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Behavioral effects of neonatal lesions on the cerebellar system

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ABSTRACT

Several rodent models with spontaneous mutations causing cerebellar pathology are impaired in motor functions during the neonatal period, including *Grid2*^{Lc}, *Rora*^{sg}, *Dab1*^{scm}, *Girk2*^{Wv}, *Lmx1a*$^{dr-sst}$, *Myo5a*^{dn}, *Inpp4a*^{wbl}, and *Cacna1a*^{rol} mice as well as *shaker* and *dystonic* rats. Deficits are also evident in murine null mutants such as *Zic1*, *Fgfr1*/*FgFr2*, and *Xpa*/*Ercc8*. Behavioral deficits are time-dependent following X-irradiated- or aspiration-induced lesions of the cerebellum in rats. In addition, motor functions are deficient after lesions in cerebellar-related pathways. As in animal subjects, sensorimotor disturbances have been described in children with cerebellar lesions. These results underline the importance of the cerebellum and its connections in the development of motor functions.

1. Specificity of cell growth in the cerebellum

The effects of lesions of the cerebellum or associated brain regions during the neonatal period in rodents (from the day of birth to weaning on postnatal day 21) provide information as to the functional impact of cell growth processes. Various behavioral effects are obtained depending on the postnatal day the lesions were made or their effects evaluated. In the rat, the cerebellar deep nuclei, Purkinje cells, and Golgi cells are formed on embryonic days 13 and 14, 14 and 15, and 19 up to the perinatal period, respectively (Altman and Bayer, 1978). Granule, basket, and stellate cells of the cerebellar cortex are formed from birth until day 21, on postnatal days 7 and 8, and on postnatal days 8–11, respectively (Altman, 1969, 1972). Besides cell birth, a possible source of behavioral variability in lesion effects is the change occurring regarding synaptic contacts. For example, rat Purkinje cells are polyinnervated by climbing fibers up to postnatal day 15 but then become mostly monoinnervated (Delhaye-Bouchaud et al., 1978; Mariani et al., 1990). Manto and Jissendi (2012) reviewed the molecular aspects of cerebellar development, including the importance of the *Math1* gene, encoding a transcription factor crucial in the development of glutamatergic neurons, as well as *Ptf1a*, encoding a transcription factor crucial in the development of neurons containing gamma-aminobutyric acid (GABA).

2. Behavioral effects of neonatal cerebellar lesions in rodents

2.1. Spontaneous mutants

2.1.1. Grid2^{Lc} mutant mouse

The neuropathology of the autosomal semi-dominant *Lurcher* mutation is caused by a gain-in-malfunction of the *Grid2* gene located on chromosome 6 and encoding an ionotropic glutamate receptor predominantly expressed on Purkinje cells (Zuo et al., 1997). While homozygous *Grid2*^{Lc} mutants die early because of defective suckling caused by brainstem damage (Resibois et al., 1997), the heterozygous can be evaluated throughout development and also as adults. The increased permeability of the mutated glutamate channel to calcium (Wollmuth et al., 2000) is likely

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responsible for the nearly complete degeneration of Purkinje cells occurring from the second to the fourth postnatal week (Caddy and Biscoe, 1979), at a time when such cells develop dendritic arbors and spines for synaptic contacts (Hatten and Heintz, 1995). The massive degeneration of granule cells is attributed to the loss in the trophic influence exerted by Purkinje cells on them (Vogel et al., 1991). Likewise, the 60–75% decrease in inferior olive cell number (Caddy and Biscoe, 1979; Heckroth and Eisenman, 1991) and the 30% decrease in deep cerebellar nuclei number (Heckroth, 1994) are secondary consequences of Purkinje cell atrophy, causing retrograde and anterograde degeneration, respectively. The overall neuropathology leads to early-onset cerebellar ataxia and deficits in motor coordination (Lalonde et al., 1996).

Grid2^{Lc} mutants were evaluated from postnatal days 0 (day of birth) to 30 in the following tests: body righting from a supine position on the back (days 0–30), negative geotropism up an inclined grid (days 0–15), motor coordination on rotating grid (days 11–14), wire suspension (days 6–16), and rotorod (days 0–30) tests, as well as swimming toward and grabbing a vertical pole (days 22–30) (Thullier et al., 1997). Wire suspension began on day 6 since the start of a potent grasping response was seen in normal mice only on day 7 (Fox, 1965). Moreover, swimming toward a vertical pole cannot reasonably be started before the eyes open on day 11. Relative to wild-type, body righting and negative geotropism were observed at their normal times in *Grid2*^{Lc} mutants. Indeed, body righting became prominent in both groups on day 5, as noted previously in normal mice of another strain (Fox, 1965). However, the mutants took longer to right themselves from days 13 to 30. In contrast, the mutants turned upward more quickly than controls on the inclined grid, presumably because of a lack in postural stability. Indeed, the mutants showed poorer motor coordination in all three tests: rotating grid (days 11–14), wire suspension (days 15 and 16), and rotorod (days 14–30). A summary of these results is provided in Table 1. As Dufour-Mallet et al. (1979) noted in mice and Bâ and Seri (1995) in rats, latencies before falling from each apparatus increased sharply in our wild-type strain. Relative to wild-type, the mutants also had slower visuomotor responses before swimming to the vertical pole throughout the testing period.

Table 1
Behavioral characteristics of cerebellar mutants.

Mutant and nature of cerebellar cell loss	Behavioral characteristics
Grid2^{Lc} mutant mouse Purkinje and granule cells	Ataxia, impaired body righting and motor coordination
Rora^{sg} mutant mouse Purkinje and granule cells	Ataxia, impaired body righting and motor coordination
Dab1^{scm} mutant mouse Granule cells	Ataxia, impaired drop-righting, negative geotaxis, and motor coordination
Girk2^{Wv} mutant mouse Granule cells	Ataxia, impaired swimming and grooming
Lmx1a$^{dr\text{-}sst}$ mutant mouse Irregular pattern of foliation	Ataxia, impaired body righting and motor coordination
Myo5a^{dn} mutant mouse Smaller with intact foliation pattern	Ataxia, impaired eyeblink conditioning and motor coordination
Inpp4a^{wbl} mutant mouse Purkinje cells	Ataxia
Cacna1a^{rol} mutant mouse Reduced calcium channel voltage	Ataxia, impaired body righting, negative geotaxis, and motor coordination
Shaker mutant rat Purkinje cells	Ataxia, impaired body righting
Dystonic mutant rat Increased noradrenergic levels	Ataxia, impaired motor coordination
Zic1 null mutant Hypoplasia and missing anterior lobe	Ataxia
Fgfr1/*FgFr2* double null mutant Dispersed granule cells	Ataxia, impaired motor coordination
Xpa/*Ercc8* double null mutant Impaired foliation	Ataxia

2.1.2. Rora^{sg} *mutant mouse*

The autosomal recessive *staggerer* mutation causes a deletion of the *Rora* gene located on chromosome 9 (Hamilton et al., 1996). This gene encodes retinoic acid-related orphan receptor, a transcription factor involved in neuronal differentiation and maturation, highly expressed in Purkinje cells (Hamilton et al., 1996; Ino, 2004; Nakagawa et al., 1997; Sashihara et al., 1996), and belonging to the steroid/thyroid hormone receptor superfamily (Koibuchi, 2008, 2013). In *Rora*^{sg} homozygotes, Purkinje cells declined in number before postnatal day 5 and, at the end of the first month, only 25% of them remained (Herrup and Mullen, 1979). Thus, the Purkinje cell loss begins earlier than *Grid2*^{Lc} mutants (postnatal day 8) but is less complete (Caddy and Biscoe, 1979). Because of its earlier start, the *Rora*^{sg} + *Grid2*^{Lc} double mutant possesses the *Rora*^{sg} phenotype in terms of cell degeneration (Messer et al., 1991). The granule cell loss in *Rora*^{sg} mutants is secondary to Purkinje cell degeneration, begins soon after their migration (Herrup, 1983), and is nearly total by the end of the first postnatal month (Landis and Sidman, 1978). Despite the Purkinje cell loss, the deep cerebellar nuclei appear present in normal numbers (Roffler-Tarlov and Herrup, 1981). However, presumably because of Purkinje cell loss, the number of inferior olive neurons decreased by 60% as early as postnatal day 24 (Shojaeian et al., 1985). Unlike the one-to-one contact seen in normal mice, Purkinje cells are multiply innervated by climbing fibers in the mutant, a sign of developmental arrest (Mariani, 1982). The overall neuropathology leads to early-onset cerebellar ataxia and deficits in motor coordination (Lalonde et al., 1996).

Rora^{sg} mutants were compared to non-ataxic controls from postnatal days 1 to 9 in body righting and cliff aversion tests (Heuzé et al., 1997). The body righting of *Rora*^{sg} mutants was slower than that of controls on postnatal days 7–9. Although *Grid2*^{Lc} mutants also showed slower body righting responses, this occurred later in development, from postnatal days 13 to 30 (Thullier et al., 1997), presumably because of their later onset of Purkinje cell degeneration (Messer et al., 1991). In addition, more *Rora*^{sg} mutants fell off a cliff on postnatal day 7, probably as a result of motor instability. Male mutants were next evaluated for motor control involved in mating during the adult period after receiving vestibular stimulation from postnatal days 1 to 21 (Guastavino et al., 1993). Mouse pups placed on a tilting turntable from 5 min to 30 min per day were better able to mate than non-stimulated controls.

2.1.3. Dab1^{scm} *mutant mouse*

The autosomal recessive *scrambler* mouse is mutated for the *Dab1* gene located on chromosome 4, which causes a deficiency in disabled-1, involved in reelin signaling (Rice et al., 1998; Sweet et al., 1996). As a result, homozygous *Dab1*^{scm} mutants possess a loss-of-function reeler-like phenotype characterized by cell malposition in cerebellar cortex, hippocampus, and neocortex (Gonzalez et al., 1997; Rice et al., 1998; Sheldon et al., 1997; Sweet et al., 1996; Weiss et al., 2003). As seen in the previous mutants, Purkinje and granule cell degeneration in *Dab1*^{scm} mutants results in early-onset ataxia and deficits in motor coordination (Jacquelin et al., 2012).

Dab1^{scm} mutants were compared to non-ataxic controls in a neurologic screen, the SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment (SHIRPA) from the day of birth to postnatal day 22 (Jacquelin et al., 2012). An abnormal gait and body tremors were detected as early as postnatal day 8. On day 15, negative geotaxic responses were incomplete and slow, the drop righting reflex was impaired, and motor coordination was