Lobe X of the Cerebellum: A Natural Neuro-resistant Region

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Abstract

The cerebellum is an encephalic region classically known for its central role in the control of movement, although recent research has revealed its involvement in other cognitive and affective tasks. Several different pathologies are known to affect this structure, causing a wide range of behavioral and gait impairments. Intriguingly, although the neurodegenerative factors affect all Purkinje cells of the cerebellum uniformly, certain neurodegeneration patterns can be distinguished, in which some Purkinje cells persist longer than other cell types. Precisely, there is a cerebellar region, lobe X, which is more resistant to different types of neurodegeneration regardless of the injury. This work will review the main models of cerebellar degeneration, with special emphasis on this region, and aims to reveal a common origin for its resistance to neuronal death.

Keywords: Cerebellum, lobe X, neurodegeneration, neuro-resistance

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Introduction. The Cerebellum

Cerebellum is a Latin word that means "small brain". This structure was initially considered to be a less significant addition to the main brain because this organ only represents 10% of the total weight of the encephalon. Its side location to different nervous centers and pathways led to the traditional belief that its role was solely focused on the coordination and refinement of motor control (G. and Thomas, 2000); however, this classical conception has changed in recent decades. The cerebellum is currently considered one of the most important encephalic structures and its involvement in several cognitive and affective tasks is widely accepted.

The cerebella in mammals and birds, the most developed among vertebrates, have several structures that can be distinguished macroscopically (Fig. 1). At a first glance, a narrow and long structure called the vermis can be observed in the most central area and throughout the entire cerebellar sagittal plane. On the sides of the vermis, the cerebellar hemispheres are placed. Two small structures called the flocculus and the paraflocculus protrude from the hemispheres, and lastly, the entire cerebellar structure is attached to the brain via the cerebellar peduncles (Voogd and Glickstein, 1998).

The outermost part of the cerebellum is called the cerebellar cortex, which is extensive but small, comprising numerous convolutions or *folia*, which, unlike other structures of the central nervous system, cross the midline completely and are perpendicular to the sagittal plane (Delgado-García, 2001). These folds of the cerebellar cortex are grouped into lobes whose separations are called fissures. In birds and mammals, up to 10 lobes can be distinguished in the most central part (i.e. vermis; Fig. 1), which are numbered rostrocaudally with Roman numerals from I to X (Kandel, 2013).

This arrangement in the lobes allows the different cerebellar regions to be distinguished. The most primitive of all is the archicerebellum or vestibulocerebellum, associated with the flocculonodular area (lobe X and cerebellar floccules and paraflocculus; Fig. 1), which is

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equivalent to the folia that only appears in the most primitive vertebrates. This structure receives vestibular and visual inputs and sends projections to the vestibular nuclei, which are related to balance, vestibular reflexes, and eye movements. The second region is the paleocerebellum or spinocerebellum, comprised of the vermis (lobes I-IX) and part of the hemispheres (Fig 1.). The term *spinocerebellum* was chosen because it receives somatosensory and proprioceptive inputs of spinal origin, and projects towards descending pathways controlling the axial muscles. Finally, the phylogenetically youngest part of the cerebellum is the neocerebellum or cerebrocerebellum, which is the most evolved in primates, and corresponds to the large lateral hemispheres of the cerebellum (Fig. 1). Its main inputs come from the cerebral cortex, and its outputs also return to the motor, premotor, and prefrontal areas of the cortex. The best-known of the neocerebellar functions is the planning and execution of fine and precise movements (Brooks and Thach, 2011).

Although the role of the cerebellum is linked to motor control, it has recently been accepted that this organ could be associated with cognitive functions and emotional control (Galliano et al., 2013). The clues suggesting the involvement of these new functions began with electrophysiology experiments, as the activation of the fastigial nucleus activated neurons of the hippocampus and *vice versa* (Newman and Reza, 1979). Later on, using new imaging techniques, it was possible to verify the activation of different cerebellar regions during different affective/cognitive tasks (Petersen et al., 1988). Moreover, similar to how damage in the cerebellum may cause motor impairments, the existence of non-motor cerebellar disorders and pathologies has been confirmed, as in the case of autism and schizophrenia.

Cell types and Cerebellar Pathways

The cerebellar cortex comprises an exquisite cellular organization, as its neurons present an arrangement and connectivity that is extremely well conserved from the most primitive vertebrates, with little variation (Fig. 2). The cortex is divided into 3 layers, from the innermost

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to the outermost part, called the granular layer, the Purkinje cell layer, and the molecular layer (Fig. 2).

The granular layer is so named because it is made up of a large number of cells. The small granule cells contain a nucleus that stains intensely and constitute the most abundant neuronal type in the brain. In this layer, other cell types can be also found, such as Golgi cells (interneurons larger than granule cells), Lugaro cells, and brush cells (Sillitoe and Joyner, 2007). Curiously, the latter cell type is only found in lobes I, IX, and X in mammals (Mugnaini et al., 1997).

The Purkinje cell layer, as its name suggests, is made up of Purkinje cells somata. Purkinje cell axons contact deep cerebellar nuclei passing through the granular layer and their dendritic trees extend through the molecular layer. Purkinje cells are one of the largest cell types in the brain and are the only efferent neurons of the cerebellar cortex. In this layer, chandelier cells (Allin et al., 2001) and the somata of the Bergmann glia (Voogd and Glickstein, 1998) can also be found. The latter cell type is related to radial glia and is responsible for guiding Purkinje and granule cells to their final location during embryonic development (Levitt and Rakic, 1980).

The outermost stratum of the cerebellar cortex is the 500-µm thick molecular layer that contains the dendritic arborizations of the Purkinje cells oriented in a sagittal plane, although the cells are narrow considering their transverse axis. In addition to these huge dendrites, other interneurons such as the stellate cells are located here, towards the surface, as well as basket cells located toward the inside of the cerebellum. The repetitive structure of the cerebellar cortex is reinforced because the dendrites of both basket and stellate cells share the orientation of the Purkinje cell dendritic arbor (Voogd and Glickstein, 1998). Lastly, parallel fibers, the axons of granule cells that branch out and make contact with the dendrites of Purkinje cells, are also located in the molecular layer (White and Sillitoe, 2013).

The connectivity of the cerebellum has also been characterized in detail (Fig. 3; Beckinghausen and Sillitoe, 2019). As previously shown, afferent information can originate

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from the spinal cord, the brainstem, and even from the cerebral cortex (Ito, 2006). This information arrives mainly via the middle peduncle as mossy or climbing fibers. Additionally, the mossy fibers, originating from different regions, arrive at the granular layer forming structures called cerebellar glomeruli. In these formations, the mossy fibers make excitatory synapses on dendrites of granule cells in a ratio of 1 fiber to 400-600 granule cells. In these same structures, Golgi cell axons make inhibitory synapses on the dendrites of granule cells (Barmack et al., 1992a; 1992b). The climbing fibers project directly to the molecular layer and contact dendrites of the Purkinje cell in a 1:1 ratio and both mossy and climbing fibers send collaterals to neurons of the deep cerebellar nuclei (Voogd and Glickstein, 1998).

The granule cells, on the other hand, emit their axons into the molecular layer where they branch and give rise to parallel fibers, which extend several millimeters and contact hundreds of Purkinje cells (White and Sillitoe, 2013). In turn, stellate and basket cells contact the dendrites and the soma of Purkinje cells, respectively (Miall, 2013).

Finally, the only efferent fibers of the cerebellar cortex are Purkinje cell axons. Thus, all the information processed here leaves these fibers and reaches the deep cerebellar nuclei, from where it travels to the different motor and cognitive centers in the brainstem and cerebral cortex (Sillitoe and Joyner, 2007).

Regions of the Cerebellar Cortex

The cerebellum has a structure that has been highly conserved throughout evolution and a histology that appears uniform throughout its different lobes (Larsell, 1952). However, beyond this uniformity, there is some heterogeneity in the form of parasagittal bands (Hawkes, 1997; Voogd and Glickstein, 1998; Apps and Hawkes, 2009). These bands can be distinguished by using certain immunocytochemical markers such as Zebrin II, named after its striped pattern in terms of its distribution across the cerebellar cortex (Brochu et al., 1990). Precisely because of these banding patterns, the four aforementioned transversal regions can be distinguished

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(Fig. 1): anterior (lobes I-V), central (VI-VII), posterior (VIII-IX), and nodular (X). These banding patterns consist of clusters of Purkinje cells that are highly immunoreactive to Zebrin II and areas with little or no immunoreactivity. The anterior zone has hardly any cells that are strongly positive to Zebrin II. However, half of the Purkinje cells in the posterior zone are usually strongly labeled with Zebrin II and the other half are not. Lastly, all Purkinje cells in the central and nodular areas are highly positive for this protein (Sarna et al., 2003). This cerebellar topography appears in prenatal development and remains stable during postnatal growth (Larouche et al., 2006; Marzban et al., 2007). Finally, similar compartmentalization exists when using other markers for granule cells (Hawkes and Turner, 1994; Ozol and Hawkes, 1997), interneurons (Sillitoe et al., 2008; Chung et al., 2009), mossy fibers (Armstrong et al., 2009), and climbing fibers (Voogd et al., 2003).

In addition to Zebrin II in mice, other parasagittal banding patterns have been generated using other markers and in several different species. For this reason, in 2011 a study was carried out on the colocalization of some of the most used markers in more than 20 different species of mammals and birds (Marzban and Hawkes, 2011). In particular, this analysis was carried out on the postero-nodular area of the cerebellum, because the largest number of Zebrin II-positive bands is known to exist in this area. Through this work, it was concluded that this banding pattern is highly conserved in mammals and in some birds. Concerning the other antigens studied, it was confirmed that the parasagittal bands revealed by the other markers were sometimes coincident with the Zebrin II-positive bands. But, on other occasions, their labeling appeared in those bands that did not express Zebrin II intensely. Finally, other markers formed mixed patterns in which they sometimes coincided, or did not, with Zebrin II expression (i.e. HSP25, as will be discussed below).

We have highlighted the heterogeneity of the cerebellar cortex, classically considered to be extremely uniform, because the different regions in which it can be divided will help us ultimately to distinguish other biological functions and characteristics. It can be anticipated that

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some of these additional expression patterns are also found by analyzing heat shock proteins (HSPs). More importantly, related to the objectives of this work, is that these regions also show a selective vulnerability to neurodegeneration.

Models of Cerebellar Degeneration

The damaging factor causing different neurodegenerative diseases is usually uniform throughout the central nervous system or in one of its regions and can be either a mutation, a toxin, or the effects of aging, among others. However, some neuronal populations rapidly degenerate in the face of these factors, while others are less vulnerable and retain their functions (Double et al., 2010). In relation to this selective vulnerability, a given tissue may be highly susceptible to certain neurodegenerative factors while not being susceptible to others. However, as we will see below, there are regions of the central nervous system that show a lower constant vulnerability to all the neurodegenerative factors mentioned; that is why we have called this phenomenon neuroresistance.

Precisely, a very striking example of neuroresistance can be found in the cerebellum (Sarna and Hawkes, 2003). Despite the similarity of all Purkinje cells and the repetitive structures in their connectivity, neurodegeneration does not occur uniformly throughout the cerebellar cortex, giving rise to patterns of neuronal death that are not specific to a particular pathology, but common among a wide range of pathologies (Chung et al., 2016). Previously, we discussed the existence of different regions exhibiting different neurochemical phenotypes, such as those positive or negative for Zebrin II staining. Furthermore, we mentioned that these regions were related to selective vulnerability to neurodegeneration. Hence, a common pattern of neurodegeneration exists depending on the cerebellar region: the anterior area is the most sensitive and generally the first to degenerate; an intermediate susceptibility can be found in the central and posterior areas; and, lastly, the greatest resistance appears in the nodular region, mainly comprising lobe X. The diseases that show this pattern of anteroposterior vulnerability

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are extremely varied. Some are derived from mutations, such as spinocerebellar ataxia (Clark et al., 1997), saposin C deficiency (one of the causes of Gaucher disease; Yoneshige et al., 2010), ataxia telangiectasia (Tavani et al., 2003), Niemann-Pick A/B and C disease (Sarna et al., 2001; Sarna et al., 2003), and multiple system atrophy (Kume et al., 1991). Others may be due to toxins, such as alcohol (Torvik and Torp, 1986), hypoxia-ischemia (Biran et al., 2011), or even normal aging (Andersen et al., 2003). For some of these diseases, there are animal models that reproduce them, such as Niemann-Pick C1 (NPC1) disease, which shows the same pattern of neurodegeneration as in humans. In addition to these pathologies, this selective vulnerability has also been found in specific rodent models such as the Leaner mouse (Heckroth and Abbott, 1994), the Toppler mouse (Duchala et al., 2004), the Robotic mouse (Isaacs et al., 2003), the shaker rat (Tolbert et al., 1995), the Lurcher mouse (Armstrong et al., 2011), and the PCD mouse (Wang and Morgan, 2007; Baltanás et al., 2021).

As we are unable to cover all animal models with cerebellar damage, we will only explain the genetic models for which the neurodegeneration patterns have been clearly described.

Tottering, Leaner, and Nagoya Models

The Tottering mutant mouse presents a pathology caused by a spontaneous mutation in the α_{1A} subunit of a Ca^{2+} channel. The mutation *tg* is recessive and there are two variants of it, which define two additional models homologous to the previous one: the tg^{la} variant corresponding to the Leaner mutant and the tg^{rol} that has given rise to the Nagoya mouse model (Fletcher et al., 1996). These three models are slightly different and overlap in some features, and although the original mutant, the Tottering mouse, is a model of absence epilepsy, they all suffer from ataxia due to Purkinje cell death. They differ in the severity of the symptoms of ataxia, with the mildest occurring in the Tottering mouse, followed by the Nagoya mouse, and, finally, the most severe symptoms appearing in the Leaner mouse (Herrup and Wilczynski,

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1982). The Leaner mouse will be described in more detail because it presents the most aggressive symptomatology of the *tg* mutation.

Like its homolog, the Leaner mutant mouse presents a pathology due to a spontaneous mutation in the α_{1A} subunit of a Ca^{2+} channel. The tg^{la} mutation is recessive and is caused by a base substitution that disrupts the transcription of the open reading frame, resulting in a truncated and nonfunctional protein (Doyle et al., 1997). The affected subunit is mainly expressed in Purkinje cells, and this mutation is the genetic basis of the Leaner mice, which suffer from ataxia. In addition, this same defect also exists in humans and is known to cause hemiplegic migraines, episodic ataxia type 2, and spinocerebellar ataxia type 6 (Lorenzon et al., 1998).

Leaner mice present cerebellar ataxia as early as postnatal day 10 (P10). Morphological analyses of the cerebellum of Leaner mice showed that the degeneration affects the Purkinje cells as well as granule and Golgi cells, especially in the anterior region. The onset of ataxia, at P10, coincides temporally with the degeneration of granule cells, although Purkinje cell loss begins at P40 (Herrup and Wilczynski, 1982). Also, a banded expression pattern of a vitamin D-dependent calcium-binding protein has been verified in this model: the anterior zones present a lower expression of this protein, while the nodular zone has the highest expression of the entire cerebellar cortex (Heckroth and Abbott, 1994). This is a pattern comparable with that of Zebrin II, which coincides with a lower vulnerability to neurodegeneration in the posterior and nodular zones compared with the more anterior regions.

Toppler Model

The discovery of the Toppler mouse is relatively recent compared to other cerebellar degeneration mutants. Hence, this model was described for the first time in 2004 as a result of a spontaneous mutation in the FVB strain of mice (Duchala et al., 2004). FVB mice had been bred for many generations without abnormalities, but suddenly four pups of the same litter began to show ataxia and abnormal postures at 4-5 weeks of age. These offspring were used as

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parents for other crosses, revealing that their pathology was hereditary. As their impairments were motor and postural, a cerebellar or demyelinating origin was suspected. Later, histological studies revealed an evident loss of Purkinje cells (Duchala et al., 2004). Surprisingly, at P30 apparently, all the Purkinje cells of lobe X survived (Fig. 4A), although their morphology was abnormal and similar to that of neurons in the regions most affected by the neurodegeneration (Duchala et al., 2004).

Robotic Model

The origin of the Robotic mouse is particular, as it is an autosomal dominant mutant model that is spontaneous in origin, but induced. Like the other models described here, the Robotic mouse has an ataxic gait and shows a loss of Purkinje cells in early adulthood, and also develops cataracts (Isaacs et al., 2003).

Mutant mice are a useful tool for understanding the functions of different genes in mammals and one way to develop these mice is by using a mutagen. In the case of the Robotic model, male C3Heb/Fej mice were injected with the mutagen *N*-ethyl-*N*-nitrosourea and were crossed with females of the same strain. The resulting mice were tested for dominant or codominant pathologies and crossed with each other to discover additional recessive traits (Hrabé de Angelis et al., 2000). This experiment gave rise to the Robotic mouse and, after carrying out genetic mapping, it was found that its pathology was due to a nonsense mutation, a base substitution that results in a stop codon, in the *Af4* gene (Isaacs et al., 2003). The involvement of *Af4* is an additional unique feature of this model, as it is a gene involved in leukemia and, although the relationship between the gene and the disease is not fully understood, *Af4* knock-out mice present an abnormal development of B and T lymphocytes (Isnard et al., 2000). In terms of gene function, the resulting protein belongs to a family of transcription cofactors that are usually translocated in childhood leukemia (Bitoun and Davies, 2009).

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To study the relationship between *Af4* and neurodegeneration, several studies were carried out to show that *Af4* was expressed mainly in Purkinje cells. Its truncated form in Robotic mice accumulates and gives rise to neurodegeneration. In addition, to study how neuronal loss progressed in Robotic mice using immunohistochemical techniques, calbindin expression was analyzed in coronal and sagittal slices of the cerebellum. Once again, a pattern of bands was observed, as in the case of Zebrin II and/or other calcium-binding proteins in other models. Moreover, an anteroposterior progression of neurodegeneration could be observed in sagittal planes, with the anterior regions degenerating much earlier than the nodular and posterior areas (Fig. 4B).

Shaker model

This is a rat model rather than a mouse model. The Shaker rat was first described in 1992 as a hereditary model that is not sex-linked and develops severe ataxia with age. After studying the cause of this disorder, an absence of Purkinje cells was found, especially in the anterior lobes, which were 52% smaller in Shaker rats than in the controls (La Regina et al., 1992).

A few years after the first publication, using the 5th and 6th generation of the original rats, two parallel research papers were published: in one of them behavioral studies were performed (Wolf et al., 1996) and in the other, sections of the cerebellum were marked with calbindin by immunoprecipitation (Tolbert et al., 1995). In the first paper, two variants in the pathology of the shaker rat were found, termed mild or strong. The mild variant corresponded to 77% of the total number of rats, being those animals that only presented ataxia. The strong variant constituted the remaining 23% suffering from both ataxia and a whole-body tremors at 3 months of age. In addition, rats with mild tremors never develop the tremor characteristic of rats with severe tremors (Wolf et al., 1996). The second article analyzed the distribution of Purkinje cells at different ages and confirmed at 11 months of age what we have already described in previous models: an anteroposterior loss of Purkinje cells in lobes I to IX, but lobe

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X remained undamaged and, the authors literally said, “In lobule X, the distribution of Purkinje Cells appeared very similar to that seen in normal rats” (Fig. 4C; Tolbert et al., 1995).

Lurcher Model

The Lurcher model presents a cerebellar degeneration caused by a mutation that was first described in 1960 (Phillips, 1960). Heterozygous Lurcher (+/*Lc*) mutants suffer the death of all Purkinje cells, among other cell types, from P10 to P65, resulting in loss of cerebellar function. The homozygous Lurcher mutation is lethal; the embryos develop apparently normal, but the pups die within a few days of birth because they are unable to suckle (Cheng and Heintz, 1997).

This murine model has a base-shift mutation in the δ2 glutamate receptor gene, which causes the receptor to behave like a small Ca^{2+} channel, a function hypothetically lost during evolution (Vogel et al., 2007). The possible mechanisms of Purkinje cell death that have been described are varied: necrotic, autophagic, apoptotic, and excitotoxicity due to high levels of glutamate derived from high levels of Ca^{2+} (Vogel et al., 2007). However, the exact cause of this neuronal loss is not known (McFarland et al., 2007).

What is known, however, is the degenerative process it undergoes. Neurodegeneration in the Lurcher mouse begins around the second postnatal week in the entire cerebellum. However, at P25-P36 this cell death is accelerated in the anterior zone (lobes I to V), becoming evident later in the central and posterior zones (lobes VI to VII and VIII to IX, respectively). Finally, the neurodegeneration of the nodular area (lobe X) is somewhat delayed with respect to the rest of the regions (Fig. 4D). In addition, and parallel to this selective neurodegeneration, the existence of parasagittal expression bands of the heat shock protein HSP25 in surviving Purkinje cells has been described (Duffin et al., 2010).

NPC1 Model

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NPC1 disease is an inherited recessive lipid storage disorder caused by a defect in intracellular cholesterol transport and homeostasis (Pentchev et al., 1986). In humans, the disease causes hepatosplenomegaly at birth and, in addition, children with the disease develop ataxia, psychomotor impairments, and/or dementia, dying at 5-15 years of age (Vanier et al., 1991). Fortunately, for the study of this pathology, there are two murine models generated by a spontaneous mutation in the same gene as in humans, the *Npc1* gene. Furthermore, the murine mutated gene corresponds to the same complementary group as the human *NPC1* gene (Akaboshi et al., 1997).

NPC1 mouse pups are indistinguishable from their wild-type littermates. However, as they reach adulthood they begin to develop ataxia, with Purkinje cells degeneration evident from P40. However, as in the other models, neurodegeneration is not uniform throughout the cerebellum: the more rostral areas of the vermis are more sensitive to neurodegeneration than the rostral areas, with the X lobe being the most resistant region of all (Fig. 4E; (Sarna et al., 2003).

As mentioned above, the nodular zone has a substantial expression of Zebrin II, and different patterns of degeneration can be defined accordingly. In the case of the NPC1 model, when analyzing the degeneration of Purkinje cells in coronal sections, it was observed that the first neurons to die are those that do not express Zebrin II (Sillitoe and Hawkes, 2002). In addition, a recent bioinformatic study cross-checked available data from other *in situ* hybridization experiments, to find genes that were more highly expressed in the nodular zone and therefore could influence the increased neuroresistance of this region (Chung et al., 2016). The result of this research showed that the expression of the heat shock protein HSP25 (referred to as HSPB1 in this review) was higher in lobe X than in the rest of the lobes of NPC1 mice. Moreover, the phosphorylation of HSP25 significantly increased its neuroprotective properties (Chung et al., 2016).

Nervous Model

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The Nervous mouse is a model that suffers from Purkinje cell degeneration due to an autosomal recessive mutation (Sidman and Green, 1970). Its degeneration starts rapidly and then slows down after two months (Sidman and Green, 1970). The *Nervous* mutation is known to be located on chromosome 8 (Mullen and LaVail, 1975), but the specific gene altered is still unknown. When this model was first studied, the membrane-bound protein P_{400} was found to be absent in nerve cells (Mallet et al., 1976), and it was then suspected that the *Nervous* mutation was related to this protein. However, as P_{400} is found in the dendrites and cell bodies of Purkinje cells (Mikoshiba et al., 1985), most of the cerebellar degeneration models show low levels of this protein (Mikoshiba et al., 1985), as well as in the Nervous model.

Although this mouse model undergoes severe neurodegeneration, about 10% of its Purkinje cells remain alive after the acute neurodegenerative phase, except for some sporadic death (Sotelo and Triller, 1979). Again, the distribution of resistant Purkinje cells is not random: in the hemispheres, 90% of Purkinje cells die, but in the vermis, only 50% are lost (Sidman and Green, 1970). Furthermore, apart from this distribution, the most ventral part of the vermis is an additional area of surviving Purkinje cells, this region being composed of the lobe I, lobe X, and the ventral part of the lobe IX (Wassef et al., 1987). These results are consistent with a later study on the compartmentalization of Purkinje cell death and Zebrin II expression (Edwards et al., 1994). Zebrin II was found to be expressed in the most vulnerable Purkinje cells in Nervous mice (Edwards et al., 1994). This explained why lobe I was less vulnerable to neurodegeneration, due to its null expression of Zebrin II. Furthermore, in this case, the posterior lobes would theoretically be more susceptible to neurodegeneration due to their higher expression of Zebrin II. Despite this presumed vulnerability, Purkinje cells of lobe X do not degenerate as expected, thus supporting an additional source of resistance of this region apart from the distribution of Zebrin II (see below).

PCD model

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This is a model of cerebellar degeneration caused by an autosomal mutation in the *Ccp1* gene. Recent research shows that this genetic defect is also present in humans, causing childhood-onset neurodegeneration with cerebellar atrophy (also called CONDCA), a disease with the same pathogenic symptoms as the animal model (Shashi et al., 2018, Karakaya et al., 2019, Sheffer et al., 2019).

The *pcd/pcd* pups are somewhat smaller than their wild-type littermates, although they show no other anomalous signs until P20 when they begin to develop ataxia due to progressive Purkinje cell death (Mullen et al., 1976). The PCD model also suffers from the death of other neuronal types such as some thalamic populations (O'Gorman and Sidman, 1985), retinal photoreceptors (Mullen and LaVail, 1975), and the mitral cells of the olfactory bulb (Greer and Shepherd, 1982). In addition, the spermatozoa of PCD males have an abnormal morphology that causes sterility. Females, on the other hand, are fertile, but unable to adequately care for their offspring (Wang and Morgan, 2007). It is also worth noting that, although cerebellar neurodegeneration is evident in PCD mice from P20 onwards, two stages of neuronal damage in the cerebellum have been shown to exist: the pre-neurodegenerative and the degenerative stage (Muñoz-Castañeda et al., 2018). The first stage starts at P15 and ends at P18 and is characterized by nuclear, cytological, and morphological alterations in the still-living Purkinje cells (Baltanás et al., 2011). Then, with the onset of death of these neurons, the degenerative stage is considered to begin (Muñoz-Castañeda et al., 2018).

The *pcd* mutation arose spontaneously in the C57BR/cdJ strain and affects a regulatory region of the *Ccp1* gene, located on chromosome 13 (Mullen et al., 1976; Fernandez-Gonzalez et al., 2002), leading to the almost complete disappearance of its transcription (Fernandez-Gonzalez et al., 2002; Chakrabarti et al., 2006). The protein affected by this mutation is called “ATP/GTP-binding protein 1” or “axotomy-induced nervous system nuclear protein 1” (AGTPB1 or NNA1, respectively). The second name is due to the origin of its discovery, as it was found to be expressed after an axotomy of the sciatic nerve, and its expression was

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associated with axonal differentiation and regeneration processes (Harris et al., 2000). Considering its function, this enzyme is a peptidase capable of hydrolyzing the carboxy-terminal ends of the glutamate chains of α and β tubulins, and because of this, it belongs to the family of cytosolic carboxypeptidases. Thus, its most frequent name is "cytosolic carboxypeptidase type 1" or CCP1. This enzyme regulates the glutamylation of tubulins and if it fails, microtubules become hyper-glutamylated thus causing an excessive instability of the cytoskeleton and ultimately Purkinje cell death (Rogowski et al., 2010; Muñoz-Castañeda et al., 2018).

Once again, in the PCD mouse, a pattern of selective neuronal degeneration is observed, such that lobe X of the cerebellum emerges as a neuroprotected region. In this sense, at P30, when the rest of the Purkinje cells have almost disappeared, lobe X remains virtually intact (Baltanás et al., 2013). Furthermore, at 9 months of age, long after the end of degeneration, some Purkinje cells can still be detected in this lobe (Mullen et al., 1976).

Tambaleante model

The Tambaleante model is another mouse that suffers a recessive mutation (see below; Wassef et al., 1987). This mouse differs from the previous models in that it does not show a clear pattern of neurodegeneration. In this case, the death of Purkinje cells occurs randomly, with some groups of these neurons remaining alive in bands at 2.5 months of age (Wassef et al., 1987). Although the resistant properties of lobe X of the Tambaleante model are not as marked as in the previous models, the surviving Purkinje cells of this lobe remain alive for a few days longer than in the rest of the cerebellar cortex.

As for the mutation, it is located on chromosome 9 and consists of an adenosine-to-guanine transition, resulting in a glycine-to-glutamate substitution that alters the function of the E3 ubiquitin ligase protein HERC1 (Mashimo et al., 2009). The mutation causes overexpression of the altered protein, the accumulation of which leads to Purkinje cell death (Mashimo et al.,

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2009). The E3 ubiquitin ligase HERC1 belongs to the ubiquitin-proteasome system, which plays a role in protein degradation, a key component in neuronal homeostasis (Ruiz et al., 2016).

Other Non-genetic Models

In addition to the aforementioned mutants, other non-genetic models also show antero-posterior neurodegeneration, with lobe X being the most resistant region of the cerebellum. The list of these examples would be excessively long, but it is worth mentioning that this resistance has been reported with toxins, some of which have the same effects in humans. For example, alcoholics have reduced Purkinje cell populations in the superior and middle regions of the cerebellum (Torvik and Torp, 1986). Cytosine arabinoside is another example: high doses of this drug were given to patients with leukemia or lymphoma and four of them developed cerebellar degeneration. Postmortem analyses revealed that Purkinje cells were relatively preserved in the posterior lower portions of the cerebellum (Winkelman and Hines, 1983). Methotrexate is another chemotherapeutic drug that was administrated to patients with acute lymphoblastic leukemia. Survivors of the disease showed hypoplasia of the cerebellar vermis caused by the drug over lobes I-VII, with lobes VI and VII being the most affected. Although no specific data on lobe X degeneration were shown, the most caudal part of the cerebellum remained undamaged (Ciesielski et al., 1994).

In addition to toxicants, another example of resistance to neurodegeneration in the posterior lobes is observed in hypoxia-ischemia models: rat pups subjected to hypoxia-ischemia developed cerebellar injuries. This degeneration was studied in two groups: lobes III-IV and lobes VIII-IX. Again, the posterior lobes were found to suffer less than the anterior lobes (Biran et al., 2011), although lobe X was not explicitly studied. Finally, the cerebella of 19 Caucasian males (19-84 years old) were studied, and the anterior lobes were found to be the most affected by aging (Andersen et al., 2003).

Possible Causes of the Neuroresistance of Lobe X

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It has been shown in these and other models of cerebellar degeneration that Purkinje cell death does not occur uniformly. Indeed, there are well-established patterns of degeneration in the cerebellum, and there are almost no examples in which Purkinje cells die randomly. Precisely, the banded patterns of neurodegeneration were first clearly described in the PCD model, together with the Nervous and the Tambaleante mice (Wassef et al., 1987). Moreover, several patterns of Purkinje cell vulnerability have been shown (Sarna and Hawkes, 2003), with the relationship between Zebrin II expression patterns and neurodegeneration being one of the most striking examples. However, this relationship is not rigorously consistent with a putative neuroprotective function for Zebrin II. Thus, in some cases, it is true that Purkinje cells that do not express Zebrin II are more sensitive to neuronal degeneration, as in the Tambaleante mouse (Fletcher et al., 1996). By contrast, there are other examples where Purkinje cells expressing Zebrin II are more vulnerable to cell death, as in the Nervous mouse described above (Wassef et al., 1987). In this sense, it seems that the presence of Zebrin II only can inform us about specific pathways of degeneration that converge (or not) in Purkinje cell death, not the presence of this protein is a strict marker of resistance (Sarna and Hawkes, 2003), as traditionally thought. This variable pattern of degeneration related to Zebrin II expression may be caused by a specific stress that acts uniformly on all Purkinje cells of the cerebellum, and depending on the nature of this weakening, some neurons are more sensitive than others to neuronal degeneration. In this case, the presence or absence of Zebrin II could be beneficial or detrimental. A clear example of uniform stress throughout the cerebellum, but with heterogeneous neurodegeneration, is found in the Tambaleante mouse. This model suffers from a mutation in a gene encoding a calcium channel (Fletcher et al., 1996). Several studies have shown that all Purkinje cells express similar levels of mRNA of this mutated gene, but not all of them suffer the same neuronal degeneration, and in this case, only those that do not express Zebrin II die (Fletcher et al., 1996). This also agrees with the case of the NPC1 mouse, in which the neurodegenerative factor, accumulations of cholesterol vesicles, is uniform in all Purkinje cells,

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and the first to degenerate are also Zebrin II-negative (Sarna et al., 2003). In these two cases, Zebrin II-positive Purkinje cells are less vulnerable to neurodegeneration, which may lead us to consider Zebrin II as a neuroprotective factor, especially considering that the Purkinje cells of lobe X express it abundantly (Sarna and Hawkes, 2003). Nevertheless, in the Nervous mouse Zebrin, II-positive Purkinje cells are more susceptible to neuronal degeneration (Wassef et al., 1987), which rules out possible neuroprotective effects of this protein, at least in general. In this sense, it would be reasonable to think that all the Purkinje cells in lobe X of the Nervous mouse would die, since they all express Zebrin II. However, there are some exceptions in areas with Zebrin II-positive cells that do not degenerate, precisely in lobes IX and X (Edwards et al., 1994), and to a lesser extent in the floccules and paraflocculles and in lobe VI. Therefore, as previously mentioned, Zebrin II expression bands define different Purkinje cells populations that are different from others, showing different susceptibilities to different neurodegenerative processes and types of cellular stress, but not a strict predisposition toward survival or cell death.

By contrast, the nodular zone always shows more resistant Purkinje cells regardless of Zebrin II-related patterns and sensitivity (see above for the case of Nervous mice). Therefore, it cannot be said that the neurons of this region are more susceptible to some impairments but not to others: lobe X is consistently more resistant regardless of the neurodegenerative factor. Hence, factors in addition to Zebrin II must be present to confer such resistance, and although not fully understood, HSP25 expression is proposed as a key mechanism for neuroprotection.

Indeed, HSP25 expression has been confirmed in the resistant regions of several models of cerebellar degeneration, such as Weaver (Armstrong and Hawkes, 2001), Lurcher (Duffin et al., 2010), and NPC1 mice (Sarna and Hawkes, 2003). Moreover, Chung et al. in 2016 confirmed the potent neuroprotective effect of HSP25 when phosphorylated at some of its serine residues. This enzymatic reaction occurs naturally in some neurodegenerative processes, especially in lobe X (Chung et al., 2016). The final evidence for the neuroprotective properties

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of HSP25 is its slight expression in the central zone of the cerebellum, in particular in lobe VI (Praggastis et al., 2015). In this lobe, a certain resistance to Purkinje cell death has been detected in models such as the NPC1 (Praggastis et al., 2015). This low vulnerability is not always detected and is not as evident as that of lobe X. Thus, there is a cause-effect relationship for HSP25 expression levels and neuroprotection. Interestingly, HSP25 has several functions, but as a small heat shock protein, one of them should be acting as a chaperone (Kostenko and Moens, 2009). We hypothesize that its constitutive expression shown in lobe X (Chung et al., 2016) might indicate that its functions in this particular lobe are more important than those of the other lobes. This idea will be developed further below.

In any case, the underlying cause of the resistance of lobe X may be complex and multifactorial. Therefore, in addition to the differential expression of either Zebrin II or HSP25, many other peculiarities differentiate lobe X from the rest. In terms of functionality, Purkinje cells in lobe X have been found to exhibit more regular impulse firing and less adaptation to repeated stimuli than lobes III-V (Kim et al., 2013). In addition, a transcriptomic study compared mRNA expression between lobes III, VI, and X in both wild-type animals and NPC1 mutants (Martin et al., 2019). It was found that between lobes III and VI there were around 180-350 differentially expressed genes. Surprisingly, when comparing lobe III or VI versus lobe X, the difference in expression increased to 1300-1500 genes (Martin et al., 2019). This gives us an idea of the myriad of possible factors why lobe X Purkinje cells survive longer. Thus, some of the additional potential reasons responsible for such neuroprotection are increased calcium signaling, increased Sonic Hedgehog signaling, and increased glutamate buffering (Martin et al., 2019). Last but not least, when comparing gene expression in NPC1 and wild-type mice, a generalized increase in immune and inflammatory response-related genes was observed, but independently of the lobes (Martin et al., 2019). These data, together with other studies, show that it is not the inflammatory response that underlies Purkinje cell resistance patterns, but some

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of the factors discussed above, which emphasizes the question of why lobe X is so different from the rest while being more protected.

Why Lobe X is Different from Other Lobes?

We have found that lobe X of the cerebellum is more resistant than the other lobes in numerous animal models suffering Purkinje cell death. As described above, some examples are the Leaner (Heckroth and Abbott, 1994), Toppler (Duchala et al., 2004), Robotic (Isaacs et al., 2003), Shaker (Tolbert et al., 1995), Lurcher (Duffin et al., 2010), NPC1 (Praggastis et al., 2015), and PCD (Mullen et al., 1976) models. In addition, in some of these animals, such as NPC1 (Sarna et al., 2003), Weaver (Armstrong and Hawkes, 2001), and Lurcher (Duffin et al., 2010) mice, an increased expression of HSP25 has been detected in lobe X, which suggests this protein is a clear candidate responsible for such resistance. However, the million-dollar question remains: Why is lobe X more protected against neurodegeneration than the rest of the lobes?

To try to answer this question, we can start by recalling the abovementioned expression of HSP25, specifically in the surviving Purkinje cells of the NPC1 mouse (Sarna and Hawkes, 2003). The functions of HSP25 in the cerebellum are unclear, but it has been shown in non-neuronal cell lines that this protein acts as a chaperone during heat stress (Jakob et al., 1993) and also regulates the organization of actin filaments during oxidative stress (Lavoie et al., 1993; Huot et al., 1996). In this sense, to date, the only functions that have been described for HSP25 point to a protective nature, similar to that of the rest of the small HSPs, which are involved in stabilizing other proteins under stress conditions (Carver et al., 1994; Boelens and de Jong, 1995; Sun and MacRae, 2005). It stands to reason, that its expression should be specifically induced by a damage/stress factor. However, the expression of HSP25 in lobe X of wild-type mice is not null, unlike in other lobes (Armstrong et al., 2000; Duffin et al., 2010; Marzban and Hawkes, 2011). Thus, if the only function of HSP25 were to act as a chaperone

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against cellular stress, it would not make sense for it to be constitutively expressed in the nodular or, to a lesser extent, in the central region (mainly in lobes X and VI, respectively) where no neurodegeneration exists. A dichotomy arises here: on the one hand, HSP25 may have other functions not described so far (besides being a chaperone) or, on the other hand, these regions need to be further protected because they may be more important than the rest of the cerebellar cortex.

In the introduction, we mentioned that lobe X, together with the cerebellar flocculi, constitute the flocculonodular zone (Brooks and Thach, 2011). This zone is the most primitive region of the cerebellum and corresponds to the only cerebellar folia possessed by the most primitive vertebrates (Brooks and Thach, 2011). In addition, the flocculonodular zone receives vestibular and visual inputs, and its outputs are projected to the vestibular nuclei, which defines its functions: it participates in balance, vestibular reflexes, and eye movements (Brooks and Thach, 2011). In this sense, it is logical to think that, evolutionarily, the protection of the flocculonodular zone has been more of a priority than that of other cerebellar areas, as it is one of the most primitive regions and responsible for the most basic functions of the cerebellum. In other words, it is possible that during the evolution the primary functions of maintaining body posture or balance (paleocerebellum) have tended to be more protected than “additional” fine motor skills (cerebrocerebellum).

Moreover, we could also attribute the resistance of lobe X to its simplicity or its different functioning. Phylogenetically more advanced cerebellar regions perform more complex processing, enabling fine and precise movements to be planned and executed (Brooks and Thach, 2011). This processing involves greater neuronal complexity and richness in gene expression, and this requirement may make the more specialized Purkinje cells phylogenetically in more advanced regions more susceptible to neuronal degeneration factors. Neuronal activity necessarily requires chromatin to be active and arranged as euchromatin to be read (Valero et al., 2006). Euchromatin is more exposed than heterochromatin, the former

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being more susceptible to DNA damage (Takata et al., 2013) and the latter more protected (Cann and Dellaire, 2011) because the compaction of DNA together with non-histone proteins acts as a shield against damage (Falk et al., 2008). Thus, more complex Purkinje cells carrying out more elaborate processing are likely to have higher gene expression. In these neurons, the euchromatin/heterochromatin ratio would be higher and they would therefore be more susceptible to different neurodegenerative factors. In fact, it has been proven that the accumulation of DNA damage in Purkinje cells of PCD mice is one of the causes of their death and that chromatin compaction is a defense mechanism, although it eventually prevents its repair (Baltanás et al., 2011). Another example of this relationship between cell complexity and vulnerability is found in the mitral cells of the olfactory bulb. These neurons are highly susceptible to DNA damage precisely because of their high metabolic and bioelectric activity (Friedman and Strowbridge, 2000; Lowe, 2003; Djurisic et al., 2004). Therefore, a future line of research could be to study the relationship between euchromatin and heterochromatin in Purkinje cells of lobe X compared with the other lobes.

The selective resistance to neuronal damage of the lobe X is especially remarkable, as it is present in many animal models of different natures. The study of its natural protection or reduced vulnerability may constitute an interesting piece of research to find putative therapies against neurodegenerative diseases, which are becoming one of the main health problems in our society.

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