

Biological clues on neuronal degeneration based on theoretical fits of decay patterns: towards a mathematical neuropathology

Lazaros C. Triarhou

Econo-Koskinas Wing for Integrative and Evolutionary Neuroscience, University of Macedonia, Thessaloniki, Greece

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Abstract

The application of the best mathematical fit to quantitative data on cell death over time in models of nervous abiotrophies can yield useful clues as to the cellular properties of degenerative processes. We review data obtained in two neurogenetic models of movement disorders in the laboratory mouse, the 'Purkinje cell degeneration' (pcd) mutant, a model of cerebellar ataxia, and the 'weaver' (wv) mutant, a combined degeneration of multiple systems including the mesostriatal dopaminergic pathway. In the cerebellum of pcd mice, analyses of transsynaptic granule cell death subsequent to the genetically-determined degeneration of Purkinje cells show that granule neuron fallout follows a typical pattern of exponential decay. In the midbrain of weaver mice, regression fits show that dopaminergic neuron fallout combines two independent components, an initial exponential decay, superceded by a linear regression, with a threshold around 100 days. The biological connotations of such analyses are discussed in light of the empirical observations and the theoretical simulation models. The theoretical connotations may link neuron loss to specific cellular idiosyncracies in elucidating the pathogenesis of chronic neurodegenerative disorders, including Parkinson's disease.

Key words: cerebellum, basal ganglia, neurological mutations, Purkinje cell degeneration (pcd), weaver (wv), mathematical models, exponential decay, linear regression.

Introduction

The present study reviews results obtained from quantitative analyses of neuron losses across the lifespan in neurogenetic mouse models of movement disorders, namely, mutant mice with cerebellar and basal ganglia defects. By applying mathematical curves to the patterns of cell decay, one can gain insight into certain biological characteristics of neuronal degeneration.

Neuron loss can be either a normal phenomenon associated with ontogeny [14,36] or a pathological

manifestation in aging and a variety of degenerative disorders [15,26]. The unavailability of human material to study neuronal death by means of standardized quantitative methods at different stages of disease progression renders the contribution of experimental animals with neurodegenerative diseases particularly valuable.

In the adult cerebellum [16,40,41] granule cells are settled in the internal granular layer beneath the Purkinje cell layer. The axons of granule cells, known as parallel fibers, bifurcate during cerebellar ontogeny

Communicating author:

Lazaros C. Triarhou, University of Macedonia, 156 Egnatia Ave., Bldg. Z-312, 54006 Thessaloniki, Greece, phone: +30 2310 891 387, fax: +30 2310 891 388, e-mail: triarhou@uom.gr

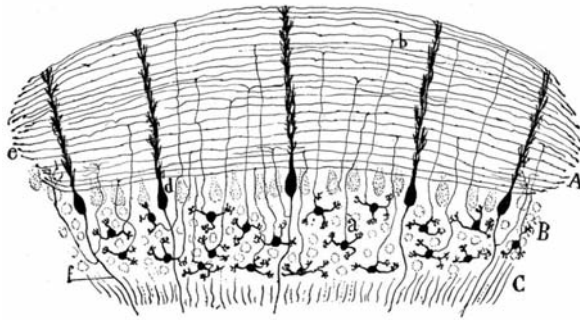


Fig. 1. Longitudinal section of a cerebellar folium. Semidiagrammatic figure. A – molecular layer, B – nuclear layer, C – white matter; a – axon of granule, b – its bifurcation in the molecular layer; e – its termination in the end-point of each cerebellar folium, d – Purkinje cell. After Ramón y Cajal and Tello y Muñoz [41].

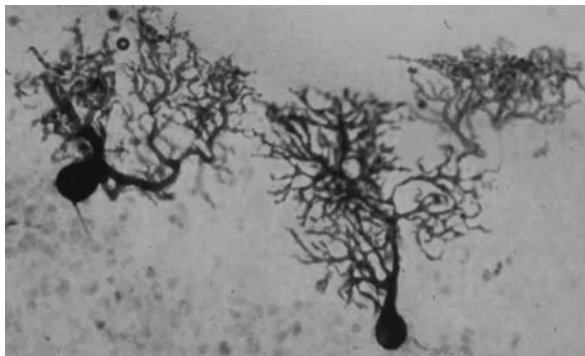


Fig. 2. Immunocytochemical labeling of extant Purkinje cells in *pcd* mouse cerebellum with antibodies against calbindin-D28k.

and establish synaptic contacts with Purkinje cell dendrites (Fig. 1). The adult mouse cerebellum contains an estimated 200 000 Purkinje cells [6] and some 20 000 000 granule cells [23].

A useful model of cerebellar degeneration is the murine mutant ‘Purkinje cell degeneration’ (*pcd*), in which cerebellar Purkinje cells (Fig. 2) are genetically programmed to die off between the third and sixth postnatal week [35]. Studies with mosaic chimaeric mice indicated that the site of action of the *pcd* gene is intrinsic to Purkinje cells [34]. The *pcd* locus has been mapped to the 5 cM interval of mouse chromosome 13, between the simple sequence repeats *D13Mit139* and *D13Mit67* [8]. *Nna1*, a gene encoding a putative nuclear protein that contains a zinc car-

boxypeptidase domain and is structurally related to the adipocyte enhancer binding protein 1, has been identified as the allele mutated in *pcd* mice [18]. It was found, in a recent *pcd* remutation (*pcd^{5l}*), that the defect results from the insertion of a GAC triplet encoding an aspartic acid residue at position 775 of the *Nna1* protein, leading to a marked decrease of its expression [9].

One specific mechanism mediating massive loss of neurons is reflected in degenerations that result from target neuron removal and are termed transsynaptic retrograde degenerations [13]. Such alterations provide compelling evidence for the importance of neuronotrophic interactions in cell maintenance [48,49]. The rapid degeneration of Purkinje cells in the *pcd* mutant is followed by a protracted degeneration of granule cells [20,56], which normally form synaptic contacts with Purkinje dendrites. Biological parameters pertinent to the onset, timing, and spatiotemporal sequence of degeneration attributes support a retrograde transsynaptic degeneration mechanism to account for the granule cell loss [20,44,48,49,56].

The second mutant mouse that the present article deals with is the weaver mutant mouse, which has been used as an animal model of progressive mesostriatal dopaminergic neuron degeneration, a useful pathophysiological phenocopy of Parkinsonism [1,3,51,53]. The *wv* allele has been mapped to the distal end of mouse chromosome 16 within a phylogenetically conserved region, highly homologous to telomeric human chromosome 21 [33]. The *wv* mutation has been identified as a missense mutation with a G→A substitution in nucleotide 953 of the inward-rectifier K⁺ channel gene *Girk2* and an ensuing Gly→Ser replacement at residue 156 of the GIRK2 protein [38]. Pathophysiological mechanisms of ionic fluxes through the weaver K⁺ channel have been investigated [46] and discussed in the perspective of the multiple systems involvement [25].

Material and methods

Counts of cerebellar granule cells in wild-type and *pcd* mice were obtained under a light microscope (Carl Zeiss) in sagittal semithin Epon sections of the cerebellar vermis, 1 μm in thickness, stained with toluidine blue from animals ranging in age from 17 postnatal days to 20 months, as described previously [52]. Granule cells were counted in cerebellar lobuli VI and

VIII, i.e. the declive and tuber vermis [45]. For the statistical analysis, the logarithmic transformation had to be applied to granule cell number in order to meet the assumption of the homogeneity of variance [47]. Logarithms of granule cell numbers were regressed upon time in order to obtain the function $\log \hat{Y} = a' + b'X$ [47].

Counts of dopamine neurons in the midbrain of wild-type and weaver mice were obtained under a light microscope (Ernst Leitz) with a mechanical counter, as described in detail elsewhere [57]. Serial paraffin sections, 10 μm in thickness, were immunocytochemically labeled with antibodies against tyrosine hydroxylase. Dopamine neuron numbers in the weaver midbrain from birth to senescence (two years of age) were regressed upon time to obtain the best mathematical function [58].

All experiments conformed with the National Institute of Health Guide (National Institute of Health Publications No. 80-23, Revised 1978) for the care and use of laboratory animals for experimental procedures.

Results

Cerebellar granule cell counts in the *pcd* mutant

A numerical analysis of granule cells was effected in *pcd* mice to determine the temporal profile of decay. Granule cells represent a 'neuronally-closed' system [13], meaning that their connections are limited to the cerebellar cortex and do not extend to other parts of the nervous system; thus, any retrograde degenerative changes of the granule cells may be mainly attributed to Purkinje cell target deprivation.

In controls, granule cell number (mean \pm SEM) in the declive and tuber vermis was 5808 ± 295 in animals younger than one month of age and 5546 ± 335 in animals older than one year of age. In *pcd* mutants, the respective figures were 5740 ± 154 and 612 ± 26 . Granule cell loss was found to follow a highly significant exponential decay ($R^2 = 0.947$, $P < 0.0001$) (Fig. 3). There was no statistically significant difference among ages in the wild-type mice.

If age t is an independent variable, granule cell count Y_t is a dependent variable, and Y_t' is the derivative of Y_t with respect to t , the relationship between the rate of neuronal degeneration and the number of viable elements [27,28] can be expressed as $Y_t' + \lambda Y_t = 0$, where λ is the constant of proportionality known as *degeneration* (or *decay*) constant.

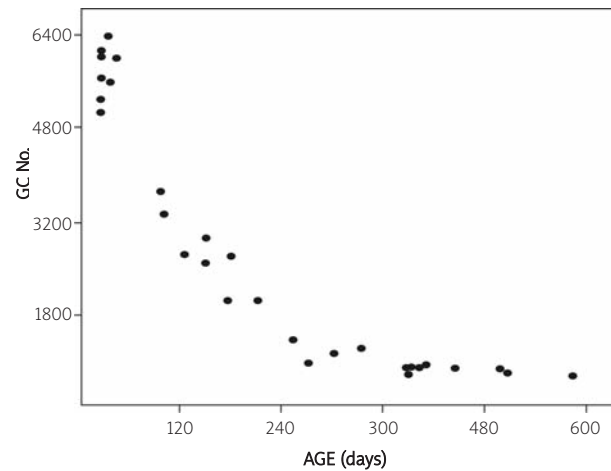


Fig. 3. Logarithmic transformation of granule cell numbers regressed upon age in *pcd* mice. Each point represents the total number of granule cells in the declive and tuber vermis in a sagittal cross-section at the given age. In the ordinate are represented the logarithmically transformed granule cell counts; in the abscissa, the days of age. Regression coefficient = -0.947 ; Y-intercept = 3.753 ; slope = -0.00224 . From Triarhou [52].

By substituting the correct values in a set of ordinary differential equations, we calculated [52] that the *initial number* of granule cells (Y_0), obtained by the Y-intercept of the regression line, is $10^{3.753}$ or 5662 neurons; the *half-life* of granule cells ($T_{1/2}$) is 135 days; and the *decay constant* λ , obtained from equation $Y_{1/2} = Y_0 e^{-\lambda T_{1/2}}$ by substituting $Y_{1/2} = 1/2 Y_0$ and taking the natural logarithm of both sides, is 0.005 per day.

Dopamine neuron counts in the weaver mutant

Quantitative immunocytochemical studies in serial paraffin sections of the weaver mouse midbrain have disclosed that the substantia nigra (or area A9, Figs. 4 and 5) has 42% fewer dopamine cells than the wild-type on postnatal day 20 and 69% fewer dopamine cells at three months of age [55,57]. A second wave of dopaminergic neuron degeneration takes place during the second year of life, which brings the total dopamine cell loss to 85% in the substantia nigra by two years of age [21].

Nigral dopamine cell numbers from birth to senescence were regressed upon age to obtain the best mathematical function in the weaver model [53,58].

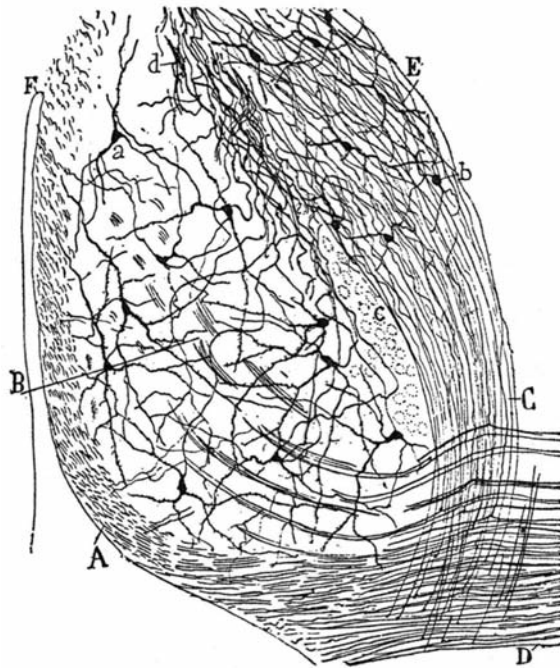


Fig. 4. Cajal's drawing of the mouse substantia nigra in the mouse midbrain; sagittal section incubated with the Golgi method. Abbreviations: A – pedunculus cerebri, B – substantia nigra, C – bundle of collaterals destined for the subthalamic region, D – continuation of pedunculus cerebri, F – pontine fissure, d – tract emanating from substantia nigra. After Ramón y Cajal [40].

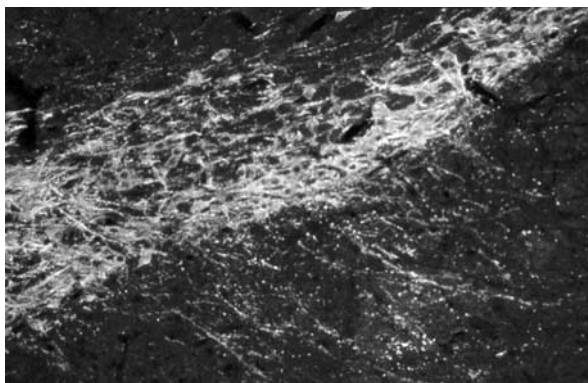


Fig. 5. Immunocytochemical labeling of normal mouse substantia nigra with antibodies against tyrosine hydroxylase, viewed under dark-field illumination. Note the characteristic arborization of dopaminergic dendrites into the pars reticulata.

The regression fits show that dopaminergic neuron fallout combines two independent components, an initial exponential decay, superseded by a linear regression, with a threshold at around 100 days (Fig. 6).

The first wave of (exponential) cell loss follows the general form $Y_t = \alpha + Y_0 e^{-\lambda t}$, where Y_t is a dependent variable representing dopamine neuron count with respect to age, Y_0 is the initial neuron number, λ is the constant of proportionality, age t is an independent variable, and constant term α represents a horizontal asymptote. The half-life $T_{1/2}$ of neurons degenerating in this phase is 58 days. The exponential pattern implies that the probability per unit time that a neuron will die is a constant (λ). This subpopulation of DA cells shares the inherent characteristic that their probability of degeneration is 0.012 per day.

In the second (linear) phase of degeneration, the probability of a neuron dying becomes a function of time and declines with advancing age, i.e., the longer a cell survives, the less likely it becomes to degenerate.

Discussion

The exponential fit of a degeneration pattern suggests that the rate of cell decay (such as the granule cells in the described cerebellar model or the dopamine cells during the first phase of degeneration in the described Parkinsonian model) at any time-point is proportional to the number of the remaining cells. The theoretical curves represent cell numbers as a function of age in an infinitely large, parametric animal population; as such, they allow predictability of cell losses at time-points other than those contained in the empirical counts.

The model of neuronal decay succinctly given by the exponential equation $Y_t = Y_0 \times e^{-\lambda t}$ allows one to infer that the probability per unit time that a neuron will die, i.e. the decay constant λ , is *constant* and *independent of age*; this is based on the law of radioactive decay, which states that the probability per unit time that a nucleus will decay is constant and independent of time [29]. Hence, granule cells of the *pcd* cerebellum share an inherent characteristic, i.e. a probability of degeneration of 0.5% per day. This is a *natural property, specific* for the cells examined.

In a system that decays exponentially, the loss of individual elements is considered to be a random effect [28]. Variables that may be operating in the

causation of the death of granule cells include the loss of their major postsynaptic target, the extensive modification of cellular environs, and an accumulation of metabolic error leading to a lethal error catastrophe [4,37]. Further, degeneration of a specific Purkinje dendrite would trigger degeneration of a granule cell subset synaptically connected to that particular dendrite. Nonetheless, such a topical 'fixed' effect can be viewed as the regional representation of a larger-scale 'random' effect, i.e. the random degeneration of any one Purkinje cell [31].

The particular cerebellar model described provides an insight into quantitative aspects of neuron death in the adult mammalian nervous system and shows that the rate of neuronal fallout follows an orderly temporal pattern simulated by a mathematical decay model with widespread applications at multiple levels of the subatomic and macroscopic world.

Based on several independent studies on the kinetics of cell loss in eighteen neurodegenerative situations of genetic or acquired origin, manifesting with cerebellar, retinal, hippocampal degeneration, as well as in Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, Clarke *et al.* [11,12] and Burns *et al.* [5] have advocated a 'one-hit' model of cell death, a hypothesis that neither requires the biochemical mechanisms participating in cell loss to be defined, nor dictates the molecular mechanism(s) by which neurons die. Instead, it proposes that affected neurons are in an abnormal 'mutant steady state' with an increased probability of a single metabolic error leading to a lethal error catastrophe [37].

In a later study, Clarke and Lumsden [10] found out that, in nine of the initial eighteen situations that they had analyzed, including our data on granule cells in the *pcd* mouse [52], the Weibull [59] lifetime distribution produced the best fit, implying that in such a distribution of one-hit risk, failure of one of many possible biochemical reactions maintaining the mutant steady state can commit a neuron to cell death. Moreover, the Weibull distribution leaves room for possible kinetic heterogeneities in 'one-hit' types of neuron death, whereby regional differences in the cellular microenvironment may modulate the kinetics of cell loss within a given affected neuronal population, thus accommodating potential neuron-to-neuron differences in death risk [10].

Cell death in hereditary degenerations is often mediated by apoptosis. However, several unresolved issues remain regarding the cellular and molecular

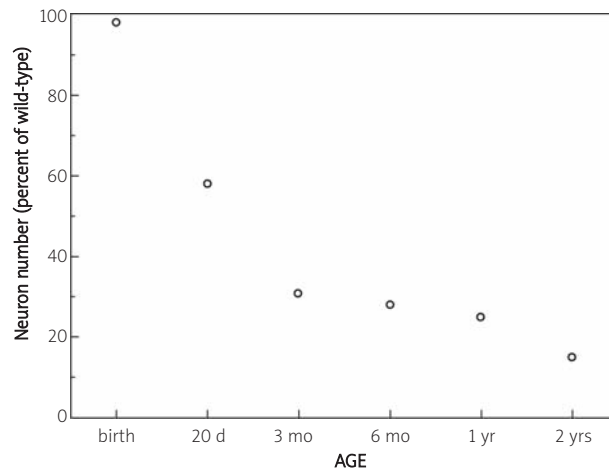


Fig. 6. Profile of dopamine neuron loss in the weaver substantia nigra. The numbers of tyrosine hydroxylase immunoreactive neurons are expressed as percent of the wild-type values over the life-span of the animals. Note that values in the abscissa are not linear, but rather represent the ages for which actual experimental data were collected. Numbers combined from several previously published articles. From Triarhou [53].

events that occur in the months, years or decades between the birth and death of a mutant neuron. A novel biochemical mechanism that attributes the exponential neuron decline in the clinical phase of Huntington's disease to the expansion of glutamine repeats [39] appears consistent with the 'one-hit' model. The exponential kinetics of neuron death, which mean that the probability of cell death remains *constant* regardless of age, argue against the age-dependent 'cumulative damage' hypothesis (associated e.g. with cumulative damage of macromolecules through oxidative stress-disrupted metabolism), in which case the probability of neuron death is expected to increase over time. Exponential kinetics, as already mentioned, further indicate that the risk of death is *constant*, that death occurs *randomly* in time, and that the death of each neuron is *independent* of other neurons.

Gjessing *et al.* [22] have pinpointed to the necessity of understanding the hazard rate and how its various shapes can arise in drawing biological conclusions from the shape of a hazard rate; thus, they propose to generalize the standard frailty models of

survival analysis as a weighted power variance function Lévy process; in this approach, quasi-stationarity implies limiting population hazard rates that are constant, in spite of the continual increase of the individual hazards.

Concerning the pattern of cell loss in the second mouse model described, i.e. the weaver mutant mouse, an independent immunocytochemical study has associated the selective vulnerability of weaver dopamine neurons with differences in their histochemical signatures; in particular, dopaminergic neurons co-expressing the 28-kDa Ca⁺⁺-binding protein appear to be more resistant to degeneration [19]. The analysis of neurogenetic timetables by means of combined [³H]thymidine dating and tyrosine hydroxylase immunocytochemistry indicate that dopamine neurons generated later in embryonic life are preferentially targeted by the weaver mutation [2]. Finally, a combination of technical approaches, including DNA gel electrophoresis, in situ end-labeling, and immunocytochemistry for the apoptosis-related proteins c-Jun and proliferating cell nuclear antigen [32], point to a co-existence of apoptotic and other types of cell death as a result of a single mutation, influenced perhaps by the suggested specific features of target neurons.

Our computational findings in the case of the dopamine system suggest the existence of two independent dopaminergic neuron subsets in the weaver midbrain with regards to degeneration, potentially pertaining to structural and developmental neuronal idiosyncrasies (such as process outgrowth, projection patterns, synaptic connectivity, etc.). In the first phase, neurons die according to an exponential decay pattern, similarly to the case of the cerebellar granule cells described above. In the second phase, the degeneration follows a linear regression, whereby the probability of a neuron dying declines with advancing age. That second phase is the reverse of the ‘cumulative damage’ scenario. Heintz [24] mentions the idea that histological abnormalities and deterioration of function may precede cell loss. In the case of the weaver mouse, it has been documented that nigral dopaminergic neurons feature a characteristic abnormality of dendritic branching from early on, which is also striking in heterozygotes, despite having normal numbers of dopamine cell somata in the midbrain [54].

The time-course of neuron losses and their mathematical analysis have received particular attention in the case of clinical Parkinsonism [7]. It appears from

previous studies in the literature, that in Parkinsonian models in both humans and experimental animals, a linear regression component of cell loss was found.

In patients with Parkinsonism, Fearnley and Lees [17] confirmed a linear fallout of pigmented neurons at a rate of 4.7% per decade in the caudal pars compacta of the substantia nigra. In a biphasic theory of aging and Parkinson’s disease, the rate of neuron loss in the second phase appears equivalent to the rate of neuron loss found in normal aging. Based on the exponential loss of pigmented neurons those authors favored the idea that Parkinson’s disease is a relatively acute monophasic illness and concluded that the age-related attrition of pigmented nigral cells is not an important factor in the pathogenesis of the disorder. In a subsequent study [30], the rate of neuronal death appeared more rapid in the earlier stages of the evolution of the pathology of idiopathic Parkinsonism and the velocity of progression slowed down to approach the rate of attrition produced by normal aging. Three prototypical mathematical models – quadratic, exponential and segmented linear – applied to the clinical data [43] seem compatible with an event that kills some neurons and damages others in such a way that their life expectation is reduced or an event that starts a process which is continuously killing healthy neurons at a constant rate.

Thiruchelvam *et al.* [50] studied transgenic mice expressing human α -synuclein and found that the number of tyrosine hydroxylase immunopositive neurons in the substantia nigra significantly declined with age, in a manner consistent with a constant or decreasing risk.

A ‘two-hit’ hypothesis has been proposed by Rando [42] to explain degenerative events observed in muscular dystrophies, with at least two biochemical consequences: a reduction in nitric oxide-mediated protection against ischemia, and an increase in cellular susceptibility to metabolic stress. Rando [42] argued that a ‘two-hit’ hypothesis may explain some of the complex spatial and temporal variations to disease expression, e.g. grouped necrosis, a pre-necrotic phase, and selective tissue involvement. Such a supposition could also explain an early apoptotic process, followed later by necrotic degeneration. It remains for future elucidation to determine whether heavy-tailed stretched exponential functions, such as the Kohlrausch-Williams-Watts function, may ultimately be able to explain the biphasic patterns of kinetic data in more complex systems [10].

Analyses of the dynamics of cellular degeneration rates over time can provide a useful complement to conventional neuropathological methods – such as tissue histochemistry, molecular genetics and light and electron microscopy – in the quest to better understand pathogenetic mechanisms causing diverse neurodegenerative phenotypes. The properties of the applied equations can offer clues on the characteristics of cell loss, which may even help better understand the underlying biochemical mechanisms. Furthermore, it has been pointed out that such approaches may have implications for therapeutic interventions in neurological disorders [24], in the sense of rescuing nerve cells from death, for example by means of pharmacological treatment, based on the dependence of their rate of degeneration on time. In all, theoretical mathematical models of cell loss in diverse neurodegenerative conditions appear as valuable tools with the potential of capturing novel principles in neuropathology.

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