ELSEVIER

NeuroImage

www.elsevier.com/locate/ynimg
NeuroImage 34 (2007) 1363 – 1374

Neuroanatomical phenotypes in the *Reeler* mouse

Alexandra Badea,[a] Peter J. Nicholls,[b] G. Allan Johnson,[a,*] and William C. Wetsel[b,c]

[a]*Center for In Vivo Microscopy, Box 3302, Duke University Medical Center, Durham, NC 27710, USA*
[b]*Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC 27710, USA*
[c]*Cell Biology, Mouse Behavioral and Neuroendocrine Analysis Core Facility, Duke University Medical Center, Durham, NC 27710, USA*

Received 6 June 2006; revised 13 September 2006; accepted 18 September 2006
Available online 20 December 2006

The *reeler* mouse (*Reln*) has been proposed as a neurodevelopmental model for certain neurological and psychiatric conditions and has been studied by qualitative histochemistry and electron microscopy. Using magnetic resonance microscopy (MRM), we have quantitated for the first time the neuromorphology of *Reln* mice at a resolution of 21.5 μm. The neuroanatomical phenotypes of heterozygous and homozygous mutant *Reln* mice were compared to those of wild type (WT) littermates using morphometry and texture analysis. The cortical, hippocampal, and cerebellar phenotypes of the heterozygous and homozygous mutant *Reln* mice were confirmed, and new features were revealed. The $Reln^{rl/rl}$ mice possessed a smaller brain, and both $Reln^{rl/+}$ and $Reln^{rl/rl}$ mice had increased ventricles compared to WT controls. Shape differences were found between WT and $Reln^{rl/rl}$ brains, specifically in cerebellum, olfactory bulbs, dorsomedial frontal and parietal cortex, certain regions of temporal and occipital lobes, as well as in the lateral ventricles and ventral hippocampus. These findings suggest that certain brain regions may be more severely impacted by the *Reln* mutation than others. Gadolinium-based active staining demonstrated that layers of the hippocampus were disorganized in $Reln^{rl/rl}$ mice and differences in thickness of these layers were identified between WT and $Reln^{rl/rl}$ mice. The intensity distributions characteristic to the dorsal, middle, and ventral hippocampus were altered in the $Reln^{rl/rl}$, especially in the ventral hippocampus. These differences were quantified using skewness and modeling the intensity distributions with a Gaussian mixture. Our results suggest that structural features of $Reln^{rl/rl}$ brain most closely phenocopy those of patients with Norman–Roberts lissencephaly.

Keywords: Magnetic resonance microscopy; Reelin; Brain; Hippocampus; Ventricles; Cerebellum

Introduction

Diagnosis of neurological and psychiatric conditions is often made according to phenomenological or behavioral criteria (American Psychiatric Association, 2000; Rowland, 2005). Postmortem studies have identified structural features in the central nervous system (CNS) that are characteristic for many neurological syndromes and the approach is somewhat successful for certain psychiatric conditions, such as schizophrenia (Roberts et al., 2005; Rowland, 2005). Unfortunately, this analysis is applicable only to deceased individuals and it cannot be used in the initial diagnosis. For this reason, investigators are combining clinical assessment with magnetic resonance imaging (MRI) and/or functional MRI to render a more concrete diagnosis of the disorder (McCarley, 2002). Although this approach has the potential to advance identification and improve the accuracy of diagnoses, in most cases it has not revealed basic mechanisms that underlie the condition so that therapies can be developed to more efficiently ameliorate the severity or expression of the syndrome. Despite this limitation, some researchers are beginning to identify genes and gene products that may underlie structural and/or behavioral features that accompany certain neurological and psychiatric conditions (Katsel et al., 2005).

Alterations in cytoarchitecture of brain may be attributed to sequelae that occur during development and/or maturation of the CNS (Rowland, 2005). Although neurodevelopmental events are often attributed to epilepsy and deficits in learning and memory, they may also underlie autism, schizophrenia, and certain neurological conditions that include hydrocephaly (Rowland, 2005). While investigators are identifying candidate or collections of genes that may predispose a given patient to present a certain syndrome, other scientists are using this information to study pre-existing mutants or to develop genetically modified animals to model neurodevelopmental morphological features of the condition.

Reln is a mutation that arose spontaneously in mice that displayed ataxia, hypotonia, and fine tremor (Falconer, 1951). Subsequently, reelin was found to be an extracellular matrix glycoprotein that is secreted by certain neurons (D'Arcangelo et al., 1995; Ogawa et al., 1995), such as Cajal–Retzius cells in the cortical marginal zones and granule cells in cerebellum (Tissir and Goffinet, 2003). Reelin appears to act as a stop signal for migrating

* Corresponding author. Fax: +1 919 684 7158.
E-mail address: gaj@orion.duhs.duke.edu (G.A. Johnson).
Available online on ScienceDirect (www.sciencedirect.com).

1053-8119/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2006.09.053

neurons where it seems to control the positioning and orientation of neurons in their respective target layers. Hence, it participates in laminar development and development of architectonic structures within the CNS (Landrieu and Goffinet, 1981; Goffinet et al., 1984; Del Rio et al., 1997; Förster et al., 2006a,b). As a result, *Reln* mice display abnormalities in neural migration that lead to distortions in the dendritic trees and axonal projections, especially in cerebral cortex, hippocampus, and cerebellum. Since these neurodevelopmental alterations bear similarities to certain aspects of those in schizophrenia, autism, and lissencephaly, $Reln^{rl/+}$ and $Reln^{rl/rl}$ mice have been proposed to represent preclinical structural models for these conditions (Fatemi, 2001).

To date, *Reln* brain has been examined by various neuroanatomical procedures (Caviness and Sidman, 1973; Mikoshiba et al., 1980). These traditional methods are prone to distortion, can cause tissue damage, are typically qualitative, and do not preserve the three-dimensional (3D) spatial relationships between anatomical structures. We have analyzed *Reln* brain using magnetic resonance microscopy (MRM). This procedure obviates many concerns of traditional methods and the sensitivity of the technique is sufficient to detect changes within 0.03% in mouse brain volume (Cyr et al., 2005). In the present study, we have used MRM with a resolution as high as 21.5 μm to assess morphometric alterations in *Reln* brain.

Materials and methods

Animal preparation and imaging protocol

The *Reln* mutation originally arose spontaneously in mice (Falconer, 1951). Male and female adult WT, heterozygous, and homozygous mutant *Reln* mice (Jackson Laboratory, Bar Harbor, Maine, USA) were used for this study. The animals were approximately 5 months of age at the time of investigation. Mice were housed in groups of 3–5 animals/cage in a temperature- and humidity-controlled room with a 14:10 light–dark cycle (lights on at 0700 h). Food and water were provided *ad libitum*. All experiments were conducted in accordance with NIH guidelines for the care and use of animals and with approved animal protocols from the Duke University Institutional Animal Care and Use Committee.

In preparation for morphology studies, mice were anesthetized with 100 mg/kg pentobarbital (i.p.) and transcardially perfused with a solution of 10% formalin and 10% ProHance (Bracco Diagnostics, Princeton, NJ) as described by Johnson and colleagues (2002). Heads were stored overnight in formalin and scanned the next day. The brains were placed in fomblin-filled tubes and were scanned in the skull to avoid distortions of brain or damage to tissue due to handling.

All imaging was performed on a 9.4-T Oxford vertical bore magnet with a GE EXCITE Console (EPIC 11.0) that has been specially adapted for MRM. Images were acquired with a radiofrequency refocused 3D spin warp sequence with TE/TR of 5.1/50 ms, a field of view of 22×11×11 mm, and zero filling was used, resulting in a 1024×512×512 matrix and an isotropic resolution of 21.5 μm. The sequence used non-uniform radial gain and asymmetric data collection resulting in a scan time of 2 h and 7 min per brain (Johnson, in preparation).

Manual segmentation

The brains were manually segmented for ventricles, hippocampus, and cerebellum using Voxstation (MRPath, Durham, NC). Only one plane of orientation was used for segmenting a specific structure; however, a 3D viewer was used to assess the accuracy of segmentation in all three cardinal planes (MacKenzie-Graham et al., 2004). The ventricles were segmented as one system comprising the lateral, third, and fourth ventricles in sagittal slices. Manual segmentation of the ventricles was facilitated by the high contrast that cerebrospinal fluid has in the MRM images, but it was made difficult, especially in WT and heterozygous mice by their thin structure. In several places, an arbitrary cut was required to separate the ventricles from clefts in the brain filled with fluid.

The hippocampus was segmented in coronal slices starting anteriorly at the level of the hippocampal commissure using the mouse brain atlas of Paxinos and Franklin (2001) as a guide. The posterior limit was established with the help of the sagittal and horizontal planes, at the level of the ventral nucleus of the lateral lemniscus. In general, the in-plane borders were established using high contrasting white matter tracts like the corpus callosum (external capsule) or alveus for the lateral side, the brachium of the superior colliculus, optic tract, or cerebral peduncles on the medial side. The ventricles provided high contrast for determining the lateral borders of the hippocampus. Aside from hippocampus, the cerebellum was segmented in two mid-sagittal slices in all brains to show the dramatic change in size of the $Reln^{rl/rl}$ brain compared to that in the WT and $Reln^{rl/+}$ mice.

Layer thickness measurements

Cortical layer measurements were performed in coronal slices at approximately −2.1 mm from bregma and in the region of the primary somatosensory cortex (Hof et al., 2000). Additionally, hippocampal length, from its upper medial to the most distal lateral aspect, and the thickness of hippocampal layers were measured in the same slice.

Image preprocessing

Brain volumes were obtained after stripping the skull using an algorithm based on mathematical morphology and connected component analysis similar to the one described by Badea et al. (2003). To increase the time efficiency of the procedure, the image data were down-sampled by a factor of two along each dimension at this step. The algorithm started with smoothing the data, and applying a number of erosions. A seed was selected automatically in the center of the volume, and region growing was started from that seed. Pixels with values between fixed thresholds and connected to the initial region were added to the brain mask. At the end, dilation was applied for the same number of times as we applied erosion. Empirically, we established that the optimal number of erosions/dilations to be used was 6 for our data, which come as a 512×256×256 matrix.

Image intensity normalization was performed in the following way. The lowest 5% and the highest 2% of the intensity values were discarded and assigned as the minimum (I_{Min}) and maximum (I_{Max}) image values. The original values (I) were mapped to I' after scaling to the same global range of image values ($I_{\mathrm{GlobalMax}}$ and $I_{\mathrm{GlobalMin}}$):

$$I' = (I - I_{\mathrm{Min}}) * \frac{I_{\mathrm{GlobalMax}} - I_{\mathrm{GlobalMin}}}{I_{\mathrm{Max}} - I_{\mathrm{Min}}} + I_{\mathrm{Min}}.$$