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Long-term reorganization of structural brain networks in a rabbit model of intrauterine growth restriction

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ABSTRACT

Characterization of brain changes produced by intrauterine growth restriction (IUGR) is among the main challenges of modern fetal medicine and pediatrics. This condition affects 5–10% of all pregnancies and is associated with a wide range of neurodevelopmental disorders. Better understanding of the brain reorganization produced by IUGR opens a window of opportunity to find potential imaging biomarkers in order to identify the infants with a high risk of having neurodevelopmental problems and apply therapies to improve their outcomes. Structural brain networks obtained from diffusion magnetic resonance imaging (MRI) is a promising tool to study brain reorganization and to be used as a biomarker of neurodevelopmental alterations. In the present study this technique is applied to a rabbit animal model of IUGR, which presents some advantages including a controlled environment and the possibility to obtain high quality MRI with long acquisition times. Using a Q-Ball diffusion model, and a previously published rabbit brain MRI atlas, structural brain networks of 15 IUGR and 14 control rabbits at 70 days of age (equivalent to pre-adolescence human age) were obtained. The analysis of graph theory features showed a decreased network infrastructure (degree and binary global efficiency) associated with IUGR condition and a set of generalized fractional anisotropy (GFA) weighted measures associated with abnormal neurobehavior. Interestingly, when assessing the brain network organization independently of network infrastructure by means of normalized networks, IUGR showed increased global and local efficiencies. We hypothesize that this effect could reflect a compensatory response to reduced infrastructure in IUGR. These results present new evidence on the long-term persistence of the brain reorganization produced by IUGR that could underlie behavioral and developmental alterations previously described. The described changes in network organization have the potential to be used as biomarkers to monitor brain changes produced by experimental therapies in IUGR animal model.

Introduction

Intrauterine growth restriction (IUGR) affects 5–10% of all pregnancies and is a major public health issue, being a prevalent condition that has been associated with a wide range of short- and long-term neurodevelopmental and cognitive dysfunctions (Arcangeli et al., 2012; Baschat, 2013), even in adulthood (Løhaugen et al., 2013). With the significant advance of magnetic resonance imaging (MRI) in the recent years, the brain alterations and reorganization underlying these neurofunctional alterations are starting to be elucidated. It has been suggested that brain reorganization starts in utero, where different patterns of cortical development (Egaña-Ugrinovic et al., 2013) and altered quantitative MRI texture predictive of altered neurodevelopment (Sanz-Cortes et al., 2013) have been shown in IUGR. At neonatal period IUGR has been reported to have decreased volume in gray matter (GM) (Tolsa et al., 2004) and hippocampus (Lodygensky et al., 2008) and discordant patterns of gyrification (Dubois et al., 2008). At one year of age, persistence of structural changes has been demonstrated, including reduced volumes of GM (Padilla et al., 2011) and decreased fractal dimension in both GM and white matter (WM) that correlate with abnormal neurodevelopment (Esteban et al., 2010). Studies on IUGR at later ages have reported changes in regional brain volumes and cortical

Abbreviations: DI, discrimination index; DWI, diffusion-weighted images; FA, fractional anisotropy; FD, fiber density; FDR, false discovery rate; GFA, generalized fractional anisotropy; GLM, general linear model; GM, gray matter; IQR, inter-quartile range; IUGR, intrauterine growth restriction; MRI, magnetic resonance imaging; OFBT, Open Field Behavioral Test; ORT, Object Recognition Task; SD, standard deviation; WM, white matter.

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thickness in 4 to 7-year-old children (De Bie et al., 2011), reduced volumes for thalamus and cerebellar white matter (Martinussen et al., 2009), and thinning of corpus callosum and general WM reduction (Skranes et al., 2005) in adolescents. There is a need to better characterize the brain reorganization underlying neurodevelopmental and cognitive dysfunctions in IUGR. Likewise, the development of imaging biomarkers is an urgent clinical and experimental need (Ment et al., 2009).

The study of brain connectivity holds great promise for the development of pathophysiological insights and biomarkers of human disease characterized by subtle brain changes that are not reflected in conventional MRI techniques (Gratacos, 2012). Indeed, one of the major recent advances in the application of new MRI modalities has been the emerging technique of "connectomics" (Hagmann, 2005), opening the possibility to extract macroscopic circuitry of the connections of the brain, in what has been called "the connectome" (Sporns et al., 2005). In particular, the use of graph theory analyses on brain networks has been demonstrated to be a useful tool to characterize brain organization by a few comprehensible parameters (Bassett and Bullmore, 2009). Different sets of data, including functional MRI and diffusion MRI, have been used to extract macroscopic brain networks and analyze network features in healthy adults, adolescents and infants (Gong et al., 2009a; Hagmann et al., 2008, 2010; Iturria-Medina et al., 2008; Yap et al., 2011) and to report altered group connectivity parameters in a wide range of neurological, neurobehavioral and neurodegenerative diseases (Alexander-Bloch et al., 2010; Liu et al., 2008; Lo et al., 2010; Shu et al., 2009, 2011; Wang et al., 2009; Wu et al., 2009). Importantly, connectomics and graph theory features have been shown to be potential tools to develop biomarkers to predict neurological outcomes in adult (He et al., 2009; Li et al., 2009; Wee et al., 2010; Wen et al., 2011) and perinatal diseases (Batalle et al., 2012, 2013; Tymofiyeva et al., 2012). Particularly, brain networks of one-year-old infants obtained from diffusion MRI have been reported to have reduced level of weighted organization and a pattern of altered regional network features that is associated with latter neurodevelopmental problems (Batalle et al., 2012), showing their potential to develop imaging biomarkers to detect infants at high risk of having neurodevelopmental problems one year later. Nonetheless, whether persistent brain reorganization produced by IUGR persists at long-term (adolescence and adult period) and whether connectomic analysis could be a suitable tool to characterize the patterns induced by this conditions are still unknown.

Assessing long-term effects of IUGR in the human brain is a challenging task, limited by the influence of uncontrolled environmental factors (Hall and Perona, 2012) and the difficulty of obtaining sufficiently large sample sizes. The induction of IUGR in rabbit models has been proven to reproduce major features of human IUGR (Bassan et al., 2000; Eixarch et al., 2009, 2011). Furthermore, white matter maturation process in rabbit is closer to humans than other species, since it starts in intrauterine period (Derrick et al., 2007). Hence, albeit their obvious limitations, rabbit model may be a useful tool to analyze long-term brain remodeling in IUGR. They could play a key role in the definition of image biomarkers for early diagnosis that are critical to demonstrate changes after the application of experimental therapies, especially when those should be tested in fetuses or neonates. Besides the highly reproducible experimental conditions, high quality MRI with long acquisition times can be performed in isolated whole brain preparations. Using this model, regional brain changes in fractional anisotropy, correlated with poorer outcome in neurobehavioral tests have been reported in newborns (Eixarch et al., 2012), some of them persisting in preadolescent period (Illa et al., 2013), where changes in the connectivity of anxiety, attention and memory networks have been shown. Due to the recent development of an MRI rabbit brain atlas (Muñoz-Moreno et al., 2013), the possibility to obtain whole brain structural networks based on diffusion MRI arises. This opens the opportunity to assess long-term network reorganization associated with functional impairments without a priori hypothesis, taking advantage of the huge potential of graph theory measures to characterize brain functioning and organization (Bassett and Bullmore, 2009) that have been previously used to characterize one-year-old infants with IUGR (Batalle et al., 2012, 2013).

In the present study, graph theory features from diffusion MRI brain networks were calculated in 15 rabbits with surgically induced IUGR and 14 controls at equivalent preadolescent age, in order to assess the long-term impact of IUGR in brain organization that could underlie behavioral and developmental alterations. The results showed a specific pattern of global network features altered in IUGR, characterized by an impaired network infrastructure, but an increase in the relative terms of organizational efficiency that we hypothesize to be associated with a compensatory effect in IUGR. An exploratory analysis of the regional features altered by IUGR condition was also performed. Both global and regional network features were associated with neurobehavioral test results. The results here presented contribute to the knowledge on long-term brain changes associated with neurobehavioral dysfunctions in IUGR, showing the feasibility of using brain network features from diffusion MRI as biomarkers to assess and potentially monitor treatment of IUGR using experimental models.

Methods

The design of the study and each of the steps of the procedures are shown in Fig. 1. A detailed description of the methodology used is included in this section.

Animals, study protocol and surgical model

Animal experimentation of this study was approved by the Animal Experimental Ethics Committee of the University of Barcelona (permit number: 206/10-5440), and all efforts were made to avoid or minimize suffering.

Part of the animals used in this study has been previously used in a recent study (Illa et al., 2013). From 13 New Zealand pregnant rabbits provided by a certified breeder, we selected two cases and two controls of each dam at birth. At the 70th postnatal day, all surviving cases and one control for each case were included resulting in a total population of 30 rabbits (15 with induced IUGR and 15 sham controls). Dams were housed for 1 week before surgery in separate cages on a reversed 12/12 h light cycle, with free access to water and standard chow. At 25 days of gestation (term at 31 days), a ligation of 40–50% of uteroplacental vessels was performed following a previously described protocol (Eixarch et al., 2009). Briefly, after midline abdominal laparotomy, the gestational sacs of both horns were counted and numbered. Afterwards, only one uterine horn was kept outside the abdomen and the induction of IUGR was performed by ligating 40–50% of the uteroplacental vessels of all the gestational sacs from this horn. After the procedure, the abdomen was closed in two layers and postoperative analgesia (meloxicam) was administered for 48 h. After surgery, the animals were allowed free access to water and standard chow for 5 days until delivery. Cesarean section was performed at 30 days of gestation and living pups were obtained. All living newborns were weighed and identified by a subcutaneous microchip inserted in their back (Microchip MUSICC, Avid Microchip S.L., Barcelona, Spain). Cases were considered those pups delivered from the ligated horn, whereas controls were those delivered from the contralateral horn (non-ligated). Both cases and controls were housed with a wet nurse rabbit with part of the offspring until the 30th postnatal day when they were weaned. Thereafter both groups of rabbits were housed in groups of three with a reversed 12/12 h light cycle with free access to water and standard chow. On the 70th postnatal day, which is considered to be equivalent to preadolescence period in humans in terms of sexual maturity (Moorman et al., 2000), functional tests were applied and the rabbits were anesthetized and sacrificed thereafter. The left and right common carotid arteries were cannulated and the brains were perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde PBS. Then,

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