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Research Report

A critical period for omega-3 nutritional supplementation in the development of the rodent visual system

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ABSTRACT

Retinocollicular connections form precise topographical maps that are normally completed through the selective elimination of misplaced axons and the stabilization of topographically ordered axon terminals during early development. Omega-3 fatty acids, acquired exclusively through the diet, and its main metabolite, docosahexaenoic acid (DHA), are involved in brain development and synaptic maturation. We have previously shown that the nutritional restriction of omega-3/DHA results in abnormal retinocollicular topographical fine-tuning. Therefore, we studied the role of omega-3 fatty acids nutritional supplementation and the developmental time windows during which this postnatal supplementation would restore normal topographical maps in the visual system. Female rats and their litters were chronically fed with either control (soy oil) or restricted omega-3 (coconut oil) diets. Fish oil supplementation was introduced between either postnatal day (PND) 7-13, PND7-28 or PND21-42. At PND13, PND28 or PND42, animals received an anterograde eye injection of a neuronal tracer to visualize retinocollicular axons. Confirming previous observations we found that an omega-3/DHA deficiency resulted in an abnormally high innervation density of retinal axons at the visual layers of the superior colliculus (SC). Although a short-term fish oil supplementation between PND7-13 could not restore normal retinocollicular topography, an extended treatment between PND7-28 completely recovered normal innervation densities of retinotectal axons. However, a late onset supplementation protocol, between PND28-42, was no longer effective in the restoration of the abnormal topographical pattern induced by an early omega-3 nutritional malnutrition. The results suggest a critical period for omega3/DHA dietary intake for the proper development of visual topographical maps.

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1. Introduction

Retinocollicular connections form precise topographical maps that are normally achieved through the selective elimination of misplaced axons and the stabilization of topographically ordered axon terminals. In subcortical pathways, the initial development of retinocollicular topography is strongly influenced by gradients of repulsive/attractive molecules between retinal axons and target neurons (Mellitzer et al., 2000; Schulte and Bumsted-O'Brien, 2008; Yates et al., 2001). However, activity-dependent mechanisms are required to ensure the fine tuning of the correct topographical representation of retinal axons terminal fields, and thus circuitry maturation (Dhande et al., 2011; McLaughlin and O'Leary, 2005). As a result, the development of topography and visual acuity is strongly impaired under conditions such as the knockout of beta-2 nicotinic receptor subunits (Mrsic-Flogel et al., 2005) and visual deprivation (Carrasco et al., 2011).

Omega-3 fatty acids are essential fatty acids (EFAs) since their chain precursor, α-linolenic acid, cannot be endogenously synthetized and must be obtained exclusively through dietary intake (Gibson et al., 2011; Innis, 2008). These fatty acids, mainly the docosahexaenoic acid (DHA), are highly incorporated in phospholipids of the brain and retina during late pregnancy and in early stages of postnatal life (Carlson et al., 2013) and acts as a trophic factor during central nervous system (CNS) development, exerting several roles in neurogenesis, cell survival and synaptogenesis (Bazan, 2009; Eady et al., 2012; Janssen et al., 2014; Wu et al., 2008). Since DHA is preferentially incorporated in phospholipids membranes over α-linolenic acid, it has been proposed that DHA could be classified as a conditionally essential fatty acid (Bazan et al., 2011a; Parletta et al., 2013).

Previous results from our laboratory (de Velasco et al., 2012) strongly suggested a role for omega-3 fatty acids in the maturation of visual circuitry specification. It has been shown that a chronic dietary deprivation of omega-3 fatty acids induced a selective decrease in DHA levels in subcortical nuclei (de Velasco et al., 2012). This resulted in a loss of axonal topographical fine-tuning, with an abnormally high density of retinal axons from the ipsilateral retina in the visual layers of the superior colliculus, as well as a decreased eye-specific segregation in the lateral geniculate nucleus (LGN). Furthermore, it has been shown that rats raised under omega-3/DHA deficiency presented an abnormally extended critical period for lesion-induced plasticity in retinocollicular pathways (de Velasco et al., 2012) which indicated a general delay in the visual system development.

Consequences in abnormal patterns of retinal connectivity and changes in the precise time courses of CNS development could explain visual dysfunctions observed with DHA dietary deficiency in humans (Molloy et al., 2012; Mulder et al., 2014). Indeed, several studies revealed that omega-3 fatty acids supplementation, in early stages of life, enhanced visual acuity development (Birch et al., 2010), and also improved the performance in cognitive tasks (Hoffman et al., 2009; Kimura et al., 2011).

In the present study, we tested whether an omega-3/DHA supplementation protocol would restore the normal topographical fine-tuning of the uncrossed retinocollicular pathway and whether omega-3/DHA supplementation would be effective during early or late postnatal development. The results showed that an early supplementation protocol was successful in reversing the abnormal effects induced by the deprivation of omega-3. A late supplementation protocol, however, was no longer effective in restoring a normal connectivity in the superior colliculus. Therefore, we suggest a nutritional critical period for omega-3 fatty acids intake during development of the visual system.

2. Results

We have previously shown that the nutritional restriction of omega-3, mainly DHA, during gestation and early post-natal life resulted in abnormally enlarged terminal fields in the rodent visual system, as a result of either a nonspecific axonal sprouting outside the main terminal zones or to a delay in the elimination of inappropriate axons during early postnatal development (de Velasco et al., 2012). Therefore, we studied the effects of the supplementation with fish oil, rich in omega-3 fatty acids on the organization of retinocollicular terminal fields in rats previously deprived of omega-3 fatty acids during gestational and early postnatal life (Fig. 1).

.Since the ipsilateral retinocollicular pathway develops during a well characterized period that spans the first 3 postnatal weeks (Serfaty et al., 2005), we studied a series of supplementation protocols to test whether we could delimitate a corresponding critical period for omega-3 supplementation.

We evaluated an omega-3 supplementation for a short period of time, from postnatal day 7–13 (PND7-13), when spontaneous activity shapes retinocollicular topography prior to eye opening (Huberman et al., 2008). The results showed that a chronic omega-3 restriction resulted in an abnormally expanded innervation density in the superior colliculus by ipsilaterally projecting retinal ganglion cells axons (Fig. 2B) in comparison to a control diet group (Fig. 2A), where well-defined and discrete clusters of terminals were confined to

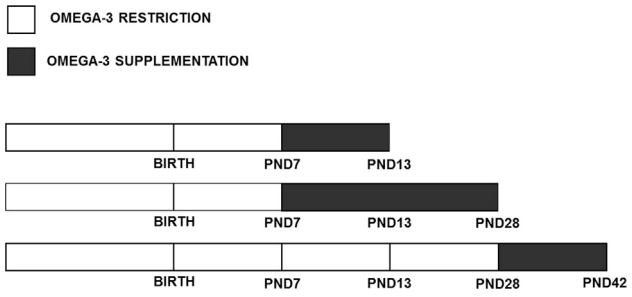

Fig. 1 – Schematic representation of nutritional omega-3 restriction and supplementation protocols. Animals under a restricted omega-3 diet (coconut oil) during 5 weeks prior to mating, received, daily, an oral supplementation with fish oil in three different stages of life: from postnatal day 7 (PND7) to PND13; from PND7 to PND28; a third group received fish oil from PND28 to PND42 ($n=4$ per group).