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Research Report

The Lurcher mouse: Fresh insights from an old mutant

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ABSTRACT

The Lurcher mouse was first discovered in 1954 as a spontaneously occurring autosomal dominant mutation that caused the degeneration of virtually all cerebellar Purkinje cells and most olivary neurons and granule cells. More recent molecular studies revealed that Lurcher is a gain of function mutation in the δ2 glutamate receptor (GluRδ2) that converts an alanine to threonine in the highly conserved third hydrophobic segment of GluRδ2. The mutation converts the receptor into a constitutively leaky cation channel. The GluRδ2 receptor is predominantly expressed in cerebellar Purkinje cells and in the heterozygous Lurcher mutant (+/Lc). Purkinje cells die due to the mutation in the GluRδ2 receptor, while olivary neurons and granule cells degenerate due to the loss of their Purkinje cell targets. The purpose of the review is to provide highlights from 5 decades of research on the Lurcher mutant that have provided insights into the developmental mechanisms that regulate cell number during development, cerebellar pattern formation, cerebellar physiology, and the role of the cerebellum in CNS function.

1. Introduction

The Lurcher mouse (+/Lc; gene symbol, Grid2^{Lc}) is a neurological mutant characterized by a wobbly, lurching gait which is caused by the extensive postnatal degeneration of key neurons in the olivocerebellar circuit, principally, the Purkinje cells and their primary afferents, granule cells and olivary neurons. The +/Lc mutant was discovered as a spontaneous mutant in the mouse colony of the Medical Research Council Radiobiological Research Unit at Harwell, England in 1954. The first description of the mutant was published in 1960 by R.J.S. Philips (Phillips, 1960). In addition to describing the ataxic characteristics of the Lurcher mutant, Dr. Philips showed that the mutated gene was on chromosome 6 and the mutation was semi-dominant, with homozygous mutants dying around birth. Heterozygous animals are viable, and, since 1960, the +/Lc mutants have provided a fertile source for a large variety of studies aimed at understanding CNS function, from development to behavior. The identification of the gene that is mutated in Lurcher, the δ2 glutamate receptor (GluRδ2; gene

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symbol, Grid2), in 1997 (Zuo et al., 1997), has inspired a fresh round of studies of the Lurcher mutation that are providing new insights into how the CNS functions, from the molecular biology of glutamate neurotransmitter receptors to the pathways of neuronal cell death and survival. The goal of this review is to highlight the role that the Lurcher mutant has played in a wide array of anatomical, physiological, behavioral, and molecular studies of the CNS, and, hopefully, to indicate promising future research directions that depend on the Lurcher mutant.

2. Early descriptions of the mutant

Once the +/Lc mutant was identified, initial studies quickly focused on the obvious degeneration of the cerebellum as a primary site of the genetic lesion in the heterozygous +/Lc mutants. The early histological studies determined that, in the adult +/Lc mutants, the cytoarchitecture of the cerebellum was severely disrupted with the loss of virtually all Purkinje cells and the vast majority of the granule cells and olivary neurons (Fig. 1; Caddy and Biscoe, 1975; Caddy and Biscow, 1976; Wilson, 1975, 1976). Swisher and Wilson's (1977) qualitative analysis of the postnatal development of the +/Lc mutant established that cerebellar development begins normally in the +/Lc mutant, but signs of Purkinje cell abnormalities and pyknotic cells in the molecular layer are apparent by 3–4 days after birth (P3–4). Depending on the lobule, some Purkinje cells appear necrotic by P4, and there are gaps in the Purkinje cell layer by P5, suggesting that Purkinje cells have started to degenerate. The most comprehensive analysis of the +/Lc mutant was published by K.W.T. Caddy and T.J. Biscoe in 1979 in the *Philosophical Transactions of the Royal Society of London Series B* (Caddy and Biscoe, 1979). In this major developmental study, Caddy and Biscoe qualitatively and quantitatively described the process of neurodegeneration of the cerebellar Purkinje cells, granule cells, and olivary neurons in the +/Lc cerebellum. Their study showed that reductions in +/Lc Purkinje cell numbers can be detected between P8 and P10 closely followed by the death of granule cells and olivary neurons. Virtually all of the Purkinje cells have degenerated by 3 months after birth, while eventually 90% of the granule cells and 75% of the olivary neurons degenerate. There is, however, no loss of deep cerebellar neurons (or a limited loss of ~20%, see Heckroth, 1994). On the basis of Golgi staining and electron microscopy, Caddy and Biscoe showed that many +/Lc Purkinje cells have an abnormal, stunted morphology with multiple primary dendrites, although, depending on the age of the mouse, some +/Lc Purkinje cells can appear normal. Somatic spines on the +/Lc Purkinje cells persist longer that in the wild type. At the ultrastructural level, many of the +/Lc Purkinje cells that are obviously degenerating show evidence of disorganized cytoplasm with free ribosomes and spherical mitochondria that fill the dendrites.

A subsequent Golgi and ultrastructural study of Purkinje cells in young +/Lc mutants by Dumesnil-Bousez and Sotelo confirmed and extended these early findings (Dumesnil-Bousez and Sotelo, 1992). +/Lc Purkinje cell abnormalities become apparent by P8: these abnormalities include perinuclear clumps of chromatin in the nuclei, a delay in the development of the cell body and dendrites, and the formation of axonal swellings on Purkinje cell dendrites in the white matter tracts. The external granule cell layer is also smaller at P8. However, synaptogenesis between Purkinje cells and parallel fibers, climbing fibers, and basket cell axons appears to proceed normally, at least through P10. After P10, the rate of parallel fiber synaptogenesis declines compared to wild type, few climbing fibers translocate from their initial soma contacts to their peridendritic locations (see also Heckroth et al., 1990), and basket cell axons fail to completely surround the Purkinje cell bodies.

In contrast to the multiple defects in +/Lc Purkinje cells, other cerebellar interneurons, including olivary neurons, appear to have a normal ultrastructure until they degenerate (Caddy and Biscoe, 1979). The number of granule cells and olivary neurons is normal in +/Lc mutants until approximately P8, when their numbers fail to keep pace with wild-type numbers and then decline to approximately 10% and 25% of wild-type values, respectively. The number of stellate and basket cells was not quantified until recently (Zanjani et al., 2002) where it was found that the density of stellate and basket cells was the same as in the wild-type molecular layer, indicating that there must be extensive loss of molecular layer interneurons since the volume of the molecular layer is reduced in the +/Lc mutant. The number of Golgi neurons has also not been quantified, though Caddy and Biscoe (1979) note that they seem to be as numerous in the +/Lc cerebellum as in the wild type. If the density of Golgi neurons appears normal in the +/Lc mutant, this may again indicate that there is extensive loss of Golgi neurons as the volume of the granule cell layer is drastically reduced in the +/Lc mutant. The sole major cell type that appears to avoid

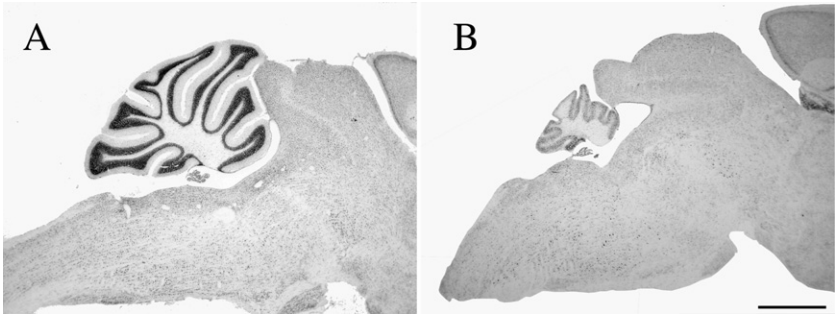

Fig. 1 – Photos of sagittal sections of the hindbrain and midbrain of adult wild-type (A) and +/Lc (B) mutant mice. Scale bar is 1 mm.

destruction in the +/Lc mutant is the deep cerebellar nuclei neurons. Their numbers are not affected in the +/Lc mutant, although they are the primary target of the degenerating +/Lc Purkinje cells.

3. Chimeric analyses of the +/Lc mutant: regulation of neuronal number in the CNS

The qualitative and quantitative descriptions of neuron degeneration and survival in the +/Lc cerebella could not be interpreted with respect to the mechanism of cell death without knowing where the mutant Lurcher gene is acting—the site of gene action. This deficit was addressed by a series of studies by Wetts and Herrup (1982b,c). They analyzed +/Lc ↔ wild-type chimeras and determined that Purkinje cells are a primary site of gene action in the +/Lc mutant, whereas olivary neurons and granule cell are not directly affected by the mutant gene. Chimeras are mosaic animals that combine wild-type and mutant cells within the same environment (McLaren, 1976; McLaren and LeDouarin, 1984; Mintz, 1962, 1965). They are constructed by incubating two preimplantation embryos (one wild type and the other with the mutant genotype) overnight during which time the cells of the two embryos co-mingle to form a double sized morula or blastocyst. The chimeric embryo is transplanted back into the uterus of a pseudopregnant host female where it undergoes a size adjustment and a normal sized pup is born at the end of the normal term. The cellular composition of each individual chimera can vary from 0 to 100% of cells of either genotype. If there are independent markers for the genotype of the mutant and wild-type cells, it is possible to determine if a gene is acting extrinsically (mutant cells can be rescued by interactions with wild-type cells) or intrinsically (mutant cells express the mutant phenotype despite interactions with wild-type cells). Using independent markers for the genotype of Purkinje cells, olivary neurons, and granule cells, Wetts and Herrup (1982b,c) showed that +/Lc Purkinje cells still died in +/Lc ↔ wild-type chimeras, whereas +/Lc olivary neurons and granule cells could be rescued in the chimeric environment. Analysis of the morphology of +/Lc Purkinje cells in P20 +/Lc ↔ wild-type chimeras showed that even the stunted morphology of the +/Lc Purkinje cells is an intrinsic effect of the Lurcher mutation (Soha and Herrup, 1995), although +/Lc Purkinje cell dendritic development can be modulated by the number of granule cell afferents during development (Doughty et al., 1999). Thus, the chimera studies demonstrated that +/Lc Purkinje cells are a primary site of gene action, but granule cells and olivary neurons are likely to be dying due to the loss of their primary target neurons, the Purkinje cells.

A further benefit of the +/Lc ↔ wild-type chimeras is that they provide a means of varying the size of the postsynaptic Purkinje cell population from 0 to 100% of wild-type values. Since each chimera is a variable mix of genotypes and the +/Lc Purkinje cells die by a cell-autonomous mechanism, a variable number of wild-type Purkinje cells survive in each chimera. A series of chimeras can then be analyzed in a cell dose experiment to determine how varying the number of target Purkinje cells affects the number of afferent neurons. The number of granule cells and Purkinje cells has been analyzed in a series of +/Lc ↔ wild-type and staggerer ↔ wild-type chimeras, wild-type ↔ wild-type chimera controls and four different strains of inbred mice (Fig. 2; Herrup and Sunter, 1987; Vogel et al., 1989; Wetts and Herrup, 1983). The results show that there is significant linear correlation between the number of granule cells and Purkinje cells, although the matching relationship is shifted upwards in +/Lc ↔ wild-type chimeras compared with the staggerer ↔ wild-type chimeras and controls. The difference between the two numerical matching relationships may derive from differences in the timing of Purkinje cell deficits in the two mutants (Caddy and Herrup, 1990; Soha and Herrup, 1993a; Vogel et al., 1989). As in the +/Lc mutant, Purkinje cells are a primary site of gene action in the staggerer mutant (gene symbol: sg), and granule cells are only secondarily affected by the developmental defect in sg/sg Purkinje cells (Herrup, 1983; Herrup and Mullen, 1979b). The staggerer mutation is a loss of function deletion of RORα (Retinoic acid-receptor-related Orphan Receptor) that causes the early cell-autonomous death of most sg/sg Purkinje cells (Hamilton et al., 1996; Herrup, 1983; Herrup and Mullen, 1979b). The differentiation of the few surviving Purkinje cells is blocked so they are unable to form mature synapses with granule cell parallel fibers (Herrup and Mullen, 1979a,b; Messer et al., 1991; Sidman et al., 1962; Yoon, 1972). Thus, in the staggerer mutant, Purkinje cells are never available as granule cell targets. However, in the +/Lc mutant, Purkinje cells appear to develop normal synapses until they begin to degenerate around P7, midway through the period of granule cell genesis. In +/Lc ↔ wild-type chimeras, the loss of granule cells subsequent to the death of +/Lc Purkinje cells may lead to the deafferentation of surviving wild-type Purkinje cells and a compensatory increase in the amount of trophic support available for surviving granule cells (Caddy and Herrup, 1990; Soha and Herrup, 1993b, 1995; Vogel et al., 1989).

The results of the site of gene action and cell dose studies in both the +/Lc and sg/sg ↔ wild-type chimeras have important

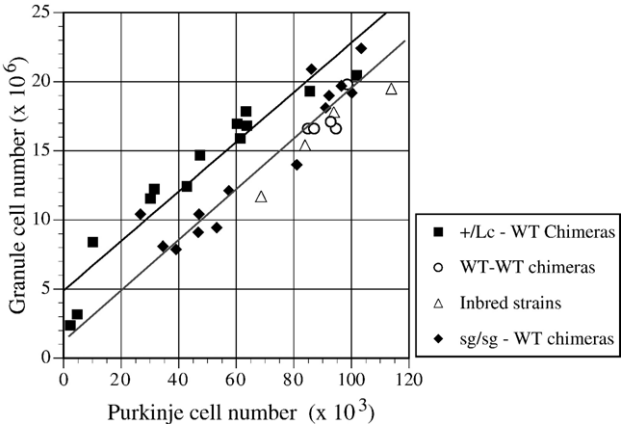

Fig. 2 – Numerical matching between Purkinje cells and granule cells in the cerebella of +/Lc ↔ wild-type, sg/sg ↔ wild-type, and wild-type ↔ wild-type chimeras and 4 different inbred strains of mice with naturally occurring polymorphisms in Purkinje cell number. The data is taken from (Herrup and Sunter, 1987; Vogel et al., 1989; Wetts and Herrup, 1983).

implications for understanding the regulation of neuronal cell death and survival not only in the +/Lc mutant, but also in the wild-type cerebellum. While +/Lc Purkinje cells are dying due to the cell-autonomous actions of the mutant +/Lc gene (see below for more details), granule cell and olivary neuron cell death is secondary to the loss of their postsynaptic target. Previous studies, primarily in the chick or frog spinal cord or peripheral nervous system, had shown that the survival of motoneurons was dependent on the presence of their target muscle fibers, and the addition of target tissue could rescue some motoneurons from naturally occurring cell death (reviewed in Williams and Herrup, 1988). The studies of the +/Lc mutant were among the first to show that the specific deletion of a target neuron in the CNS (Purkinje cells) results in the death of their afferents. The death of most granule cells and olivary neurons in the +/Lc mutant supports the concept that afferent neurons depend on their target for trophic support and that there is a critical period during which afferent survival can be stabilized by interactions with their target neurons. For not all granule cells and olivary neurons die in the +/Lc mutant and this is likely to be due to the delayed death of +/Lc PCs. In the mouse cerebellum, Purkinje cells and olivary neurons are generated by E14 during embryogenesis (Miale and Sidman, 1961; Goffinet, 1983). Granule cells are generated postnatally from P0 to P14, and the major period of synaptogenesis spans the first 2 to 3 weeks of postnatal development. Birthdate labeling studies in wild-type and +/Lc mutants with [^{3}H]-thymidine suggest that the majority of granule cells that survive in the +/Lc cerebella are those that were generated before +/Lc Purkinje cells begin to degenerate around P8 (Vogel and Herrup, 1989). The implication is that these early generated granule cells derive sufficient trophic support from early contacts with +/Lc Purkinje cells to survive once the Purkinje cells start to degenerate. It is clear from the staggerer mutant that no granule cells will survive if there are no Purkinje cells available to form mature synaptic contacts.

The linear correlation between the number of granule cells and Purkinje cells in +/Lc and staggerer ↔ wild-type chimeras and control mice further indicates that Purkinje cells tightly regulate the number of granule cells in the cerebellum. This is likely to be accomplished by Purkinje cell regulation of both granule cell genesis and survival during the period of naturally occurring granule cell death. Purkinje cell regulation of neurogenesis was suggested by studies showing that the external granule cell layer is reduced in mutants where the number of Purkinje cells is reduced during the period of granule cell genesis, including the +/Lc mutant (Sonmez and Herrup, 1984; Swisher and Wilson, 1977). In particular, Smeyne et al. (1995) showed that granule cell proliferation was specifically reduced in regions of the external granule cell layer where deeper Purkinje cells were killed by ectopic expression of diphtheria toxin using the L7 promoter. More recent studies have shown that Purkinje cells express sonic hedgehog, which acts as a powerful mitogen for granule cell precursors (Dahmane and Ruiz-i-Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999). While it is not yet clear how the cessation of granule cell genesis is regulated (e.g. see Doughty et al., 1998), the emerging model for the regulation of granule cell numbers suggests that Purkinje cells stimulate granule cell genesis with sonic hedgehog and regulate granule cell survival by limiting the amount of trophic support or synaptic sites.

Purkinje cell regulation of olivary neuron number appears to be more complex compared with granule cells. In mice, olivary neurons undergo their period of naturally occurring cell death during postnatal development, though the exact timing is still controversial (Delhaye-Bouchaud et al., 1985; Zanjani et al., 1994; see also Cunningham et al., 1999; Madalosso et al., 2005). Nevertheless, 75% of the olivary neurons in the +/Lc mutant die, indicating that their survival is dependent on their Purkinje cell targets. Counts of olivary neurons and Purkinje cells in the contralateral cerebella of +/Lc ↔ wild-type chimeras show that there is a linear relationship between the two populations, suggesting that, like granule cells, Purkinje cells tightly regulate olivary numbers (Herrup et al., 1996). However, this correlation does not hold for sg/sg ↔ wild-type chimeras and different strains of inbred mice with polymorphisms in Purkinje cell number. These results suggest that the regulation of olivary neurons may be more complex with afferents, other targets (e.g. deep cerebellar neurons), and even different matching relationships between subsets of olivary neurons and their Purkinje cell targets possibly playing a role (Herrup et al., 1996).

If granule cell and olivary neuron number is regulated by the number of Purkinje cells, what then regulates the number of Purkinje cells? Counts of the surviving wild-type Purkinje cells in +/Lc ↔ wild-type chimeras using three different wild-type inbred strains suggested that Purkinje cell number is established by a lineage-related mechanism. The lineage model suggests that a small set of progenitor cells is selected early in development to give rise to all Purkinje cells, and each progenitor cell produces the same number of Purkinje cells (Herrup, 1986; Herrup and Sunter, 1986; Herrup et al., 1984; Vogel and Herrup, 1993; Wetts and Herrup, 1982a). This model is based on the findings that the number of wild-type Purkinje cells in all of the chimeras varied by quantal increments, suggesting that there was a limited founder cell population and each founder cell contributing equal numbers of Purkinje cells. These results and their interpretation have been questioned based on a possible founder cell effect for the whole embryo (Soriano and Jaenisch, 1986) and the statistical analysis of the original data (Jennings, 1988). However, subsequent analyses of Purkinje cell lineage using different techniques agree with the basic principle that all Purkinje cells are descended from a limited number of progenitor cells, each contributing a set number of Purkinje cells, although there are likely to be more Purkinje cell progenitor cells than those suggested by the initial +/Lc ↔ wild-type chimera studies (>100 vs. 8 to 11; Baader et al., 1996; Hawkes et al., 1998; Mathis et al., 1997). However, none of the analyses of the number of Purkinje cell progenitors takes into account the effects of naturally occurring Purkinje cell death. There was little hard evidence for developmental Purkinje cell death until analyses of transgenics that overexpressed the anti-apoptotic gene Bcl-2 or deleted the proapoptotic gene Bax showed that the number of Purkinje cells is increased if cell death is blocked during development (Fan et al., 2001; Zanjani et al., 1996). While it seems likely that there are cell lineage mechanisms that

regulate Purkinje cell numbers in part, it is still not known what role Purkinje cell afferents and targets may play in regulating the final number of Purkinje cells.

4. The δ2 glutamate receptor and the mechanisms of +/Lc Purkinje cell death

4.1. *δ2 glutamate receptors and cerebellar function*

The Lurcher mutation was identified as a gain of function in the δ2 glutamate receptor by Zuo et al. (1997). There are two δ glutamate receptors (GluRδ1 and GluRδ2) that were isolated on the basis of their sequence homology to the NMDA and AMPA/kainate receptor subunits (~20–30% homology; Araki et al., 1993; De Jager and Heintz, 1998; Lomeli et al., 1993; Yamazaki et al., 1992). GluRδ1 expression is relatively low throughout the adult rat CNS, except for high levels in inner hair cells and in all spiral and vestibular ganglion neurons as well as in their satellite glial cells (Lomeli et al., 1993; Safieddine and Wenthold, 1997). However, expression levels of GluRδ1 are relatively higher at early postnatal stages, suggesting that it may play an important role during CNS development (Lomeli et al., 1993). GluRδ2 is preferentially expressed at high levels in cerebellar Purkinje cells and at lower levels in some hindbrain neurons (Araki et al., 1993; Lomeli et al., 1993; Takayama et al., 1996). Within Purkinje cells in the adult rodent, GluRδ2 is preferentially co-localized with AMPA receptors at PC-parallel fiber synapses (Landsend et al., 1997). GluRδ2 is first expressed early in Purkinje cell development, and GluRδ2 immunoreactivity is initially diffusely spread within the cytoplasm of Purkinje cell dendrites (Takayama et al., 1996). Immunogold EM labeling studies in the rat show that GluRδ2 is initially localized to climbing fiber synapses during the first week of postnatal development (Zhao et al., 1998). GluRδ2 do not appear at parallel fiber synapses until P10 in folia III–V. After this time, the number of GluRδ2 at parallel fiber synapses increases to adult levels, while they disappear from climbing fiber synapses.

The function of GluRδ1/2 remains unknown as GluRδ1/2 do not bind glutamate or glutamate agonists (Araki et al., 1993; Lomeli et al., 1993; Mayat et al., 1995). However, there is growing evidence that GluRδ2 plays an important role in Purkinje cell-granule cell synapse stabilization and the regulation of long-term depression (LTD) (Hirai et al., 2003; Kashiwabuchi et al., 1995; Lalouette et al., 2001), which is thought to underlie a form of information storage in the cerebellum (Ito, 1989). A number of recent studies have shown that GluRδ2 interacts with other synaptic proteins (PTPMEG, S-SCAM/MAGI-2 shank, Delphilin; Hironaka et al., 2000; Miyagi et al., 2002; Uemura et al., 2004; Yap et al., 2003) as well as with n-PIST and Beclin (Yue et al., 2002). GluRδ2 may play an important role in cerebellum-dependent motor learning since Grid2 knock-out mutant mice have defects in the formation of parallel fiber-PC synapses, the removal of climbing fiber multiple innervation, LTD induction, and motor coordination (Hashimoto et al., 2001; Kashiwabuchi et al., 1995; Katoh et al., 2005; Lalouette et al., 2001; Yoshida et al., 2004). Functional studies with a blocking antibody recently showed that GluRδ2 may regulate LTD by controlling AMPA receptor endocytosis (Hirai et al., 2003).

4.2. *The Lurcher mutation in GluRδ2*

The Lc mutation in GluRδ2 is a base-pair substitution that changes an alanine to threonine in a motif at the end of the third hydrophobic segment highly conserved in all ionotropic glutamate receptors. The mutation results in large constitutive cationic currents in the absence of ligand in the cells that express the subunit. Examination of the current–voltage relationship revealed that constitutive currents were doubly rectified: currents flowed more easily at membrane potentials lower than +20 mV or higher than +70 mV. Such feature is similar to those of glutamate-induced currents through AMPA or kainate receptors that have a glutamine (Q), but not arginine (R), residue in the channel pore region. Indeed, GluRδ2 has a glutamine residue at the corresponding site, and introduction of a Q → R mutation abolished rectification properties of the GluRδ2^{Lc} leak current. In addition, like heteromeric AMPA or kainate receptor complexes, when GluRδ2^{Lc} (Q) and GluRδ2^{Lc} (R) are coexpressed, the resultant leak current showed no rectification. These results indicate that GluRδ2^{Lc} forms an oligomeric channel, whose properties are similar to those of AMPA and kainate receptors (Kohda et al., 2003; Wollmuth et al., 2000).

NMDA receptors are associated with channels that are highly permeable to Ca^{2+}, which plays crucial roles in excitotoxicity. Moderate Ca^{2+} permeability of AMPA receptors is associated with glutamine at the Q/R site. Although it is difficult to accurately measure the Ca^{2+} permeability of constitutively open channels, GluRδ2^{Lc}, which has glutamine at the Q/R site, seems to be associated with a modest level of Ca^{2+} permeability, even lower than that of AMPA receptors (Kohda et al., 2003; Wollmuth et al., 2000). Although characterization of the Lurcher mutation has indicated that GluRδ2 can act as ion channels, it needs to be restated that it is still unclear whether wild-type GluRδ2 also functions as an ion channel. The ability of GluRδ2 with the Lurcher mutation to form channel pores may just be a function that was lost during evolution.

In acute cerebellar slices, +/Lc Purkinje cells are depolarized by the chronic cation leak (Zuo et al., 1997). Leak currents observed in +/Lc Purkinje cells did not show prominent rectification. Similarly, in HEK293 cells expressing both wild-type GluRδ2 and GluRδ2^{Lc}, the limited rectification was observed. Thus, the leak current in +/Lc Purkinje cells is likely to be mediated by heteromeric GluRδ2 and GluRδ2^{Lc} channels. The leak current appears to develop in tandem with the translocation of GluRδ2 receptors to synaptic sites; the leak current first appears at P5–6 and then strengthens through P9–12 (Selimi et al., 2003).

The mechanism of GluRδ2^{Lc}-induced neuronal death has yet to be definitively established (Heintz et al., 1999). Once reliable molecular markers were available for the Lc mutation to genotype embryos, it was found that the death of homozygous mutants is caused by massive neuronal death in the mid- and hindbrain in late embryonic development (Cheng and Heintz, 1997; Resibois et al., 1997), but nothing is known about their mechanism of cell death.

In +/Lc heterozygotes, cell-autonomous Purkinje cell death has been alternately described as necrotic, apoptotic, and autophagic based on a variety of criteria. Evidence for necrotic cell death is based on morphological evidence (Dumesnil-Bousez and Sotelo, 1992), but it has also been proposed that GluRδ2^{Lc} may trigger an autophagic cell death pathway through its failure to sequester n-PIST and Beclin (Yue et al., 2002). Autophagosome-like vesicles have been described in +/Lc Purkinje cells, but there is no evidence yet of how the autophagy in +/Lc Purkinje cells causes their death (Selimi et al., 2003; Yue et al., 2002). There is, however, considerable evidence for apoptotic pathways of cell death in +/Lc Purkinje cells. TUNEL labeled +/Lc Purkinje cells have been detected in three (Norman et al., 1995; Selimi et al., 2000a; Wullner et al., 1995) out of four (Herrup and Busser, 1995) studies. BAX and Bcl-x expression are increased in dying +/Lc PCs (Wullner et al., 1995) and Mariani and colleagues (Selimi et al., 2000a) have shown that pro-caspase-3 levels are increased in approximately 25% of +/Lc PCs at P12 to P20. Activated caspase-3 is expressed in a few PCs that are presumably dying, although it has not been possible to double label +/Lc PCs for activated caspase-3 and TUNEL labeling. In addition, there is an increase in c-Jun phosphorylation in +/Lc PCs along with increases in caspase-8 and -9 expression (Lu and Tsirka, 2002). The caspases are key executioners in apoptosis (Green and Kroemer, 1998; Thornberry and Lazebnik, 1998), so caspase activation in +/Lc PCs suggests that at least some elements of apoptotic cell death pathways are activated in +/Lc PCs.

There are at least two competing hypotheses for the trigger mechanisms involved in the GluRδ2^{Lc}-mediated Purkinje cell death: autophagy and excitotoxicity. Wild-type GluRδ2 interacts with Beclin at its C-terminus through n-PIST, a novel PDZ/coiled domain protein (Yue et al., 2002). Beclin 1 was originally identified as a Bcl-2 interacting protein (Liang et al., 1998), and it was quickly identified as a mammalian ortholog of the yeast autophagy gene, Atg6/Vps30 (Liang et al., 1999). Beclin 1 associates with a Class III PI3 kinase complex that participates in autophagosome formation by regulating the aggregation of other autophagy proteins to the pre-autophagosomomal membrane (Kihara et al., 2001). In the model of cell death via autophagy, the Lc mutation constitutively activates GluRδ2 receptors so that Beclin is released to activate autophagy, leading to cell death. Coexpression of Beclin 1 and the Grid2^{Lc} receptor in heterologous HEK293 cells in vitro leads to the formation of Beclin-1-positive autophagosome-like vesicles and induces increased cell death (Yue et al., 2002). Coexpression of the wild-type GluRδ2 does not induce autophagy and cell death.

The role of autophagy in +/Lc Purkinje cell death is supported by studies of Purkinje death in a Lurcher:hotfoot double mutant (Selimi et al., 2003). Hotfoot (gene symbol, ho) is a null allele for GluRδ2, so only GluRδ2^{Lc} is expressed in Lc/ho Purkinje cells. Morphological and electrophysiological studies of Lc/ho double mutants have shown that Purkinje cells die prematurely in this mutant, before the GluRδ2^{Lc} leak current can be measured (Selimi et al., 2003). In addition, there is ultrastructural evidence for increased autophagy. The results of this study have been interpreted to show that the induction of autophagy through release of Beclin 1 is the key initiator of cell death of +/Lc PCs, while the leak current is less important (Rubinsztein et al., 2005). However, the interpretation of the Lc/ho results is controversial. For example, it is not yet possible to rule out the possibility that there are early undetected leak currents in Lc/ho Purkinje cells that stimulate autophagy nor has the role of apoptotic proteins been analyzed in the Lc/ho Purkinje cells. In addition, a mutant kainate receptor GluR6^{Lc}, in which alanine to threonine (Lc) mutation was introduced at the corresponding position in GluR6, also caused constitutive currents and cell death associated with Beclin-positive vacuoles in HEK293 cells (Yamada et al., 2003b). Moreover, when extracellular Na^+ ions were substituted with non-permeable cations, both autophagy and cell death were prevented in cells expressing GluRδ2^{Lc}. Because GluR6 lacks the C-terminus that binds to n-PIST and Beclin 1, these preliminary results indicate that the constitutive leak current is necessary and sufficient to induce autophagy and cell death, at least in heterologous cells (Yamada et al., 2003a). Therefore, further studies are warranted to clarify the role of GluRδ2 in autophagy and cell death in Lurcher Purkinje cells in vivo.

However, in addition to the evidence for autophagy, there is also evidence for an excitotoxic mode of cell death in the form of increased oxidative stress in +/Lc Purkinje cells (Vogel et al., 2004). The chronic cation leak induced by GluRδ2^{Lc} channels is sufficient to activate voltage-sensitive Ca^{++} channels (Mouginot et al., 1997), so it is likely that both Na^+ and Ca^{++} levels are increased in +/Lc Purkinje cells. Once a cell is depolarized, ATP is required for the ion pumps that restore intracellular Na^+ and Ca^{++} levels; for example, about 50% of CNS ATP is used for the outward transport of Na^+ by NaK-ATPase (Ames, 1997). The distal dendrites of +/Lc Purkinje cells are filled with dystrophic mitochondria (Caddy and Biscoe, 1979; Dumesnil-Bousez and Sotelo, 1992), and mitochondrial cytochrome oxidase activity is significantly increased in Purkinje cells (Vogel et al., 2001). These data suggest that mitochondrial oxidative respiration is increased in response to the increased demand for ATP in depolarized +/Lc Purkinje cells. There are likely to be metabolic costs to increased mitochondrial respiratory activity. Approximately 1–2% of the oxygen not consumed by mitochondrial cytochrome c oxidase is reduced to O_2^- and H_2O_2 at mitochondrial and extra-mitochondrial sites (Radi et al., 1997). Therefore, any activity that increases cellular respiration rates may increase reactive oxygen species (ROS) production as a by-product. Increased Ca^{++} levels and ROS production may also stimulate the production of nitric oxide (NO) by nitric oxide synthase (NOS), leading to the formation of peroxynitrite (Beckman and Crow, 1993; Bredt, 1999; Kamii et al., 1996; Keller et al., 1998). Peroxynitrite is a powerful oxidizing and nitrating agent that will, among other reactions, nitrate protein tyrosine residues. Purkinje cells only express neuronal NOS (nNOS) early in postnatal development, but nNOS may also be induced in Purkinje cells in response to traumatic injury or excitotoxic lesions (Bruning, 1993; Chen and Aston-Jones, 1994; Ikeda et al., 1999; O'Hearn et al., 1995). In support of the hypothesis that +/Lc Purkinje cells are subject to increased oxidative stress, we have preliminary evidence that nitrotyrosine immunolabeling and NOS activity is increased in +/Lc Purkinje cells from P10 through the death of most Purkinje cells (Vogel et al., 2004). In addition, the expression of

manganese super oxide dismutase (MnSOD) is increased in +/Lc Purkinje cells (unpublished observations). MnSOD is the principal scavenger of super oxide in mitochondria (Fridovich, 1975) and is induced by a variety of cytotoxic and proapoptotic agents. One potential link between increased oxidative stress and cell death is that peroxynitrite may trigger apoptotic cell death by nitrating key second messenger proteins to activate pathways that promote cell death (e.g. stress activated MAP kinase pathways (Borsello et al., 2003; Savinainen et al., 2001; Yang et al., 1997) or to inactivate pathways that inhibit cell death (e.g. PI/AKT; Klotz et al., 2002; Minetti et al., 2002; Monteiro, 2002).

A number of studies have attempted to probe the mechanisms of +/Lc Purkinje cell death by overexpression or deletion of key cell death pathway genes, with mixed results. Overexpression of a human Bcl-2 transgene will delay +/Lc Purkinje cell death, but they will eventually die (Zanjani et al., 1998a,b). Similarly, deletion of the proapoptotic genes, Bax and Bim, does not prevent +/Lc Purkinje cell death (Bouillet et al., 2003; Doughty et al., 2000; Selimi et al., 2000b). Interestingly, deletion of Bax expression does slightly delay +/Lc Purkinje cell death, and activated caspase-3 is no longer detected in +/Lc-Bax$^{-/-}$ Purkinje cells, which indicates that the mechanism of PC death is altered in the absence of BAX expression (Doughty et al., 2000; Selimi et al., 2000b). The +/Lc:Bax$^{-/-}$ experiments have been interpreted as evidence for a caspase-independent program of cell death in +/Lc Purkinje cells (Rubinsztein et al., 2005). However, the expression of other effector caspases has not been examined in the +/Lc:Bax$^{-/-}$ double mutants, so it is premature to rule out an apoptotic mechanism of cell death in the +/Lc-Bax$^{-/-}$ Purkinje cells.

In light of the redundancies in the Bcl-2 and caspase family of apoptotic genes, it is likely that there are multiple pathways of cell death that can be stimulated in +/Lc Purkinje cells. If one pathway is blocked, then a second can take its place. For example, Lu and Tsirka (2002) blocked the expression of tissue plasminogen activator (tPA) in +/Lc mutants and again found that there is a delay in +/Lc:tPA$^{-/-}$ Purkinje cell death. Analysis of the expression of activated caspase-8 and -9 showed that deletion of tPA expression reduced the expression of activated caspase-8, but activated caspase-9 is still expressed at P30 at the same levels in +/Lc and +/Lc:tPA$^{-/-}$ cerebella. The authors argue that at least two cell death pathways are activated in +/Lc Purkinje cells: (1) a cell-autonomous pathway mediated by mitochondrial dysfunction and activation of caspases-9 and -3 and (2) an extracellular pathway that involves the secretion of tPA from depolarized +/Lc Purkinje cells and the initiation of an extracellular tPA/plasmin cascade that leads to the induction of a receptor-mediated cell death pathway. The later pathway may involve activation of caspase-8 and phosphorylation of Jun.

An interesting corollary of the experiments to investigate the mechanisms of cell death in +/Lc Purkinje cells is that they have provided important insights into the mechanisms of olivary and granule cell death in the +/Lc mutant. Overexpression of a human Bcl-2 transgene (Hu-Bcl-2) rescues olivary neurons from target related cell death in the +/Lc mutant (Zanjani et al., 1998b). The number of olivary neurons in the +/Lc:Hu-Bcl-2 double mutant is restored to wild-type levels, but this means that not all olivary neurons are rescued by overexpression of Bcl-2 since olivary neuron number is increased by over 20% above wild-type levels in Hu-Bcl-2 transgenics. Furthermore, despite their survival without target neurons, the rescued +/Lc olivary neurons are atrophic, and the olivary nucleus in +/Lc:Hu-Bcl-2 double mutants is no larger than in +/Lc mutants. The difference in olivary neuron survival between Hu-Bcl-2 transgenics and +/Lc:Hu-Bcl-2 double mutants may indicate either that Bcl-2 overexpression will not rescue all olivary neurons from target related cell death or that the Bcl-2 transgene is not expressed in high enough levels in all olivary neurons to provide sufficient protection. The atrophy of the rescued olivary neurons further indicates that blocking apoptosis may prevent cell death, but if the target tissue is not available to provide appropriate trophic support, then the rescued neurons may still be impaired. Similar results have been obtained from studies of other neuronal populations where overexpression of Bcl-2 or deletion of Bax expression rescues neurons from cell death, but they remain atrophic, presumably from lack of target-derived trophic support (Deckwerth et al., 1996; Greenlund et al., 1995; Sun et al., 2003).

Although Bcl-2 and Bax interact to regulate cell death (Danial and Korsmeyer, 2004), deletion of Bax expression in +/Lc olivary neurons does not prevent their death (Doughty et al., 2000; Selimi et al., 2000b). In contrast, deletion of Bax expression does significantly reduce the amount of +/Lc granule cell death (Doughty et al., 2000; Selimi et al., 2000b). The later result is surprising given that deletion of Bax expression does not rescue granule cells from naturally occurring cell death (Fan et al., 2001). These results highlight evidence from a variety of experimental systems that there are multiple pathways of cell death that may vary by cell type and even between different cell death stimuli within the same cell type. In particular, there may be distinct differences between naturally occurring cell death and cell death following target removal (Chu-Wang and Oppenheim, 1978; Lanser and Fallon, 1984, 1987; Pilar and Landmesser, 1976; Vogel and Herrup, 1989).

5. Immuno-endocrinological abnormalities in +/Lc mutant mice

The neurodegeneration in +/Lc mice is accompanied by a chronic inflammatory state, which is evident both at the periphery and in the brain. Stimulation of peritoneal macrophages of +/Lc mice by the active fragment of endotoxin, lipopolysaccharide (LPS), induces higher expression of the proinflammatory cytokines IL-1α, IL-1β, and TNFα at the mRNA and protein levels, compared with wild-type mice while the level of IL-6 remains normal (Kopmels et al., 1990, 1991; Vernet-der Garabedian et al., 1998). In addition to their possible role in neural degeneration, proinflammatory cytokines modulate immune as well as endocrine functions. One of their targets is the hypothalamo–pituitary–adrenal (HPA) axis, which plays a major role in adaptation to stress. Peripherally released IL-1, IL-6, and TNFα act on the brain via either blood-borne mediators or a neural pathway involving activation of peripheral sensory nerves, leading to the synthesis of IL-1, IL-6, and TNFα in the brain. IL-1, IL-6, and

TNFα stimulate the hypothalamic release of CRH, which ultimately leads to an adrenal release of glucocorticoids. Glucocorticoids in turn down-regulate macrophage IL-1 production and exert a negative feed-back on ACTH and CRH secretion.

To assess whether the increased responsiveness to inflammatory stimuli is accompanied by a higher pituitary–adrenal response, the adrenocorticotropic hormone (ACTH) and corticosterone response to intraperitoneal (i.p.) administration of lipopolysaccharide (LPS) was compared in +/Lc and wild-type mice. +/Lc mice display resting levels of ACTH and corticosterone similar to those of wild-type mice, but LPS induces a corticosterone surge 2-fold higher in Lurcher than in wild-type mice. By contrast, the response to IL-1 is similar in both genotypes, suggesting that a differential reactivity of the hypothalamo–pituitary adrenal axis to IL-1 does not account for the higher reactivity of +/Lc mice to LPS. To test whether the increased responsiveness of the pituitary–adrenal axis of +/Lc mice generalizes across stressors, mice were exposed to a novel environment. This condition also induced a surge of ACTH and corticosterone 3.5- and 2-fold higher in +/Lc than in wild-type mice. Prior blockade of IL-1 receptors by injection of IL-1 receptor antagonist failed to block the response to LPS injection and exposure to novelty. In contrast, immunoneutralization of hypothalamic corticotropin-releasing hormone (CRH) significantly attenuated the ACTH surge and abrogated the difference between +/Lc and wild-type mice in their responses to a novel environment, suggesting that hypothalamic CRH neurons are involved in this excessive response (Frederic et al., 1997).

Besides the possible effects of hyperproduction of inflammatory cytokines, the question arises as to whether the cerebellar pathology of +/Lc mice plays a role in the enhanced response to novelty. The imbalance and falls that +/Lc mice make during the exploration of a novel environment may by themselves be a stressor, though independent from cytokine production. Furthermore, neuroanatomical and electrophysiological studies in rodents and other mammals have identified direct reciprocal connections between the cerebellum and the hypothalamus, the CRH neurons receiving inputs from the cerebellar nuclei. It is possible, therefore, that, in +/Lc mice, the degeneration of most of the Purkinje cells, whose axons constitute the only output of the cerebellar cortex to the cerebellar nuclei, modifies the modulation exerted by the cerebellar cortex on hypothalamic function and participates in the neuroendocrine abnormalities depicted in the +/Lc mutant together with the abnormal proinflammatory cytokine production.

Proinflammatory cytokines have also potent behavioral effects. Peripheral and central administration of LPS and IL-1 induces lethargy and anorexia and decreases social activities. Since these effects are mediated by the synthesis of endogenous cytokines in the brain in response to exogenous cytokines, +/Lc mutants suffering from chronic inflammation should be more sensitive to the behavioral effects of LPS and IL-1. Thus, the behavioral responses of adult male +/Lc and wild type to an i.p. or i.c.v. injection of recombinant IL-1 and lipopolysaccharide (LPS) were assessed. IL-1 or LPS (i.p. or i.c.v.) decreased social exploration measured 2, 4, and 6 h later, and this decrease was significantly more pronounced in +/Lc than in wild-type mice. These results suggest that the chronic inflammatory state which characterizes +/Lc mice renders these animals more sensitive to the effects of cytokines such as IL-1 and LPS (Bluthe et al., 1997). This difference may be due to the higher reactivity of brain macrophages and glial cells to LPS and IL-1 in +/Lc mice than in wild type.

6. The Lurcher mutant as a model system for investigating cerebellar function

As the carrier of a mutation that specifically affects the development of the olivocerebellar circuit, +/Lc mutant mice have proved to be valuable models for investigating not only neuron–target interactions in the developing cerebellum, but larger questions of how the cerebellum interacts with the rest of the nervous system, including its influence on behavior. This section will provide selected examples of the use of the +/Lc mutant to investigate developmental interactions in the cerebellum and the effect of the degeneration of the olivocerebellar circuit on behavior.

6.1. *Afferent connectivity and cerebellar pattern formation in the +/Lc cerebella*

The Purkinje cell has been ascribed a defining role in regulating cerebellar development both for its role in regulating the number of afferents and for providing a molecular map that organizes the topography of afferent projections (Arsénio-Nunes et al., 1988; Chédotal et al., 1996, 1997; Madalosso et al., 2005; Sotelo and Wassef, 1991). The cell-autonomous death of +/Lc Purkinje cells midway through the period of synaptogenesis in the cerebellar cortex has provided a system that can be used to probe the role of Purkinje cells in organizing cerebellar development. For example, analysis of the developmental expression of the cerebellar compartment marker, Zebrin, in +/Lc mutants revealed a novel mediolateral and anteroposterior boundary in the mouse cerebellum that cuts across lobule VIII (Tano et al., 1992). The developmental expression of Zebrin proceeds normally in +/Lc cerebella until P7, when the normal upregulation of Zebrin expression in all Purkinje cells fails in +/Lc Purkinje cells leaving Zebrin positive neurons caudal to a boundary along the dorsal surface of lobule VIII. The failure of more rostral +/Lc Purkinje cells to express Zebrin is not due to their immediate death or a failure to continue to differentiate. Analysis of the developmental expression of a second Purkinje cell compartmental marker, HSP25, suggests that the defect in Zebrin expression is specific for Zebrin since the characteristic expression pattern of HSP25 rostral and caudal to the lobule VIII is not affected by the +/Lc mutation (Armstrong et al., 2005).

Although it has been recognized for over 40 years that cerebellar compartments can be defined by the temporal and spatial expression of a variety of Purkinje cell enzymes or antigens, it is still not clear how the compartmental boundaries guide afferent innervation (Arsénio-Nunes et al., 1988; Gravel et al., 1987; Hawkes and Gravel, 1991; Herrup and Kuemerle, 1997; Lannoo et al., 1991; Leclerc et al., 1992; Sotelo and Wassef, 1991). Studies of climbing fiber and mossy fiber innervation in the +/Lc mutant have at least shown that the

maintenance of appropriate afferent connectivity is not dependent on the presence of Purkinje cells (Heckroth and Eisenman, 1988, 1991; Vogel and Prittie, 1994). Despite the loss of their Purkinje cell targets, the surviving olivary neurons in +/Lc mutants maintain appropriate topographic projections. +/Lc Purkinje cell bodies are initially innervated normally by climbing fibers, but they fail to climb the +/Lc Purkinje cell dendrites at the end of the normal perisomatic "nest" stage of differentiation (Heckroth et al., 1990). In addition, +/Lc Purkinje cells remain multiply innervated by climbing fibers until their death (Rabacchi et al., 1992). Spinocerebellar mossy fibers in the +/Lc cerebellum project to the appropriate anterior and posterior lobules and form relatively normal parasagittal zones, within the constraints of a much smaller cerebellum (Vogel and Prittie, 1994). Both studies indicate that, if Purkinje cells are responsible for establishing the cues for the topography of afferent projections, these cues must be expressed in embryonic or neonatal +/Lc Purkinje cells, but they are not necessary to maintain the appropriate projection patterns after the +/Lc Purkinje cells die.

6.2. *Behavioral studies in +/Lc mutant mice*

Behavioral studies in the +/Lc mutant mouse, started 20 years ago, have demonstrated motor disabilities in various paradigms and, more recently, spatial alterations and anxious-like abnormalities. Therefore, the mutation does not only affect motor skills but also cognitive and emotional functions. Thus, these studies provide insights into the role of the cerebellum not only in motor coordination, but also in higher CNS functions like cognition and emotion.

6.2.1. *Motor skills and motor learning*

+/Lc mutant mice develop more slowly than control mice of the same strain. Their body weight is lower until the end of the first postnatal month and most of the postnatal reflexes are delayed (Thullier et al., 1997). When adults, they exhibit motor skill deficiencies. Their muscular strength is weaker than that of control mice as demonstrated by their shorter latency before falling when the mice are hung by their forepaws in the middle of a thin string (Hilber and Caston, 2001). They are also impaired in tasks requiring climbing skills, such as the coat-hanger and grid tasks (Lalonde et al., 1992; Thifault et al., 1996). Their static equilibrium is impaired as shown by poor performances, compared to controls, on the static beam test (Le Gal La Salle, 1993; Le Marec et al., 1997) and an unstable elevated platform (Hilber and Caston, 2001). Dynamic equilibrium is also impaired in +/Lc mice as demonstrated by their performance on the rotorod where the latency before falling is shorter than that recorded in control mice (Caston et al., 1995; Le Marec et al., 1997). Moreover, their strategy to maintain balance on the rotorod is different: while control mice can walk or run on the rotorod, +/Lc mutants grasp the rotorod while being passively rotated, even 100% of the time during the very first trials (Hilber and Caston, 2001). These results show that the mutants are impaired in their motor coordination since they are unable to walk synchronously with the rotation of the rod (Caston et al., 1998b). Such impairments in motor coordination are also demonstrated on the hole board, a task requiring an accurate motor coordination since the animals have to walk on the board without slipping into a hole. In this task, the slip frequency is higher in Lurcher mutants than in controls (Hilber and Caston, 2001).

When these tests are repeated over time, +/Lc mice are able to improve their score (Lalonde, 1994; Lalonde et al., 1996a,b; Lalonde and Thifault, 1994; Le Marec et al., 1997; Thifault et al., 1996). Particularly, on the rotorod, their performance is significantly enhanced with training, and they adopt a walking strategy which tends to resemble that of controls (Caston et al., 1995; Hilber and Caston, 2001; Lalonde et al., 1995), demonstrating that, in spite of their motor disabilities, they are able to learn an adapted strategy to maintain balance and to improve their motor coordination. However, when the task is difficult (when rotation of the rotorod is high, i.e. 30 rev/min), the strategy used by the +/Lc mutant mice returns to grasping the rotorod. Such a strategy does not decrease with training, indicating that, when the task is more difficult, the animals do not learn to walk and do not improve their motor coordination abilities with repeated experience, suggesting that the cerebellar cortex is necessary for the animals to acquire accurate motor coordination (Caston et al., 1995). Indeed, Ivry and Keele (1989) have suggested that one of its roles could be to organize temporally the muscular contractions needed for the execution of skilled movements, which is consistent with the idea that the cerebellar cortex may function as an internal clock (Braitenberg, 1967; Pellionisz and Llinas, 1982) where the neurophysiological substrate would be the olivocerebellar pathway (Llinas and Sasaki, 1989). More generally, the cerebellum seems to be involved in time perception, and particularly in timed active avoidance, given that, compared to controls, +/Lc mice need a high number of trials to learn the timing task, and some are even unable to learn such a task (Monfort et al., 1998).

6.2.2. *Exploration and spatial abilities*

When the hole board is used to test exploration behavior (as measured by frequency of hole poking) instead of motor coordination, +/Lc mutant mice have fewer hole pokes than control mice (Caston et al., 1998a; Lalonde et al., 1993b). Such a low level of exploration is not due to a deficit of locomotor activity, but rather to both a decreased motivation to explore a novel environment and to spatial deficits (Caston et al., 1998a). The spatial impairment of +/Lc mice is demonstrated by their behavior in the Morris water maze, a widely used paradigm to test spatial abilities wherein animals have to find a submerged and invisible platform to escape from water by using spatial cues. While the escape latency from water decreases with training, the scores of +/Lc mutants are always worse than those of control mice. The probe test (removing the platform and measuring the time spent in the quadrant where it was located during the learning test) shows that they behave at random (Hilber et al., 1998; Lalonde, 1998). They are similarly impaired in a spatial learning task in a Z-maze filled with water (Lalonde et al., 1996b). They have also difficulty in guiding themselves in the water towards a visible goal (Lalonde et al., 1988), which is probably due to the fact that they are impaired in visual discrimination learning (Lalonde et al., 1993a). Moreover, they have deficits in long-term spatial memory as demonstrated by low scores during the retention

test done 7 days after the learning session in the Morris water maze, which demonstrates that they have partly forgotten the spatial location of the platform (Hilber et al., 1998). Spatial memory deficits are also demonstrated by the fact that they alternate less often in a two-trial procedure of spontaneous alternation (Lalonde et al., 1986), especially when the delay between the forced and the choice trials is 1 h long (Caston et al., 1997). Their working memory and their reference memory requiring the use of a cognitive map (as it is the case in the Morris water maze) are impaired, but not the reference memory requiring a left–right discrimination (Belzung et al., 2001). All these results suggest that +/Lc mutant mice are unable to construct a cognitive map as normal mice do but that their "spatial" abilities are rather associative in nature (the animals would associate a proximal item with the platform location). They mainly use a route strategy rather than a true spatial strategy, as suggested by the better scores they obtain when, in the Morris water maze, the starting point is fixed than when it is variable (Hilber et al., 1998). However, in chimeras between heterozygous (+/Lc) mutant embryos and +/+ embryos (which have Purkinje cells ranging from zero to normal values), no spatial working memory deficit was revealed, while such deficits were obvious in +/Lc mice (Martin et al., 2004).

6.2.3. *Emotional behavior*

Relatively fewer studies have been done on the emotional status of +/Lc mutant mice. However, it has been demonstrated that +/Lc mutants spend more time than controls in the open arms of the elevated plus-maze (Hilber et al., 2004; Monnier and Lalonde, 1995), which is considered to measure state anxiety (Lister, 1987). The open arms are considered the aversive space so these results can be interpreted as a reduced anxiety level, especially since the tendency to stay in the open arms longer is due neither to an inability to perceive the height nor to an unawareness of the stressful nature of the open arms (Hilber et al., 2004). Indeed, following their stay in the elevated plus-maze, their plasma corticosterone level is much higher than that of controls (while the basal level is similar in both groups), suggesting that they are more stressed than the controls are (Frederic et al., 1997; Hilber et al., 2004). Therefore, compared to controls, +/Lc mutant mice placed in an aversive and conflicted situation in which they have to resolve an "avoid-approach" conflict (Lister, 1990) are more stressed but less anxious. In the Porsolt test, which measures depression-like behavior, Lurcher mutants spend less time in an immobile floating posture than controls, suggesting either that they are defective in inhibitory mechanisms during swimming (Lalonde, 1998) or that they are less resigned and less "depressive" than controls.

6.2.4. *Conclusion*

Motor skills, motor learning, and spatial learning are impaired in +/Lc mutant mice, and their emotional status seems to be different from that of control mice of the same strain. The explicit or implicit conclusion of these studies is that the behavioral impairments in the +/Lc mutant reveal the contribution of the cerebellum to the regulation of these behaviors. This conclusion is supported by recent studies where behavioral deficits are found in mice with more subtle deficits in cerebellar function caused by deletion of the GluRδ2 receptor, thereby blocking LTD at the Purkinje cell-parallel fiber synapse (Kato et al., 2005; Sacchetti et al., 2004; Takatsuki et al., 2003; Yoshida et al., 2004). However, not all of the behavioral deficits can be attributed solely to cerebellar dysfunction since there may be compensatory functional changes in the rest of the CNS. For example, many of the behavioral impairments in +/Lc mutants are partly reversible if the animals are reared from birth in a sensorimotor and social-enriched environment (Caston et al., 1999). This suggests either that the early enriched experience has decreased or slowed the degenerative process in the olivocerebellar system or that there is compensatory plasticity in the remaining cells in the olivocerebellar system or in the extracerebellar regions taking over from lost cerebellar function. The later hypothesis is supported by a study of cytochrome oxidase (COX) activity in adult +/Lc mutants showing changes in regional brain metabolism in diverse CNS areas with direct or indirect connections to the cerebellum (Strazielle et al., 1998). The changes in COX activity in diverse CNS regions suggest that the pattern of neuronal activity in these regions has altered in response to changing demands following the degeneration of most neurons in the olivocerebellar circuit. The long-term structural and functional consequences of neonatal neuronal degeneration have not been adequately studied; therefore, the +/Lc mutant mouse may represent a good model to study the pathological evolution of progressive neurodegeneration in the central nervous system during aging (Hilber and Caston, 2001).

7. Conclusion

In the past 50 years of analysis, the +/Lc mutant has proved to be a valuable model for studying the mechanisms of cerebellar development, cerebellar physiology, and the role of the cerebellum in CNS function. Studies of +/Lc Purkinje cell, granule cell, and olivary neuron cell death have contributed insights into the developmental mechanisms that regulate cell number in the CNS. The behavioral studies in the +/Lc mutant have emphasized that the cerebellum plays a larger role in CNS function beyond the regulation of fine motor coordination. Although cerebellar function and the +/Lc mutant have been extensively characterized, there are still many unanswered questions whose resolution will continue to involve the Lurcher mutant. In particular, the cell-autonomous death of +/Lc Purkinje cells is likely to continue to be a useful system for studying the mechanisms of neuronal cell death since +/Lc Purkinje cells can serve as a model for the types of cell death programs that degenerating neurons undergo. The advantage of the +/Lc model for studying excitotoxic and autophagic cell death is that the initiating insult is a well-defined genetic lesion that affects a single cell type (Purkinje cells) that are easily studied in vivo and in vitro. Furthermore, there are relatively few animal models for chronic neurodegenerative diseases as opposed to acute models of stroke and trauma. In the area of cerebellar physiology, studies of the GluRδ2 receptor and the Lc mutation are beginning to provide important insights into the mechanisms of cerebellar learning. These studies may eventually

provide an important link to behavioral studies in the +/Lc mutant where it may eventually be possible to better understand the role that the cerebellum plays in higher CNS cognitive and emotional functions and to gain a better appreciation for plasticity in the CNS in response to the degeneration of a major component of the brain, the cerebellum.

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