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COMPARTMENTATION OF THE REELER CEREBELLUM: SEGREGATION AND OVERLAP OF SPINOCEREBELLAR AND SECONDARY VESTIBULOCEREBELLAR FIBERS AND THEIR TARGET CELLS

J. VIG,[a] D. GOLDOWITZ,[b] D. A. STEINDLER[c] AND L. M. EISENMAN[d]*

[a]*Neurobiology Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest H-1094, Hungary*

[b]*Department of Anatomy and Neurobiology, University of Tennessee, Medical College, Memphis, TN 38163, USA*

[c]*Department of Neuroscience, University of Florida, Gainsville, FL 32611, USA*

[d]*Department of Pathology, Anatomy and Cell Biology, Jefferson Medical College, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107, USA*

Abstract—The cerebellum of the reeler mutant mouse has an abnormal organization; its single lobule is composed of a severely hypogranular cortex and a central cerebellar mass (CCM) consisting of Purkinje cell clusters intermixing with the cerebellar nuclei. As such the reeler represents an excellent model in which to examine the effect of the abnormal distribution of cerebellar cells on afferent-target relationships. To this effect we studied the organization of the spinocerebellar and secondary vestibulocerebellar afferent projections in homozygous reeler mice (rl/rl) using anterograde tracing techniques. Spinal cord injections resulted in labeled spinocerebellar mossy fiber rosettes in specific anterior and posterior regions of the cerebellar cortex. Some vestiges of parasagittal organization may be present in the anterior projection area. Within the CCM, labeled fibers appeared to terminate on distinct groups of Purkinje cells. Thus, the spinocerebellar mossy fibers seem to form both normal and heterologous synapses in the reeler cerebellum. Secondary vestibular injections resulted in both retrograde and anterograde labeling. Retrograde labeling was seen in clusters of Purkinje cells and cerebellar nuclear cells; anterograde labeling was distributed in the white matter and in specific regions of the anterior and posterior cortex of the cerebellum. The labeled spinocerebellar and secondary vestibulocerebellar afferents overlapped in the anterior region but in the posterior region the vestibulocerebellar termination area was ventral to the spinocerebellar area. An area devoid of labeled terminals was also observed ventral to the posterior secondary vestibulocerebellar termination field. Using calretinin immunostaining it was determined that this area contains unipolar brush cells, a cell type found primarily in the vestibulocerebellum of normal mice.

Our data indicate that despite of the lack of known landmarks (fissures, lobules) the spinocerebellar and vestibulocerebellar afferent projections in the reeler cerebellum do not distribute randomly but have specific target regions, and the position of these regions, relative to each other, appears to be conserved. Two caveats to this were the finding of overlapping terminal fields of these afferents in the anterior region, and a posteroventral region that contains unipolar brush cells yet is devoid of secondary vestibulocerebellar afferents. The distribution of Purkinje cells and cerebellar nuclear cells is not random either; those that give rise to cerebellovestibular efferents form distinct groups within the central cerebellar mass.

Key words: reeler, spinocerebellar afferent, vestibulocerebellar afferent, Purkinje cells, unipolar brush cells, vestibular nuclei.

The cerebellum has two major afferent systems: the olivocerebellar climbing fiber projection and the mossy fiber projections. In the present study we examine two different mossy fiber projections, the spinocerebellar (sc) and the secondary vestibulocerebellar afferents (svc) in the reeler mutant mouse. In the normal mouse sc afferents form a precise topographic map within the cerebellum (Ito, 1984; Gravel and Hawkes, 1990; Sotelo and Wassef, 1991). These fibers project to specific lobules of the anterior and posterior lobe (lobules II–V, and VIII–IX, respectively) where they segregate to parasagittal bands. Terminal fields are ordered with respect to biochemically different Purkinje cell compartments (Gravel and Hawkes, 1990; Ji and Hawkes, 1994; Ji et al., 1997). The arrival of sc mossy fibers occurs early during development [embryonic day 13/14 (E13/14) in the mouse; Grishkat and Eisenman, 1995] and precedes the development of their final cortical postsynaptic targets, the granule cells (Arsenio-Nunes and Sotelo, 1985). Vestibulocerebellar projections consist of primary and secondary vestibulocerebellar fibers (pvc and svc, respectively) and each terminates in discrete areas of the cerebellum. Pvc project to the ipsilateral nodulus and ventral uvula whereas svc from the vestibular nuclei project bilaterally to the nodulus, ventral uvula, flocculus and paraflocculus, and to the ventral part of the anterior lobe (Voogd et al., 1996). The pvc afferents are the earliest to arrive during development [E13 in the rat; Ashwell and Zhang, 1992, 1998]. There is some very recent evidence that the pvc projection may exhibit mediolateral periodicity, at least regarding canal afferents (Maklad and Fritzsch,

*Corresponding author. Tel: +1-215-503-1686; fax: +1-215-923-3808.
E-mail address: leonard.eisenman@mail.tju.edu. (L. M. Eisenman).

Abbreviations: CCM, central cerebellar mass; ECM, extracellular matrix; IGL, internal granular layer; IVN, inferior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; NHS, normal horse serum; PBS, phosphate buffer saline; pvc, primary vestibulocerebellar; rl/rl, homozygous reeler; sc, spinocerebellar afferents; svc, secondary vestibulocerebellar afferents; UBC, unipolar brush cell; WGA-HRP, wheat germ agglutinin–horseradish peroxidase.

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2003). Secondary vestibular afferents also appear to exhibit some degree of clustering in the medio-lateral plane in the nodulus and ventral uvula (Thunnissen et al., 1989). The efferent projection from Purkinje cells and nuclear cells to the vestibular system arises from distinct parasagittal zones of Purkinje cells that project to different vestibular nuclei (Voogd et al., 1996).

Numerous cerebellar mouse mutants have been used to study the effects of different target abnormalities on the organization of sc mossy fiber projections [weaver (Arsenio-Nunes et al., 1988); meander tail (Eisenman and Arlinghaus, 1991); lurcher (Vogel and Prittie, 1994); staggerer (Ji et al., 1997)]. These studies have suggested that both Purkinje cells and granule cells are involved in organizing this afferent system. Purkinje cells appear to have an important role in establishing the gross topography by forming temporary connections with mossy fibers parentally (Mason and Gregory, 1984). Granule cells, the final postsynaptic targets, are suggested to be required for the secondary refinement that occurs as a result of an activity-dependent process (Tolbert et al., 1994; Ji and Hawkes, 1996). In the present work we studied the organization of sc and svc projections in adult homozygous reeler mice (rl/rl).

The reeler mouse (Falconer, 1951) shows an abnormal organization of all laminated cerebral structures, i.e. the cerebral cortex, the hippocampus and the cerebellum, and some non-cortical structures such as the inferior olive, the facial nucleus and other brainstem nuclei (Caviness and Rakič, 1978; Goffinet, 1984). The reeler cerebellum is greatly reduced in size and has an abnormal gross morphology consisting of a single cerebellar lobule (Goffinet et al., 1984). There is an extensive reduction in the number of granule cells (Mariani et al., 1977) and Purkinje cells (Heckroth et al., 1989). The mutant phenotype is due to an autosomal recessive mutation (Caviness and Rakič, 1978) affecting the reelin gene (Hirotsune et al., 1995; D'Arcangelo et al., 1995). The product of this gene, the reelin protein [recognized by the CR-50 monoclonal antibody (D'Arcangelo et al., 1997; Curran and D'Arcangelo, 1998)] is a glycoprotein of the extracellular matrix (ECM), expressed in a temporal and spatial pattern during development (D'Arcangelo et al., 1997; Alcántara et al., 1998). The presumed function of the reelin protein is to arrest the migration of neural cells (e.g. Purkinje cells) by enabling their detachment from radial glia (Yuasa et al., 1993; Dulabon et al., 2000). In the developing cerebellum the reelin gene is expressed in deep nuclear cells, before their outward migration from the ventricular epithelium between E13 and P14 (Miyata et al., 1996), and in the external granular layer inducing the linear alignment of Purkinje cells along that zone. Premigratory Purkinje cells may already be responsive to reelin (Miyata et al., 1997).

The reeler mutant mouse has been a useful model system to study the establishment of afferent-target connections in various parts of the mammalian brain [cerebellum (Wilson et al., 1981; Goffinet et al., 1984; Blatt and Eisenman, 1988); hippocampus (Borrel et al., 1999; Deller et al., 1999a,b); visual system (Simmons and Pearlman, 1982; Simmons et al., 1982; Schiffmann et al., 1997; Rice et al., 2001); thalamocortical afferents (Yuasa et al., 1994)]. The purpose of this study was to examine the effects of the abnormal cerebellar cytoarchitecture in the reeler mutant mouse on the distribution and organization of the sc and svc mossy fiber projections.

EXPERIMENTAL PROCEDURES

Adult rl/rl from the B6C3Fe a/a-Reln<rl>/J strain were used for this study (n=29 animals). The animals were obtained from a colony maintained at Thomas Jefferson University Central Animal Facility. The colony was developed from animals obtained from Jackson Laboratories (Bar Harbor, ME, USA). The experiments in this study were conducted so as to ensure that animals did not experience more than momentary pain or discomfort. The Institutional Animal Care and Use Committee of Thomas Jefferson University reviewed and approved the experiments described in this report.

Spinal cord and vestibular injections

The animals were anesthetized with Avertin (20 ml/kg). Surgery was performed to expose a small region of the lumbar spinal cord. After visualizing the spinal cord, 0.03–0.04 μl of 4% (w/v) wheat germ agglutinin–horseradish peroxidase (WGA-HRP; Vector, Burlingame, CA, USA) was injected into the lumbar spinal cord (20 animals). In other animals, surgery was performed on the posterior aspect of the head to expose the dorsal aspect of the medulla and the posterior aspect of the cerebellum. Using this approach, injections were made into the vestibular nuclei (four animals). Three days after spinal cord injections and 24 h after vestibular nuclei injections, the animals were deeply anesthetized with sodium pentobarbital (60 mg/kg) and transcardially perfused with normal saline followed by 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2–7.4), and postfixed in the same fixative omitting the glutaraldehyde for 1–2 h. The brain (vestibular nuclei injections) or the brain and spinal cord (spinal cord injections) were dissected from each animal and stored at 4 °C overnight in phosphate buffer saline (PBS; pH 7.4) containing 30% sucrose. The brainstems and cerebella were frozen sectioned at 40 μm in the coronal or sagittal plane and collected in PBS. The spinal cords were sectioned at 40 μm in the horizontal plane. Every third section was processed for the visualization of HRP using tetramethylbenzidine as the chromogen (Mesulam, 1978) and mounted on gelatin-coated slides. After air-drying, coverslips were applied with Permount. An adjacent series of sections was processed similarly and counterstained with Neutral Red.

Calbindin and calretinin immunostaining

Adult reelers (n=5) were used for calbindin and calretinin immunohistochemistry. Each animal was deeply anesthetized with Avertin and perfused transcardially with normal saline followed by Bouin's fixative. The brains were dissected, blocked, and postfixed overnight in the same fixative. The blocks were then dehydrated in increasing concentrations of ethanol (70% overnight, 95% overnight, two changes of 100% for 1 and 2 h, respectively). After immersion in ethanol–toluene mixture (100% ethanol–toluene, 1:1) for 30 min and in toluene for 1.5 h the blocks were embedded in paraffin. Serial 10 μm coronal or parasagittal sections were cut using a rotary microtome, and selected sections were mounted onto gelatin-coated slides. After deparaffinization and rehydration these mounted sections were processed for immunocytochemistry according to the following protocol. After rinsing in PBS for 3×5 min sections were incubated in 10% normal horse serum (NHS) in PBS for 30 min to block nonspecific protein binding sites in the tissue. The tissue was then incubated with the primary antibody [anti-calbindin D-28K,