Box. 100180 Niterói, RJ CEP 24001-970, Brazil.
E-mail addresses: claudio.serfaty@gmail.com, cserfaty@id.uff.br (C. Alberto Serfaty).

http://dx.doi.org/10.1016/j.neuroscience.2017.06.001
0306-4522/

administration, essential omega-3 fatty acids and extracellular matrix molecules such as metaloproteinase-9 appear to modify both the fine tuning and plasticity of retinal axons in the superior colliculus during early postnatal development (Bastos et al., 1999; Oliveira-Silva et al., 2007; Gonzalez et al., 2008; Cabral-Miranda et al., 2011; de Velasco et al., 2012).

Reelin is a large extracellular matrix glycoprotein able to affect several steps in brain development, regulating neuronal migration, positioning and lamination in the early developing cortex (D'Arcangelo et al., 1995, 1997; Frotscher et al., 2009), maturation of dendrites and dendritic spines (Niu et al., 2004, 2008) and axon guidance/targeting to the dorsal lateral geniculate nucleus (dLGN) (Su et al., 2011). Regarding the use-dependent maturation of neuronal circuits in the hippocampus, reelin accumulation is accompanied by reduced GluN2B-containing NMDA receptors in active synapses (Sinagra et al., 2005) and added exogenous reelin induces the developmental shift of synaptic receptors to GluN2A-containing NMDARs (Groc et al., 2006, 2007). Thus, it appears that reelin may be involved in several mechanisms leading to synaptic development, stabilization and plasticity.

In fact, over the last decade, altered reelin expression has been associated with multiple neurodevelopmental disorders such as schizophrenia and autism (Guidotti et al., 2000; Fatemi et al., 2009; Folsom and Fatemi, 2013). Autism has been related to errors in synapse elimination during development (Pfeiffer et al., 2010) and also to an imbalance in excitatory and inhibitory circuits (Fatemi et al., 2002; Yizhar et al., 2011). In the present study, we propose a role for reelin on the fine tuning of the ipsilateral retinocollicular pathway topography suggesting that reelin signaling may also influence the stabilization of developing visual topographical maps.

EXPERIMENTAL PROCEDURES

All experiments were performed under general anesthesia and in strict accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experimental protocols were approved by the local Animal Care Committee (CEUA-UFF/ 0012009). Lister Hooded rats at ages ranging from PND 5 (postnatal day 5) to PND 28 were used for *in vivo* neuroanatomical, immunofluorescence and biochemical experiments. Litters with no more than eight animals were maintained in a temperature- and humidity-controlled room under a 12–h/12–h light/dark cycle with free access to food and water.

Western blot

Following euthanasia with an overdose of isoflurane, superior colliculi of animals at PND 5, 7, 10, 14, 21 and 28 were dissected and homogenized in ice-cold lysis buffer containing 20 mM Tris, 10 mM $MgCl_2$, 0.6 mM $CaCl_2$, EGTA, 0.5 mM DTT, 5 μg/ml aprotinin, 2 μg/ml leupeptin, 0.05% TX-100 and 1 mM PMSF. Protein concentration analysis was carried out through the Bradford method and the final concentrations were normalized with a lysis buffer containing 0.1% SDS. Samples (40 μg protein/lane) were separated by SDS–PAGE (5% or 10% with 4% concentrating gel) and electrotransferred to PVDF membranes (Amersham Biosciences®). Unspecific sites were blocked for 2 h at room temperature with 5% non-fat milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T pH 7.6). Membranes were then incubated overnight at 4 °C with either mouse G10 anti-reelin (1:2000, Millipore®), rabbit anti-pDab1 (1:100, Sigma®), mouse anti-GAD65 (1:1000, Cell Signaling®), goat anti-p-GAP43 (1:800, Santa Cruz®), mouse anti-GAP43 (1:1000, Millipore®), mouse anti-GluN1 (1:1000, Millipore®), rabbit anti-GluN2A (1:1000, Millipore®), mouse anti-GluN2B (1:1000, Millipore®). Next, membranes were incubated with secondary HRP-conjugated antibodies (donkey anti-rabbit, 1:5000; donkey anti-mouse, 1:5000; donkey anti-goat, 1:5000; all from Amersham Bioscience®) in TBS-T for 1 h. Then, membranes were revealed using ECL plus chemiluminescence kit (Amersham Bioscience®). The detection of chemiluminescence was performed using a ChemiDoc system (BioRad®) and the densitometry analysis by Image Lab™ software. To ensure that all lanes were loaded with the same amount of protein, membranes were stripped and reprobed with anti-vinculin (1:2500, Sigma®) or mouse anti-β-tubulin (1:50,000, Millipore®) primary antibodies. Densitometric analysis was done taking the ratio of the primary antibody to its corresponding loading control in each lane.

Immunohistochemistry

At PND 10, animals were deeply anesthetized with an overdose of isoflurane before transcardial perfusion with saline (0.9%NaCl) containing 0.1% heparin followed by a 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH = 7.4) for 20 min. After brain removal and cryoprotection (20% sucrose in the same buffer), coronal sections of 10-μm thickness were cut in a cryostat and mounted on gelatinized slides. The sections were washed in PBS pH 7.4 and blocked in 10% normal goat serum (NGS) in PBS plus Triton-X 0.2% (PBS-T) for two hours. Next, sections were incubated overnight with the primary antibody (1:800 G-10 anti-reelin, Millipore®) diluted in PBS-T containing 5% NGS. After several washes in PBS-T, the sections were incubated in AlexaFluor488-conjugated goat anti-mouse (1:300, Invitrogen®) made up in PBS-T containing 5% NGS for 2 h. Dapi (Sigma®) was used for nuclear labeling. Superior colliculus images were acquired using a Leica DM2500 fluorescence microscope coupled to a digital camera (Leica®).

Anterograde tracing of retinocollicular projections

The uncrossed retinocollicular projections were accessed by an intravitreal injection of horseradish peroxidase (30% HRP type VI, Sigma) in 2% dimethylsulfoxide (DMSO) in 0.9% NaCl, in the right eye at PND 13. After 24 h, the animals were deeply anesthetized with isoflurane and transcardially perfused with saline (NaCl 0.9%) containing 0.1% heparin followed by a mixture of aldehydes (1% paraformaldehyde and 2%

Please cite this article in press as: Antonioli-Santos R et al. The fine tuning of retinocollicular topography depends on reelin signaling during early postnatal development of the rat visual system. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.06.001