Published in final edited form as:

Dev Neurosci. 2012 ; 34(2-3): 129–139. doi:10.1159/000336828.

The Role of Central Nervous System Development in Late Onset Neurodegenerative Disorders

Amy M. Palubinsky^{1,2,3}, Jacob A. Martin^{3,4}, and BethAnn McLaughlin^{3,5,6}

¹Neuroscience Graduate Program, Vanderbilt University, Nashville, TN 37232, USA

²Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN 37232, USA

³Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN 37232, USA

⁴Department of Neurological Surgery, Vanderbilt University, Nashville, TN 37232, USA

⁵Department of Neurology, Vanderbilt University, Nashville, TN 37232, USA

⁶Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA

Abstract

The human brain is dependent upon successfully maintaining ionic, energetic and redox homeostasis within exceptionally narrow margins for proper function. The ability of neurons to adapt to genetic and environmental perturbations and evoke a ‘new normal’ can be most fully appreciated in the context of neurological disorders in which clinical impairments do not manifest until late in life, although dysfunctional proteins are expressed early in development. We now know that proteins controlling ATP generation, mitochondrial stability, and the redox environment are associated with neurological disorders such as Parkinson’s disease and Amyotrophic Lateral Sclerosis. Generally, focus is placed on the role that early or long term environmental stress has in altering the survival of cells targeted by genetic dysfunctions; however, the CNS undergoes several periods of intense stress during normal maturation. One of the most profound periods of stress occurs when 50% of neurons are removed via programmed cell death. Unfortunately, we have virtually no understanding of how these events proceed in individuals who harbor mutations that are lethal later in life. Moreover, there is a profound lack of information on circuit formation, cell fate during development and neurochemical compensation in either humans or the animals used to model neurodegenerative diseases. In this review, we consider the current knowledge of how energetic and oxidative stress signaling differs between neurons in early versus late stages of life, the influence of a new group of proteins that can integrate cell stress signals at the mitochondrial level, and the growing body of evidence that suggests early development should be considered a critical period for the genesis of chronic neurodegenerative disease.

Correspondence should be addressed to: Dr. BethAnn McLaughlin, 465 21st Avenue South, MRB III Room 8110A, Nashville, TN, 37232-8548 Tel: (615) 936-3847. Fax: (615) 936-3747. bethann.mclaughlin@vanderbilt.edu.

Conflict of interest: The authors do not declare any conflict of interest.

Keywords

Neural development; Neurodegeneration; Programmed cell death (PCD); Stress sensors; Parkinson's disease (PD); Reactive oxygen species (ROS); Oxidative stress; Mitochondria

Introduction

Over the past 20 years, we have witnessed a transition from high-end, time intensive genomic testing for a handful of devastating diseases, to a market targeting at-home users with kits that can identify not simply diseases, but risk factors for developing diseases as well. The astounding growth of bioinformatics has increasingly enabled the general public to access affordable genetic information so that individuals can investigate their risk of developing diabetes, Alzheimer's disease, Parkinson's disease, cancer, and a rapidly expanding number of additional conditions.

As scientists, we are particularly limited in considering age of onset in the context of genetic risk factors. The scientific community has long been aware that mutations in neuroprotective genes play a major role in CNS diseases that present later in life. These mutant alleles are present throughout development and in early life; however, their role in early disease processes remains unknown.

The purpose of this review is to introduce the emerging theory that neurodegenerative diseases are a result of disordered neural development. Understanding the relationship between genetic risk factors as well as genes that invariably result in neurodegeneration within the context of development will help us take an active approach in addressing neurodegenerative diseases before they present with debilitating symptoms. Our ability to intercede against neurotoxic events is currently restricted to periods following which disease has already caused significant neurodegeneration and clinical manifestations and we must re-examine this practice given our understanding of devastating diseases like Parkinson's and Alzheimer's. From a clinical perspective, there is remarkable inconsistency in monitoring those who harbor genetic deficits for these disorders. Our clinical colleagues lack diagnostic and prognostic tools to assess CNS functioning and compensation. Patients are generally into their 3rd or 4th decade of circuit compensation, neurochemical adjustments and vascular and cellular remodeling, processes we simply do not yet fully understand, before baseline blood work or imaging is performed.

Understanding developmental neuroadaptation to these diseases is essential for developing high value therapeutics as many neuroprotective mechanisms, such as the regulation of protein trafficking and degradation, antioxidant defenses and changes in energetic sensing proteins may have already been employed in the initial decades of life. Therefore, one would predict that re-targeting these proteins with small molecule therapies or supplementation would likely have limited efficacy. As we begin to address the gaps in knowledge that exist in our understanding of developmental compensation to stress, we can be guided by lessons provided by schizophrenia and autism, both of which offer excellent examples for how to consider early developmental stress on prospective neural dysfunction.

While not a neurodegenerative condition, data has increasingly shown that schizophrenia is likely rooted in development. Clinical, epidemiological, imaging, and post-mortem studies have linked dysregulation of genes integral to CNS connectivity and synaptogenesis, with the acquisition of disease which is fulminant in late adolescence and early adulthood [1–5]. Driven heavily by significant changes in RNA and protein expression, the goal of experiments in this field has become focused on not simply identification of biomarkers, but also to uncovering metrics for clinical utility. In this regard, brain asymmetries and anomalies in morphometric measurements detected early in life may help further refine our understanding of disease risks beyond genetic predispositions [3].

This point is underscored by recent CNS post mortem analyses of individuals with autism which demonstrate that neuronal cell numbers are significantly higher in the prefrontal cortex and other brain regions within this patient population [6]. During the last decade, autism spectrum disorders (ASDs) were shown to have some correlation with larger head circumference [7]; therefore, the recent study demonstrating an average increase of 700 million neurons in the prefrontal cortex in the ASD group as well as an associated increase in brain weight [6] may provide further explanation of this previously recognized correlation. It will be of particular interest to determine how higher numbers of neurons in areas of executive function correlate with behavioral manifestations of autism given the massive heterogeneity that exists within this patient population.

These data support the hypothesis that neurodegenerative diseases should be thought of as having roots in neural development. However, there are currently no studies in which patients with mutations in genes associated with late in life neurodegenerative disorders have been evaluated using the same straightforward strategy of genetic risks and brain size/head circumference used in the ASD and schizophrenia populations. Such a study may provide keys to understanding the mechanisms by which the brain compensates for long term stressors and could provide valuable insight regarding novel neuroprotective strategies.

Neurodevelopmental Pruning: Getting the Landscape Just Right

Large numbers of neurons are systematically culled during CNS maturation. Cell proliferation occurs at a near exponential rate between 10 and 20 weeks of gestation [8]. Initially, mitotic progenitor cells in proliferative regions of the brain generate neurons in excess. These cells are directed by the local milieu of growth factors to specific regions where they then compete for synaptic targets. This redundancy maximizes the probability that necessary connections will develop. After connections are made, large numbers of superfluous neurons remain. The number of neurons in humans peaks between 3 months and 3.5 years, depending on the brain region [9]; however, as early as the third trimester, neurons are culled via mechanisms of PCD which have long been recognized as essential in normal nervous system development [10]. This process continues in young children as synaptogenesis moves to the forefront in proper development.

Cellular elimination processes are primarily regulated via signaling molecules released at targets of innervation. These proteins and amino acids suppress intrinsic suicidal programs in migrating cells and are secreted in a limited concentration that restricts the number of

neurons that continue to synapse in a given region [11]. If a fiber does not receive essential chemical signals, indicating that it has failed to reach a supportive target and establish appropriate connectivity to facilitate efficient electrical coupling, it may pare back a process or initiate PCD [12]. Based upon work with neurotrophins, the first type of these signals to be discovered, the survival signal theory has become well established in the CNS and many other cell types and organs throughout development [13].

Further linkage between neurodevelopmental processes and cell death can be found in the eloquent work of Karl Herrup, Lloyd Green and David Park who have all demonstrated that an apparent re-entry of post mitotic neurons into the cell cycle contributes to both acute and chronic models of neurodegeneration [14] [15,16].

From the programmed cell death field evolved the cellular and molecular identification of proteins and protein modifications that regulate cell degradation. While caspases were shown to play a major role in neuronal culling, they were also noted as essential regulators of neuronal growth, differentiation, migration, synaptogenesis and neuroprotection. Indeed, blocking proteolysis by caspases causes growth cone collapse [17] and blocks self-renewal of embryonic stem cells [18]. Similarly, processes typically associated with neurodegeneration including high levels of protein degradation and ubiquitination, caspase 3 activation and organelle autophagy are essential for neural circuit formation [19,20]. Consequently, protein markers of these processes are abundantly expressed in development (Figure 1).

Developmental windows may represent periods of profound stress for patients harboring genetic mutations associated with late in life neurodegeneration, which manifest as subtle abnormalities in CNS wiring and connectivity in early life. At this point, many of the essential circuits have been established and a high rate of elimination occurs via mechanisms of PCD. While the ‘textbook’ answer for how much neuronal cell death occurs as the brain develops is generally cited as 50%, this number is worth some scrutiny given that it is generally derived from chick and rat studies of regions of topographic reorganization to control motor function and sensation [21]. Moreover, there is ongoing controversy as to markers of active and passive cell death suggesting that the ultra-structural components of neurodegeneration are highly variable and may require reexamination beyond using markers such as cell swelling or protease activation to determine the nature and biochemical underpinnings of neurodegeneration [22].

In this regard, landmark studies were pioneered by Ron Oppenheim, Rita Levi Montalcini and Victor Hamburg, who undertook the meticulous dissection of factors influencing axon guidance and synapse stabilization. Oppenheim noted in a retrospective analysis that “Although it seems plausible that by modifying neuronal numbers, altered cell death could be involved in the etiology of many congenital nervous system anomalies, as well as in relatively benign species, strain, or individual differences in neurobehavioral development, there is regrettably little concrete data on this point” [8]. Indeed, two decades later, we are only now poised to address the role of neurodevelopment in diseases associated with late in life onset. This endeavor would require detailed analysis of developmental cell death in regions of critical interest such as the striatum, substantia nigra or upper and lower motor

neurons in humans – areas exquisitely sensitive to some of the most devastating neurodegenerative diseases.

Neurotrophins as Therapy: Too Little or Too Late?

Neurotrophins provide a logical therapeutic target for the treatment of neurodegenerative disorders and were an area of intense biotechnology and research investment in the 1990s with trials directed at Alzheimer's (AD), Parkinson's (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) [23,24]. However, initial setbacks occurred as a result of inadequate delivery of neurotrophins to target areas [24]. In some cases, the half-life of the neurotrophin was too quick while in other instances, receptors for the trophic factor were present in non-target cells, binding neurotrophins before they could reach their target [25].

In cases of severe neurodegeneration, retrograde axonal transport, which is the mainstay of neurotrophic trafficking, is not possible and target acquisition is again prevented. Gene therapies and advancements in gene delivery methods have proven critical in the improvement of such studies yet with over a decade of trials and no breakout targets or treatments, enthusiasm has waned in this field which provided pioneering advancements in drug delivery, disease modeling and outcome measures both in animal and human models [24,26]. It should be noted that the appeal of long term treatments using guided neurotrophic factors warrants further examination in the newly identified genetic models of PD and AD to determine if these proteins can increase the age to symptom onset.

The Role of Mitochondria in Controlling Energetic and Redox Tone in Development: Life at the Epicenter

While the brain accounts for just 2% of adult body weight, it receives 20% of the body's total blood flow. A human weighing 155 pounds is estimated to generate 20 pounds of ATP within the brain each day. Moreover, 40–60% of this ATP is used to maintain ion homeostasis across the plasma membrane [27]. Failure to maintain energetic status has grave and rapid consequences for the CNS. The loss of oxygen and glucose necessary to fuel mitochondria results in measurable dysfunction within a minute and death of the most vulnerable neurons after periods of hypoxia lasting longer than 5 minutes.

The populations of cells most heavily dependent upon oxygenation are unique early in development as neurons rely heavily on anaerobic respiration to generate ATP, primarily via pyruvate. In the first few years of human CNS development, mitochondrial numbers are low; however, both energetic demands and mitochondrial quantity increase steadily into adulthood [27,28].

One of the earliest markers of neural dysfunction is loss of ionic balance, which is critical in neurons where the tightly regulated movement of charged particles dictates changes in the membrane electrical potential. This potential is responsible for signaling events such as graded potentials at the synapse and the firing of action potentials. The most important pump for ion movements is the Na^+/K^+ -ATPase, which drives Na^+ outward and K^+ inward at the expense of ATP. In the developing brain, as migration increases and neurons begin to form

active synapses, the expression of the Na^+/K^+ -ATPase increases 4–12 fold in the first few post natal weeks [27,29,30]. In response to changes in energy demands, the metabolic profile of the developing brain shifts dramatically. One of the best examples occurs in the developing rat inferior colliculus, where essential connections will form for the relay and processing of audition. During the first postnatal week, mitochondrial capacity in the inferior colliculus is low followed by an approximately 11-fold increase by post-natal day 25 [27,31]. These numbers are paralleled by increases in the expression and activity of electron transport chain (ETC) protein complexes.

Clarke's study of metabolic capacity of neuronal cell bodies during rat development demonstrated that the activity of Complexes II and III are the last components of aerobic respiration to come online as late as post-natal day 60 [32]. Such increases in aerobic metabolic capacity are thought to correlate with increases in synaptic activity and the large resultant energy demands of active ion transport that are essential for proper development. Although significant changes occur in metabolism during CNS development, glucose remains the preferred substrate for energy production into adulthood [27].

This tight reliance on glucose and metabolic coupling for processes including cell migration, synaptogenesis, protein trafficking, and communication during development is particularly intriguing given that mitochondrial dysfunction has been identified as a key component of a number of CNS disorders which carry germline mutations (mutations present since birth).

The Fate of Neurons During Energetic Failure

Perturbations in energy, especially in the form of mitochondrially-generated ATP, are of extreme importance. In neurons, significant decreases in ATP can lead to the dysregulation of energy-dependent membrane pumps and result in depolarization and a subsequent influx of calcium ions (Ca^{2+}) [33]. As mitochondria depolarize, ATP-dependent uniporters, which sequester Ca^{2+} within the endoplasmic reticulum and mitochondria fail, resulting in the activation of damaging proteases and kinases. In addition, excess intracellular Ca^{2+} promotes the generation of free radicals (derived from oxygen and nitrogen) [34,35].

ROS: Essential Molecules in Signaling and Development

Reactive oxygen species (ROS) have historically been considered detrimental to the survival of neurons although research in the last decade has demonstrated that rather than acting as random destructive molecules, ROS can elicit discreet changes in protein structure and function that are neuroadaptive and essential to controlling external stress and promoting development [35,36]. During normal mitochondrial functioning, 0.15–2% of electron flow “leaks” from the ETC before it reaches cytochrome oxidase [37]. Leakage, or premature transfer of single electrons to molecular oxygen, produces superoxide anions ($\text{O}_2^{\bullet-}$), a type of free radical which, if not captured by superoxide dismutase or other antioxidants, results in the formation of highly damaging hydroxyl radicals.

While ROS can irreversibly alter proteins, lipids, and DNA, the reversible modification of disulfide bonds, and tyrosine crosslinks, for example, play essential roles in normal signal transduction. Similar to the addition of a polar phosphate group by a kinase, covalent

modifications of amino acids by ROS is a highly selective process that can change the conformation, binding and kinetics of proteins. Histidine, arginine, lysine, proline, cysteine, and methionine the most 'oxidizable' amino acids; however, 12 amino acids are known to be ROS-sensitive [38]. Due to the high reactivity of many free-radical species, it is thought that microenvironments, which facilitate the production and maintenance of high ROS concentrations, provide both temporal and spatial selectivity to control post translation modification and signaling via ROS.

Cellular antioxidants are at the front line of defense against oxidative stress in the developing brain. These molecules reduce ROS before they can modify proteins, lipids, or nucleic acids and cause damage. Subsequently, they also help to maintain the cellular redox potential at a dynamic equilibrium.

Glutathione (GSH) is arguably the most important cellular antioxidant in the CNS. Its low redox potential (-240mV at pH 7.0) makes it ideally suited for oxidative environments. GSH is synthesized in an ATP dependent manner from glutamatic acid, cysteine and glycine, with cysteine being the limiting amino acid in both developing and adult CNS [39]. Glutathione is oxidized as a result of chronic stress and normal aging processes [40,41]. Mitochondria typically aim to maintain GSH and NADPH at rather high levels, between 2 and 14mM and between 3 to 5mM, respectively [35]; however, endogenous antioxidant defenses can only curtail damage for limited periods of time.

During CNS development, peak GSH levels coincide with high levels of synaptogenesis. In the rodent brain, for example, the highest GSH concentrations are found during the highly active period at the end of post-natal week one [42]. Peripheral administration of inhibitors of GSH synthesis result in rapid cerebral cortical cell death in newborn rats, underscoring the importance of maintaining redox homeostasis throughout neural development [39]. Levels of other key antioxidants show similar patterns in the developing brain. The concentration of catalase, for instance, reaches three times its embryonic level shortly after birth before declining again to a relatively low level in maturity [43].

Given the central role of energetic and ion homeostasis in ROS production as well as the temporal changes in antioxidant systems during development, it is essential to understand the forms of compensation in antioxidant defenses as a result of long-term exposure to mutated genes at times of intense bioenergetic and redox stress, such as synaptogenesis.

Mitochondrial Dysfunction and Neurodegeneration

The role of mitochondrial signaling in response to stress and in relation to diseased states of the brain has been the object of intensive study for three decades. Primary dysfunction in genes controlling mitochondrial biogenesis and function have, until recently, only been studied in developing circuits as it was assumed that dysfunction of proteins involved in these essential pathways would be highly toxic and incompatible with the survival of long lived cells such as neurons. We now appreciate that late in life diseases including PD, AD, and ALS [44,45] all have primary dysfunction of mitochondrial dynamics and degradation.

In order to maintain an optimal mitochondrial population within a cell, mitochondria continuously undergo fission and fusion events. These events occur in response to environmental stimuli, developmental status, and cellular energy requirements [46]. Fusion can occur in response to stress wherein injured mitochondria fuse with healthy mitochondria as a means of complementation. Some of the key factors involved in fusion are the outer mitochondrial membrane (OMM) proteins, mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), and the inner mitochondrial membrane (IMM) protein, optic atrophy 1 (OPA1). The fusion of outer and inner mitochondrial membranes is exquisitely coordinated and rapidly results in the development of new mitochondria.

The process of mitochondrial fission relies heavily upon Dynamin related protein 1 (Drp1) and ganglioside-induced differentiation-associated protein 1 (GDAP-1) [46]. Unlike fusion, fission is an essential, early event in the removal of damaged mitochondria via mitophagy underscoring the importance of autophagic containment in neuronal cell fate decisions. Under severe stress, events that are normally adaptive can become dysregulated (Figure 2). A balance between fission and fusion is essential not only in determining cell fate but also in the development of appropriate circuitry [44]. For example, mutations in OPA1 result in autosomal dominant optic atrophy (ADOA) [46], while mutations in the fusion protein, Mfn2 and the fission protein, GDAP-1 have both been linked to the peripheral neurodegenerative disorder, Charcot-Marie-Tooth disease type 2A (CMT2A) [46,47], further highlighting the delicate balance between these processes.

Insight from PD Genetics Highlights Mitochondrial Dynamics as Essential to Neural Fate

A growing number of loss-of-function mutations have now been linked to mitochondrial function and inherited forms of PD [48–51]. These genes not only have direct impact on mitochondrial dynamics but are also involved in the regulation of proteasome activity, mitophagy, ubiquitination and antioxidant defenses.

Of these proteins, the best understood are the E3 ubiquitin ligase Parkin, the stress associated kinase PTEN-inducible putative kinase 1 (PINK1) and the molecular chaperone DJ-1, all of which can be mutated in familial PD, and have been shown to functionally interact [52,53]. While both PINK1 and Parkin are expressed embryonically, little is known about their impact until later in life [54,55]. PINK1 and Parkin RNA are enriched in tissues with high mitochondrial capacity, such as skeletal muscle, heart, liver, kidney, testes and brain [55] and mutations in any one of these proteins causes profound mitochondrial dysregulation [56].

In zebra fish, PINK1 knockdown in embryos results in severe developmental phenotypes, including halted head/tail development, spinal curvature, and enlarged brain ventricles. These morphants demonstrate a 30% decrease in dopaminergic neurons and severely affected larvae fail to thrive, further suggesting a role for PINK1 in normal developmental processes [57]. However, morphants also present with alterations in mitochondrial function, including increased caspase 3 activity and higher levels of ROS [57], making it hard to

separate improper development and neurodegeneration. Similar data have been observed in human and other animal models including: mice, rats, and drosophila [53,58,59].

At this point, there is little to no data addressing the cause of the selective loss of dopaminergic neurons in PD or whether somatic mutations in these genes can be introduced by environmental factors. Moreover, given the tight linkage between PD mutations and caspase mediated cell death and autophagy, one would predict that developing pluripotent stem cells from individuals harboring genetic mutations for familial forms of these diseases may represent a skewed platform given the role of caspases in normal neural development as well as proliferation of embryonic stem cells. These studies would require sophisticated baseline analysis of neurite outgrowth [60,61], spine formation [62] and differentiation prior to undertaking any analysis of stress responsiveness.

PINK1 and Parkin: Involvement in Mitochondrial Quality Control and Mitophagy

The N-terminal mitochondrial targeting sequence (MTS) and the transmembrane domain (TM) of PINK1 insert into the OMM while the C-terminal serine/threonine kinase domain faces the cytosol. When directed to polarized mitochondria, PINK1 inserts into the OMM and is immediately cleaved by proteases, releasing PINK1 into the cytosol where it is taken to the proteasome for degradation [63–65]. The entire process from full-length protein synthesis to degradation only takes 3 minutes [66]. When a mitochondria is injured or depolarized, PINK1 is stabilized and rapidly accumulates in the OMM [63]. PINK1 then recruits cytoplasmic Parkin to damaged mitochondria resulting in the ubiquitination of substrates [64].

Elegant proteomic studies by Chan and colleagues demonstrate that upon stress, a large number of OMM proteins are targeted for proteasomal degradation and that this process is key in the induction of mitophagic processes [67]. Interestingly, OMM proteins targeted for degradation are involved in mitochondrial fusion such as Mfn1 and Mfn2, both of which are Parkin substrates. These data support a model in which Parkin recruitment via PINK1 acts as part of a quality control mechanism blocking mitochondrial fusion as a means of protecting overall cellular integrity (Figure 3). Given that mitophagy occurs throughout the lifespan of a cell and is essential to energetic compensation suggests that mild stress which normally could be contained might promote neurotoxic mitochondrial failure in those harboring mutations in either PINK1 or Parkin and that exposures to environmental stress early in development may dramatically change the trajectory of these at risk cells.

Genes and Environment: The Untangle able Knot

When considering the role of environmental stress in contributing to early onset neurodegeneration in individuals with similar genetic backgrounds, the best understood disease is PD. The majority of PD cases are sporadic, exhibiting no genetic inheritance while only 15% of PD cases are familial [68]. The discrepancy between the number of genetic versus sporadic cases has led to the consensus that PD develops from multiple risk factors which may include genetics, aging and exposure to environmental triggers.

Initial support for an environmental trigger for PD began with a group of drug users whom were unintentionally exposed to 1-methyl-4-phenyl-1,2,3-tetrahydropyridine (MPTP), a byproduct of illicit heroin synthesis. These patients presented with symptoms so similar to PD that they were clinically indistinguishable [69]. Subsequent work highlighted herbicides, fungicides, pesticides and manganese as molecules which could capture some of the features of PD [68]. One of the most significant issues in understanding environmental risk factors is the sheer number of man-made chemicals to which humans are currently exposed. The Center for Children's Health and the Environment noted that toxic chemicals of human origin contribute to diseases in children and concluded that the environmentally attributable portion was 30% for asthma, 5% for cancer, and 10% for neurobehavioral disorders which is perhaps not surprising given that some 70,000 different chemicals are on the market and approximately 1500 new ones are introduced every year [70]. Moreover, an average >280 man-made chemicals can be found in non-institutionalized United States civilian populations, making the complexity of understanding these chemical interactions with genetic risks staggeringly difficult [71].

Conclusions

Neurodegenerative disorders affect millions of patients worldwide. Although science has made great strides in identifying the molecular mechanisms and pathways involved in these disorders, therapeutic interventions often remain elusive. As we continue to search for therapeutics for neurological diseases, interesting new insights into the relationship between abnormal neural development and susceptibility to disease have been proposed.

Development, characterized by both new life and widespread death, puts a great strain on the mechanisms that select cells for survival. Data from the schizophrenia and autism literature supports the hypothesis that neurodegenerative diseases can, and should be, thought of as having roots in neural development.

We currently lack retrospective studies in which patients with mutations in PD-related genes have been evaluated using the same straightforward strategy of genetic risks and brain size/head circumference used in the ASD and schizophrenia literature. One would predict that those harboring mutations in genes which will cause diseases like Parkinson's might have smaller head circumferences and brain weights before symptom onset. Moreover, understanding the mechanisms by which the brain compensates to these long-term stressors could provide valuable tools for acute neuroprotective strategies and more rational therapies for chronic neurodegenerative conditions.

The activation of neuronal programmed cell death is almost certainly favorable during developmental periods of elimination; however, it is also during these times in which perturbations in trophic factors can place the survival of essential circuits in jeopardy. Moreover, massive increases in the energy needs of the developing brain demand that large numbers of new mitochondria come online. Antioxidant production and redox-sensing systems must be adequate in order to quench ROS production and remove damaged mitochondria in order to limit runaway oxidative stress.

Mitochondrial dysfunction has become increasingly linked to neurodegenerative diseases. As a result, stress-related mitochondrial signaling has become a growing target of new research. Indeed, major players in normal mitochondrial dynamics, including during periods of development, have been uncovered. These insights may provide key links between energy-intensive developmental processes and the acquisition of neurodegenerative diseases later in life.

Acknowledgments

The authors would like to thank Dr. Pat Levitt for helpful conversations, Ms. Lauren Koenig and Ms. Stacy Yanofsky for editorial support as well as Ms. Kylie Beck for graphical assistance. This work was supported by NIH grant NS050396 (BM) and a Vanderbilt Brain Institute Scholars Award (AMP). Statistical and graphical support was provided by P30HD15052 (Vanderbilt Kennedy Center).

Abbreviations

PCD	Programmed cell death
AD	Alzheimer's disease
ASD	Autism spectrum disorder
PD	Parkinson's disease
ALS	Amyotrophic lateral sclerosis
HD	Huntington's disease
ADOA	Autosomal dominant optic atrophy
CMTD	Charcot-Marie-Tooth disease
ROS	Reactive oxygen species
GSH	Glutathione
ETC	Electron transport chain
IMM	Inner mitochondrial membrane
OMM	Outer mitochondrial membrane
Mfn1	Mitofusin 1
Mfn2	Mitofusin 2
OPA1	Optic atrophy 1
Drp1	Dynamin related protein 1
GDAP-1	Ganglioside-induced differentiation-associated protein 1
PINK1	PTEN-inducible putative kinase 1.

References

1. Levitt P, Ebert P, Mirnics K, Nimgaonkar VL, Lewis DA. Making the case for a candidate vulnerability gene in schizophrenia: Convergent evidence for regulator of g-protein signaling 4 (rgs4). *Biological psychiatry*. 2006; 60:534–537. [PubMed: 16860780]
2. Faludi G, Mirnics K. Synaptic changes in the brain of subjects with schizophrenia. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience*. 2011; 29:305–309. [PubMed: 21382468]
3. Raedler TJ, Knable MB, Weinberger DR. Schizophrenia as a developmental disorder of the cerebral cortex. *Curr Opin Neurobiol*. 1998; 8:157–161. [PubMed: 9568403]
4. Berretta S. Extracellular matrix abnormalities in schizophrenia. *Neuropharmacology*. 2011
5. Schmitt A, Hasan A, Gruber O, Falkai P. Schizophrenia as a disorder of disconnectivity. *Eur Arch Psychiatry Clin Neurosci*. 2011
6. Courchesne E, Mouton PR, Calhoun ME, Semendeferi K, Ahrens-Barbeau C, Hallet MJ, Barnes CC, Pierce K. Neuron number and size in prefrontal cortex of children with autism. *JAMA: The Journal of the American Medical Association*. 2011; 306:2001–2010. [PubMed: 22068992]
7. Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, Chisum HJ, Moses P, Pierce K, Lord C, Lincoln AJ, Pizzo S, Schreibman L, Haas RH, Akshoomoff NA, Courchesne RY. Unusual brain growth patterns in early life in patients with autistic disorder. *Neurology*. 2001; 57:245–254. [PubMed: 11468308]
8. Oppenheim RW. Cell death during development of the nervous system. *Annual Review of Neuroscience*. 1991; 14:453–501.
9. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol*. 1997; 387:167–178. [PubMed: 9336221]
10. Chan WY, Lorke DE, Tiu SC, Yew DT. Proliferation and apoptosis in the developing human neocortex. *Anat Rec*. 2002; 267:261–276. [PubMed: 12124904]
11. Oppenheim R, Milligan C. Programmed cell death; in Editor-in-Chief: Larry RS (ed) *Encyclopedia of neuroscience* Oxford: Academic Press; 2009 11171124
12. Huang EJ, Reichardt LF. Neurotrophins: Roles in neuronal development and function. *Annu Rev Neurosci*. 2001; 24:677–736. [PubMed: 11520916]
13. Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD. Programmed cell death and the control of cell survival: Lessons from the nervous system. *Science*. 1993; 262:695–700. [PubMed: 8235590]
14. Park DS, Obeidat A, Giovanni A, Greene LA. Cell cycle regulators in neuronal death evoked by excitotoxic stress: Implications for neurodegeneration and its treatment. *Neurobiology of Aging*. 2000; 21:771–781. [PubMed: 11124421]
15. Herrup K, Neve R, Ackerman SL, Copani A. Divide and die: Cell cycle events as triggers of nerve cell death. *The Journal Of Neuroscience*. 2004; 24:9232–9239. [PubMed: 15496657]
16. Busser J, Geldmacher DS, Herrup K. Ectopic cell cycle proteins predict the sites of neuronal cell death in alzheimer's disease brain. *J Neurosci*. 1998; 18:2801. [PubMed: 9525997]
17. Campbell DS, Holt CE. Apoptotic pathway and maps differentially regulate chemotropic responses of retinal growth cones. *Neuron*. 2003; 37:939–952. [PubMed: 12670423]
18. Fujita J, Crane AM, Souza MK, Dejosez M, Kyba M, Flavell RA, Thomson JA, Zwaka TP. Caspase activity mediates the differentiation of embryonic stem cells. *Cell stem cell*. 2008; 2:595–601. [PubMed: 18522852]
19. McLaughlin BA, Hartnett KA, Erhardt JA, Legos JJ, White RF, Barone FC, Aizenman E. Caspase 3 activation is essential for neuroprotection in ischemic preconditioning. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:715–720. [PubMed: 12522260]
20. McLaughlin BA. The kinder side of killer proteases: Caspase activation contributes to neuroprotection and cns remodeling. *Apoptosis*. 2004; 9:111–121. [PubMed: 15004508]
21. Brown MC, Booth CM. Postnatal development of the adult pattern of motor axon distribution in rat muscle. *Nature*. 1983; 304:741–742. [PubMed: 6888543]

22. Stoica BA, Faden AI. Cell death mechanisms and modulation in traumatic brain injury. *Neurotherapeutics*. 2010; 7:3–12. [PubMed: 20129492]
23. Turner MR, Parton MJ, Leigh PN. Clinical trials in als: An overview. *Seminars in neurology*. 2001; 21:167–175. [PubMed: 11442325]
24. Rangasamy SB, Soderstrom K, Bakay RA, Kordower JH. Neurotrophic factor therapy for parkinson's disease. *Progress in brain research*. 2010; 184:237–264. [PubMed: 20887879]
25. Brodski C, Vogt Weisenhorn DM, Dechant G. Therapy of neurodegenerative diseases using neurotrophic factors: Cell biological perspective. *Expert review of neurotherapeutics*. 2002; 2:417–426. [PubMed: 19810873]
26. Kernie SG, Parada LF. The molecular basis for understanding neurotrophins and their relevance to neurologic disease. *Arch Neurol*. 2000; 57:654–657. [PubMed: 10815130]
27. Erecinska M, Cherian S, Silver IA. Energy metabolism in mammalian brain during development. *Prog Neurobiol*. 2004; 73:397–445. [PubMed: 15313334]
28. Erecinska M, Cherian S, Silver IA. Brain development and susceptibility to damage; ion levels and movements. *Current Topics In Developmental Biology*, Vol 69. 2005; 69:139–186.
29. Fukuda A, Prince DA. Postnatal development of electrogenic sodium-pump activity in rat hippocampal pyramidal neurons. *Dev Brain Res*. 1992; 65:101–114. [PubMed: 1372539]
30. Schmidt TA, Larsen JS, Kjeldsen K. Quantification of rat cerebral cortex na⁺,k⁽⁺⁾-atpase: Effect of age and potassium depletion. *J Neurochem*. 1992; 59:2094–2104. [PubMed: 1331324]
31. Pysh JJ. Mitochondrial changes in rat inferior colliculus during postnatal development: An electron microscopic study. *Brain Res*. 1970; 18:325–342. [PubMed: 5511217]
32. Bates TE, Almeida A, Heales SJR, Clark JB. Postnatal development of the complexes of the electron transport chain in isolated rat brain mitochondria. *Developmental Neuroscience*. 1994; 16:321–327. [PubMed: 7768212]
33. Iadecola C, Anrather J. The immunology of stroke: From mechanisms to translation. *Nat Med*. 2011; 17:796–808. [PubMed: 21738161]
34. Hazell AS. Excitotoxic mechanisms in stroke: An update of concepts and treatment strategies. *Neurochem Int*. 2007; 50:941–953. [PubMed: 17576023]
35. Adam-Vizi V, Starkov AA. Calcium and mitochondrial reactive oxygen species generation: How to read the facts. *J Alzheimers Dis*. 2011; 20(Suppl 2):S413–426.
36. O'Rourke B, Cortassa S, Aon MA. Mitochondrial ion channels: Gatekeepers of life and death. *Physiology (Bethesda)*. 2005; 20:303–315. [PubMed: 16174870]
37. Turrens JF, Alexandre A, Lehninger AL. Ubisemiquinone is the electron donor for superoxide formation by complex iii of heart mitochondria. *Arch Biochem Biophys*. 1985; 237:408–414. [PubMed: 2983613]
38. Stadtman ER. Protein oxidation and aging. *Free Radic Res*. 2006; 40:1250–1258. [PubMed: 17090414]
39. Martensson J, Jain A, Stole E, Frayer W, Auld PAM, Meister A. Inhibition of glutathione syntethesis in the newborn rat - a model for endogenously produced oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America*. 1991; 88:9360–9364. [PubMed: 1681551]
40. Hazelton GA, Lang CA. Glutathione contents of tissues in the aging mouse. *Biochem J*. 1980; 188:25–30. [PubMed: 7406884]
41. Lang CA, Mills BJ, Mastropaolo W, Liu MC. Blood glutathione decreases in chronic diseases. *J Lab Clin Med*. 2000; 135:402–405. [PubMed: 10811055]
42. Nanda D, Tolputt J, Collard KJ. Changes in brain glutathione levels during postnatal development in the rat. *Brain Res Dev Brain Res*. 1996; 94:238–241. [PubMed: 8836583]
43. McQuillen PS, Ferriero DM. Selective vulnerability in the developing central nervous system. *Pediatric neurology*. 2004; 30:227–235. [PubMed: 15087099]
44. Chan DC. Mitochondria: Dynamic organelles in disease, aging, and development. *Cell*. 2006; 125:1241–1252. [PubMed: 16814712]
45. Navarro A, Boveris A. Brain mitochondrial dysfunction in aging, neurodegeneration, and parkinson's disease. *Front Aging Neurosci*. 2010; 2

46. Palmer CS, Osellame LD, Stojanovski D, Ryan MT. The regulation of mitochondrial morphology: Intricate mechanisms and dynamic machinery. *Cell Signal*. 2011; 23:1534–1545. [PubMed: 21683788]
47. Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evgrafov O, Jonghe PD, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timmerman V, Schroder JM, Vance JM. Mutations in the mitochondrial gtpase mitofusin 2 cause charcot-marie-tooth neuropathy type 2a. *Nat Genet*. 2004; 36:449–451. [PubMed: 15064763]
48. Matsumine H, Saito M, Shimoda-Matsubayashi S, Tanaka H, Ishikawa A, Nakagawa-Hattori Y, Yokochi M, Kobayashi T, Igarashi S, Takano H, Sanpei K, Koike R, Mori H, Kondo T, Mizutani Y, Schaffer AA, Yamamura Y, Nakamura S, Kuzuhara S, Tsuji S, Mizuno Y. Localization of a gene for an autosomal recessive form of juvenile parkinsonism to chromosome 6q25.2-27. *Am J Hum Genet*. 1997; 60:588–596. [PubMed: 9042918]
49. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW. Hereditary early-onset parkinson's disease caused by mutations in pink1. *Science*. 2004; 304:1158–1160. [PubMed: 15087508]
50. Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, Frontali M, Albanese A, Wood NW. Localization of a novel locus for autosomal recessive early-onset parkinsonism, park6, on human chromosome 1p35-p36. *Am J Hum Genet*. 2001; 68:895–900. [PubMed: 11254447]
51. Valente EM, Brancati F, Ferraris A, Graham EA, Davis MB, Breteler MM, Gasser T, Bonifati V, Bentivoglio AR, De Michele G, Durr A, Cortelli P, Wassilowsky D, Harhangi BS, Rawal N, Caputo V, Filla A, Meco G, Oostra BA, Brice A, Albanese A, Dallapiccola B, Wood NW. Park6-linked parkinsonism occurs in several european families. *Ann Neurol*. 2002; 51:14–18. [PubMed: 11782979]
52. Deng H, Dodson MW, Huang H, Guo M. The parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in drosophila. *Proc Natl Acad Sci U S A*. 2008; 105:14503–14508. [PubMed: 18799731]
53. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ. The pink1/parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci U S A*. 2008; 105:1638–1643. [PubMed: 18230723]
54. Kuhn K, Zhu XR, Lubbert H, Stichel CC. Parkin expression in the developing mouse. *Brain Res Dev Brain Res*. 2004; 149:131–142. [PubMed: 15063093]
55. Unoki M, Nakamura Y. Growth-suppressive effects of bpoz and egr2, two genes involved in the pten signaling pathway. *Oncogene*. 2001; 20:4457–4465. [PubMed: 11494141]
56. Vives-Bauza C, de Vries RL, Tocilescu M, Przedborski S. Pink1/parkin direct mitochondria to autophagy. *Autophagy*. 2010; 6:315–316. [PubMed: 20200476]
57. Anichtchik O, Diekmann H, Fleming A, Roach A, Goldsmith P, Rubinsztein DC. Loss of pink1 function affects development and results in neurodegeneration in zebrafish. *J Neurosci*. 2008; 28:8199–8207. [PubMed: 18701682]
58. Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H, Jiang H, Hirano M, Rampersaud E, Jansen GH, Donkervoort S, Bigio EH, Brooks BR, Ajroud K, Sufit RL, Haines JL, Mugnaini E, Pericak-Vance MA, Siddique T. Mutations in ubqln2 cause dominant x-linked juvenile and adult-onset als and als/dementia. *Nature*. 2011; 477:211–215. [PubMed: 21857683]
59. Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, Miller D, Maric D, Cedazo-Minguez A, Cookson MR. Dj-1 acts in parallel to the pink1/parkin pathway to control mitochondrial function and autophagy. *Hum Mol Genet*. 2011; 20:40–50. [PubMed: 20940149]
60. Stanwood GD, Levitt P. Prenatal exposure to cocaine produces unique developmental and long-term adaptive changes in dopamine d1 receptor activity and subcellular distribution. *Journal of Neuroscience*. 2007; 27:152–157. [PubMed: 17202482]
61. Powell EM, Campbell DB, Stanwood GD, Davis C, Noebels JL, Levitt P. Genetic disruption of cortical interneuron development causes region- and gaba cell type-specific deficits, epilepsy, and behavioral dysfunction. *Journal Of Neuroscience*. 2003; 23:622–631. [PubMed: 12533622]

62. Svitkina T, Lin W-H, Webb DJ, Yasuda R, Wayman GA, Van Aelst L, Soderling SH. Regulation of the postsynaptic cytoskeleton: Roles in development, plasticity, and disorders. *The Journal Of Neuroscience*. 2010; 30:14937–14942. [PubMed: 21068295]
63. Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. Mitochondrial membrane potential regulates pink1 import and proteolytic destabilization by parl. *J Cell Biol*. 2010; 191:933–942. [PubMed: 21115803]
64. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, Tanaka K. Pink1 stabilized by mitochondrial depolarization recruits parkin to damaged mitochondria and activates latent parkin for mitophagy. *J Cell Biol*. 2010; 189:211–221. [PubMed: 20404107]
65. Meissner C, Lorenz H, Weihofen A, Selkoe DJ, Lemberg MK. The mitochondrial intramembrane protease parl cleaves human pink1 to regulate pink1 trafficking. *J Neurochem*. 2011; 117:856–867. [PubMed: 21426348]
66. Lin W, Kang UJ. Characterization of pink1 processing, stability, and subcellular localization. *J Neurochem*. 2008; 106:464–474. [PubMed: 18397367]
67. Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL, Hess S, Chan DC. Broad activation of the ubiquitin-proteasome system by parkin is critical for mitophagy. *Hum Mol Genet*. 2011; 20:1726–1737. [PubMed: 21296869]
68. Gao HM, Hong JS. Gene-environment interactions: Key to unraveling the mystery of parkinson's disease. *Prog Neurobiol*. 2011; 94:1–19. [PubMed: 21439347]
69. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science*. 1983; 219:979–980. [PubMed: 6823561]
70. Bertollini R, Danzon M. A dialogue on chemicals and children: Bulletin of the World Health Organization. 2004:811–890.
71. Control CfD: Fourth national report on human exposure to environmental chemicals. 2009:1–11.

Figure 1. Markers of cellular stress often associated with neurodegeneration are also expressed early in neuronal development and are essential for proper circuit formation

Primary neuronal cortical cultures prepared from embryonic rat pups were fixed and processed for immunocytochemistry on day *in vitro* 3. **(A)** Neurons stained positive for cleaved caspase 3 (blue) and tubulin (green). Note the presence of cleaved caspase 3 in neuronal processes and growth cones, further supporting the role of caspases in normal growth and development. **(B)** Neurons also stained positive for microtubule-associated protein light chain 3 also known as LC3 (blue) and tubulin (green), indicative of autophagic processing during development.

Figure 2. Model of energetic- and redox-based cell fate decisions at the level of the mitochondria
Endogenous and exogenous stressors can decrease ATP production and generate reactive oxygen species (ROS). While some proteins have the capacity to respond to both oxidative modification and energetic deprivation, there is a growing consensus that each stress signal modifies a select group of gatekeeping molecules which ultimately determine mitochondrial and, in turn, overall neuronal cell fate. As depicted in this diagram, conditions of mild stress causes activation of a subset of mitochondrial gatekeeping molecules which reinforce a neuroprotective phenotype. Severe stress; however, pushes the cell towards death. Known substrates of free radical modification include proteins governing mitochondrial fission and fusion as well as ion transporters required to maintain mitochondrial and cell membrane

potential. Redox activation of these proteins at low levels can promote either selective degradation of the organelle by a process known as mitophagy or irreversible ionic disequilibrium. Also subject to energetic and redox modification are the caspase proteases which can promote survival, differentiation and outgrowth when activated at low levels or apoptosis when temporal and spatial constraints are lost.

Figure 3. Model of the regulation of fission and fusion, the machinery of mitochondrial homeostasis

(Fission) Growth factor withdrawal, nutrient deprivation, and other cell stressors can promote the separation of mitochondria or fission. Proteins which are central regulators of this process include Drp1 which is regulated by both reactive oxygen species (ROS) as well as ubiquitination. These post translational modifications promote the recruitment of Fis1 which is essential for forming functional daughter mitochondria. The process of determining which daughter mitochondria are energetically and biochemically sound is poorly understood, but mitochondria can be degraded by LC3-mediated autophagy or ‘mitophagy’ if they are dysfunctional. **(Fusion)** The selective joining or ‘fusion’ of mitochondria is an

essential process to achieve greater biochemical and membrane stability and is governed by Mfn and Opa1. These proteins function at the level of the OMM and IMM, respectively.

(PINK1/Parkin Involvement) Mitochondrial depolarization leads to the accumulation and stabilization of PINK1 in the OMM. PINK1 can then recruit Parkin to the mitochondria resulting in poly-ubiquitination of substrates including, mitofusins. This process inhibits mitochondrial fusion and promotes mitophagy when both proteins are functional. Mutations in these genes have been linked to inherited forms of PD.