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Review Article

Alzheimer's Disease Selective Vulnerability and Modeling in Transgenic Mice

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Abstract. Neurodegenerative diseases are characterized by 'hot spots' of degeneration. The regions of primary vulnerability vary between different neurodegenerative diseases. Within these regions, some neurons are lost whereas others that are morphologically indiscriminate survive. The enigma of this selective vulnerability is tightly linked to two fundamental problems in the neurosciences. First, it is not understood how many neuronal cell types make up the mammalian brain; estimates are in the order of more than a thousand. Second, the mechanisms by which some nerve cells undergo functional impairment followed by degeneration while others do not, remain elusive. Understanding the basis for this selective vulnerability has significant implications for understanding the pathogenesis of disease and for developing treatments. Here, we review what is known about selective vulnerability in Alzheimer's disease, frontotemporal dementia, and Parkinson's disease. We suggest, since transgenic animal models of disease reproduce aspects of selective vulnerability, that these models offer a valuable system for future investigations into the physiological basis of selective vulnerability.

Keywords: Alzheimer's disease, amygdala, amyloid-β, frontotemporal dementia, hippocampus, neurofibrillary tangles, Parkinson's disease, tau

THE ENIGMA OF SELECTIVE VULNERABILITY

The factors that determine which neurons degenerate in neurodegenerative diseases are largely unknown. At issue are brain areas that may be referred to as 'hot spots' as they undergo selective degeneration. Within these hotspots, some individual neurons are spared and others are lost as the disease progresses. This phenomenon has been termed 'selective vulnerability' [1]. It is tightly linked to two fundamental problems in the neurosciences. Firstly, it is not understood how many cell types there are in the mammalian brain partly due to a lack of definitive criteria as we outline further below [2]. Secondly, the mechanisms by which nerve cells first undergo a functional impairment and eventual degeneration have remained elusive.

It could be speculated that different molecular mechanisms exist in different neural subtypes that underpin their survival or, conversely, vulnerability to death. It may even be possible that subtle differences between neurons have quite significant effects. In this review, we suggest that identifying the molecular signatures of different neural subtypes in the central nervous system will be essential to elucidate the mechanisms that underlie their selective vulnerability. This will be impor-

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tant, both for an understanding of the disease process and ultimately, for developing treatments.

HOW ARE NEURONAL CELL-TYPES DEFINED?

Brain function is orchestrated by a highly diverse array of neuronal cell types, many of which are not well defined. There may be several hundred or tens of thousands of types of neuronal cells. In addition to neuronal cell types, there are microglia and astrocytes. The complexity is enormous when considered in the context of the vast number of processes and dendritic spines that establish an intricate network of connectivity effectively linking the informational and operational domains of these different cell types. Brain function is impaired when the number of cells of particular types in a brain area falls below a critical threshold or when they fail to communicate properly.

But are the current criteria sufficient to define neuronal cell types, even in the context of neurodegeneration? In defining cell types, the ultimate goal has always been to single out a group of neurons that carry out a distinct function, although the strategic path has traditionally been first to identify cell types and then to discover their function. Morphology has been regarded as the prime criterion by which neuronal cell types are distinguished, in which neuronal shape is the main criterion because it directly reflects synaptic connectivity. The criterion of shape allows not only one cell to be distinguished from another, but it is a first step towards understanding the underlying wiring. Cell types have been further defined on the basis of location, electrophysiological properties, synaptic physiology, and marker gene expression. Clearly, however, a simple well defined neuronal taxonomy remains elusive.

For the mammalian brain, extrapolating numbers in the experimentally more readily accessible retina provides some idea of the scale we can expect. In mammals, the retina contains five major classes of neurons, together represented by around 60 individual cell types; for example, there are projection neurons (around a dozen in most mammalian species), and intrinsic neurons, which have been subdivided into horizontal, bipolar, and amacrine cells, using a simple letter code such as A1, A2, or A3 to define cell types based on morphological criteria and anatomical location [3,4]. The different shapes can often be associated with distinct, defining combinations of proteins, of which those that are involved in synaptic transmission have been particularly helpful. For example, bipolar cells that depolarize in response to light have axons that form arborizations deep in the inner plexiform layer; these cells express the metabotropic glutamate receptor isoform mGluR6. Bipolar cells that hyperpolarize to light form arborizations high in the inner plexiform layer and express ionotropic (AMPA/kainite) receptors; this helps in the identification of functional cell types. By extrapolation, it is possible to speculate that with around 60 cell types in the retina, there may be 1,000 neuronal cell types in the cortex alone, integrating knowledge of neuronal spacing, cell numbers, and the diameter of the dendritic field [5]. However, most of these cell types are not defined.

To address this issue, more recently a number of highly sophisticated tools have become available. Ways to mark different neurons include the 'Cre' recombination technology and 'Green Fluorescent Protein (GFP)' staining, to simultaneously stain neighboring neurons so that they are spectacularly isolated, exhibiting up to 166 different "color shades" based on four types of fluorescent proteins [6]. While the technology requires refining, the hope exists that it will be possible to use such technology to assist in identifying neuronal subtypes. It seems reasonable to suggest that combining such approaches with gene-expression profiling could be a useful alternative strategy in dividing neurons into functional subtypes [7,8]. We suggest this approach offers great hope for creating a molecularly defined taxonomy of neuronal subtypes that will, in turn, establish the molecular basis of selective vulnerability.

SELECTIVE VULNERABILITY IN ALZHEIMER'S DISEASE AND FRONTOTEMPORAL DEMENTIA

Neurodegenerative diseases of the human brain comprise a variety of disorders that, for demographic reasons, affect an increasing percentage of the aging population [9]. Alzheimer's (AD) and Parkinson's disease (PD) are examples of such late-onset diseases [10]. AD is the most common form of dementia, whereas PD is the most common movement disorder. The pathological changes in the AD and PD brain precede the onset of clinical symptoms by decades [11]. Histopathologically, the AD brain is characterized by abundant amyloid plaques, neurofibrillary lesions, and the concomitant loss of nerve cells and synapses [12,13]. This neurodegeneration spreads in a predictable, non-random

manner across the brain [14]. The neurofibrillary tangles (NFTs) contain massively phosphorylated, aggregated forms of the microtubule-associated protein tau and represent a hallmark lesion of the disease, in addition to amyloid-β (Aβ)-containing amyloid plaques, and develop in specific predilection sites [15–17]. The fact that there is selective vulnerability to NFT formation is beyond question. In the basal forebrain, all neurons that die appear to contain NFTs [18]. In contrast, up to 20% of the neuronal loss in the CA1 region cannot be explained by NFT formation, which is a slow process [19–21].

The spreading of tau pathology is subject to little inter-individual variation and provides a basis for distinguishing six stages: the transentorhinal stages I and II representing silent cases; the limbic stages III and IV; and the neocortical stages V and VI [17]. The cellular and molecular foundation of this staging is not at all understood. Several hypotheses have been put forward. For example, a correlation has been proposed with the pattern of consecutive myelination in the course of the development of the nervous system. More specifically, it has been claimed that neurons in association areas with minimal myelination are more vulnerable than those in primary cortices that are characterized by a more extensive and developmentally delayed myelination [22]. For the hippocampus, neuronal loss has been correlated with the distribution of glucocorticoid receptors [23].

There is a second level of vulnerability: remarkably, within brain areas susceptible to neurodegeneration, some neurons that appear morphologically indistinguishable from neighboring neurons that die can be spared for decades. Wherever neurons are lost in affected brain areas, protected neurons are found in their immediate vicinity [19,24,25]. Similar findings have been reported for other dementias, such as frontotemporal dementia (FTD) that is characterized by a tau pathology, in the absence of amyloid plaques [26]. Together this raises the major question as to whether the differences in selective vulnerability reflect molecular differences between cells that are not obvious based on current definitions of 'similar neurons'. If true then these different cells may potentially have slightly different intracellular signaling mechanisms that either lead to, or resist, cell death.

SELECTIVE VULNERABILITY IN PARKINSON'S DISEASE

PD is the most frequent neurodegenerative disorder with impaired motor functions. The PD brain is characterized by a selective loss of a subset of dopaminergic neurons. Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of dopamine, and TH-reactivity is used as marker for dopaminergic neurons. The clinical features of parkinsonism such as tremor, bradykinesia, and rigidity become evident only after around 80% of the TH-expressing neurons in the Substantia Nigra pars compacta (SNpc) have died [27–30]. It has been shown that upon the loss of TH-positive SNpc neurons in the rodent 6-hydroxy-DA (6-OHDA) model of PD, TH-positive neurons can partially recover [31]. There is evidence that this recovery occurs via a phenotype 'shift' from TH-negative to TH-positive cells [32]. Given that 80% of dopaminergic neurons must be lost from the SN before profound symptoms occur, just some preservation or restoration of dopaminergic neurons will have a dramatic therapeutic impact. There is therefore every reason to hope that innovative therapeutic strategies such as gene or cell replacement therapy may work for PD.

The PD brain is marked by fibrillar cytoplasmic inclusions that are abundant in degenerating dopaminergic neurons of the SN [33]. The lesions are known as Lewy bodies (LBs) and neurites (LN); they are ubiquitin-positive and mainly contain α-synuclein. In PD, neuronal vulnerability is known to characterize the SNpc, but abnormal protein deposition extends far beyond. It includes additional, specific neurons in autonomic ganglia, the spinal cord, brainstem, basal forebrain, limbic lobe, and even the neocortex [34].

While there is a selective neuronal loss of the TH-positive A9 dopaminergic neuron group in the SNpc, with a survival rate of 10%, the TH-positive A10 group in the medial and ventral tegmentum is largely spared, with a survival rate of 60%, even in severe cases [28, 35]. A9 neurons mainly project to the dorsolateral striatum involved in motor control, whereas A10 neurons connect to the ventromedial striatum, thalamus, and cortex and are involved in reward and emotional behavior.

Again, there is a second level of vulnerability: within the A9 group, the caudally and laterally located ventral TH-positive neurons are the most vulnerable [28,36]. This is unlikely a random process but may reflect distinct characteristics of TH-producing neurons that are not picked up using conventional criteria. Vulnerability of SN neurons may be caused by the greater susceptibility of dopamine and its metabolites in their production of reactive oxygen species (ROS) that eventually kill neurons [37]. Differential protein expression has been implicated in selective vulnerability, such as the pres-

ence of the K^+ channel GIRK2, which is exclusively expressed in vulnerable A9 neurons [38,39]. A role for GIRK2 is further supported by the loss of dopaminergic neurons in the *weaver* mouse model, which carries a spontaneous *GIRK2* mutation [40]. This indicates that differential gene expression patterns may determine selective vulnerability not only in the PD, but also AD brain.

It is therefore likely that the vulnerability of different subtypes of dopaminergic neurons depends on their specific molecular signatures, as this will in turn determine the intracellular signaling pathways that define their vulnerability to cell death. The process of therapeutic development for PD will require understanding the basis for this selective vulnerability for two reasons. Firstly, understanding the reasons for lack of vulnerability of some neurons may allow the development of approaches to protect the neurons that are vulnerable. Secondly, with the current suggestions that cell replacement strategies may eventually work, it likely will be important to replace the correct subtypes of dopaminergic neurons if these are to generate therapeutic benefits. It is therefore critical to develop an effective and robust taxonomy of dopaminergic neurons and to subsequently elucidate the basis of their selective vulnerability.

SELECTIVE VULNERABILITY IN TRANSGENIC MOUSE MODELS

Patterns of selective vulnerability comparable to the human AD brain also occur in rodents [41,42]; however, nerve cell loss, the end-point, has only been reproduced in a small subset of transgenic mouse models [43,44]. We will mention just a few of these models here, to elaborate on our point that selective vulnerability of neurons to cell loss is observed in mouse models of neurodegeneration.

One of these mouse models is the K3 strain [45], which expresses human tau together with the pathogenic K369I mutation found in Pick's disease [46]. Pick's disease belongs to the FTD complex that is often characterized by parkinsonism [47]. K3 mice express the K369I tau transgene in neurons within the hippocampus (CA1 pyramidal), cortex (pyramidal), amygdala, striatum, and SNpc, among other brain areas. Transgene expression affects the dopaminergic system, causing early-onset parkinsonism (rigidity, tremor, bradykinesia, and postural instability) in these mice. We identified an underlying molecular mechanism: the phenotype is caused by an impaired anterograde transport of distinct cargos, as shown for the nigrostriatal system and sciatic nerve [45,48]. Importantly, functional impairment has an early onset and occurs in the absence of overt nerve cell loss. However, in the absence of better cell-type-specific markers, it is unclear whether at this age one or more neuronal cell types have already been lost that would account for the phenotype. Eventually, as the mice age, up to 60% of TH-positive neurons in the SNpc are lost – but again, whether there is a selectivity for specific neuronal cell types is still in question [45].

In the K3 mice, two questions arise: 1) K369I tau expression of TH-positive neurons is moderate compared with other brain areas, but not all SNpc neurons degenerate, and CA1 neurons, for example, do not. What makes SNpc neurons particularly vulnerable compared with neurons in the CA1 region? 2) The loss of TH-positive, K369I tau-expressing neurons in the SNpc is only partial (60% loss by 24 months of age). What protects a subset of morphologically indiscriminable neurons within this brain area while others degenerate? Clearly the K3 mice we have generated offer a valuable tool to address these questions.

Meanwhile, different mice carrying similar mutations can exhibit different vulnerabilities. For example, to understand tau hyperphosphorylation and aggregation in AD [49], we established P301L mutant tau transgenic pR5 mice that are characterized by tau-containing NFT formation in the hippocampus and amygdala, as well as memory impairment [50–56]. In the absence of an unbiased stereological analysis, we found no obvious cell loss. In contrast, a different line of mice that expresses P301L mutant tau under inducible control (the $rTg(tauP301L)_{4510}$ line) shows an NFT pathology similar to that of the pR5 mice, but is characterized by massive brain weight loss and gross atrophy of the forebrain [57]. In fact unlike our pR5 mice, in the $rTg(tauP301L)_{4510}$ mice, 60% of CA1 hippocampal pyramidal neurons have already been lost by 5.5 months and only 23% remain by 8.5 months [57]. Reducing transgene expression in the latter mice using doxycycline in the drinking water partly rescues brain atrophy and nerve cell loss (as shown for CA1 neurons) and improves the phenotype. The explanation for the different vulnerability to cell loss observed between the two strains of mice is most likely that the two strains of mice express different levels of the P301L mutant tau transgene. Additionally, genetic background issues could be important as different strains of mice show differential vulnerability to cell loss. This points to the fact that a range of factors in any given cell, such as ex-

pression levels of a 'toxic' gene and the genetic context in which a 'toxic' gene is expressed, will determine the vulnerability of that neuron to cell loss.

When the Aβ pathology is combined with the tau pathology, this causes an increased NFT formation [58]. Interestingly, this enhanced pathology is restricted to specific brain areas. When P301L tau-expressing JNPL3 mice were crossed with Aβ-forming Tg2576 mice, these showed a more than seven-fold increase in NFT numbers in restricted brain areas, the olfactory bulb, entorhinal cortex, and amygdala, compared to P301L single transgenic mice; plaque formation, in comparison, was unaffected by the presence of the tau lesions [59].

We used an alternative approach to reveal the phenomenon of selective vulnerability: we stereotaxically injected synthetic preparations of fibrillar Aβ_{42} into the somatosensory cortex and the CA1 region of the P301L tau transgenic pR5 mice described above [50], wild-type human tau transgenic ALZ17 mice [60,61], and nontransgenic littermate controls, causing a five-fold increase of NFTs specifically in the amygdala of pR5, but not at all in ALZ17 or control mice [51]. This implies that not all brain areas are similarly susceptible to Aβ-mediated NFT induction. In both studies, the amygdala turns out to be a 'hot spot' of NFT induction. Unless in both mouse models tau levels in the amygdala are particularly high compared to other brain areas, a different transcriptional profile may account for the observed differences [62]. Support for the latter is provided by data obtained with long-term neuronal cultures from wild-type mice, as cortical neurons are less susceptible to Aβ- and staurosporine-induced toxicity than hippocampal neurons [63].

Other AD mouse models with selective neuronal loss, to name several prominent examples, include P301S tau transgenic mice [64,65], the N279K tau transgenic strain T-279 [66], ΔK280 tau transgenic TauRD/ΔK280 mice with an inducible tau expression [67], Pin1 knockout mice [68,69], APP transgenic APP23 mice [70], APP(SL)PS1 knock-in mice [71], and apoE4Δ(272–299) mice [72], as reviewed recently [44]. Among the neuronal populations that are lost are motor neurons, pyramidal CA1, or dopaminergic neurons. In all these model mice, selective vulnerability is clearly evident, as only a subset of all neurons are lost in any of the affected brain regions. The identity of these selectively vulnerable neurons is unknown.

In conclusion, aspects of selective vulnerability known to characterize the AD brain, in particular its susceptibility to specific toxic insults such as the Aβ peptide and selective neuronal cell loss, have been reproduced in transgenic animal models. These model systems are therefore excellent tools for longitudinal studies that aim to define neuronal cell types and to obtain a transcriptomic and proteomic profile of vulnerable compared to protected brain areas.

TRANSCRIPTOMICS IN DEFINING CELL TYPES AND DISSECTING SELECTIVE VULNERABILITY

The advent of increasingly sophisticated transcriptomic and proteomic techniques made it possible to identify differentially expressed genes and proteins in the AD and PD brain, and to pinpoint pathogenic mechanism, such as mitochondrial dysfunction or an impairment of the unfolded protein response (UPR) [73–75]. We used the tools of functional genomics to characterize our AD and FTD mouse and tissue culture systems and to dissect pathogenic mechanisms that not only operate in the transgenic mouse but also in human diseased brain [76–82].

Functional genomics not only assisted in dissecting disease mechanisms, but also emerged as a powerful tool in identifying cell-type-specific gene expression (i.e., obtaining a transcriptomic profile under physiological conditions) that may ultimately assist in identifying neuronal cell types [83]. However, thus far only a few instances of truly cell-type-specific gene expression profiles have been reported [84]. For example, serial analysis of gene expression (SAGE) was used to identify gene markers in the developing retina [85].

In an impressive, laborious study, Sugino and colleagues obtained 11 fluorescently-labeled neuronal populations from different brain areas using four GFP-expressing transgenic mouse lines [2]. They triturated the neurons and, by a panning process, manually isolated 30–120 neurons from each brain area that they had before characterized via current-clamp recordings. The subsequent analysis of the transcriptomic profile using the *Gene Ontology* software allowed them to construct a taxonomic tree that showed clear distinctions between neuronal cell types such as cortical interneurons and projection neurons [2]. As the authors point out, this dataset should be useful for the classification of unknown neuronal subtypes, the investigation of specifically expressed genes, and the genetic manipulation of specific neuronal circuit elements [2].

Transgenic mice such as the K3 model of parkinsonism described earlier in this review lose a signifi-

cant subset of TH-positive neurons in the SNpc as they age. These mice are therefore an excellent system to apply an integrative functional genomics approach; it will link differential gene expression in the SNpc to the identification of novel cell types in this brain area. We believe that studies such as these will shed light on the selective vulnerability that characterizes diseases such as AD and PD and that this new knowledge can be used to develop treatment strategies [12,86]. Ultimately, it may be able to equip neurons that are prone to degenerate with genes or combinations of genes that will protect them from neurodegeneration.

Of course the complexity of this approach will be profound. The gene sets expressed in different neurons will differ depending on the brain area that needs to be protected, the patient's burden of disease-associated mutations and risk alleles, the disease variant, and the type of toxic insult.

SUMMARY AND OUTLOOK

In summary, the hypothesis we put forward is that subtle differences in molecular signaling pathways between different neurons define the neuronal subtype and this in turn will define their selective vulnerability to death [87]. It is not hard to imagine that different cell types may show a predisposition for, or a differential protection from, alternative death mechanisms such as apoptosis, which is relatively fast, disruption of the Golgi apparatus, oxidative damage, or other mechanisms of death [88–90]. Defining the molecular signatures of different neurons and relating it to their vulnerability will be a major undertaking but an important one. It will not be easy, however; unraveling all of this is likely to be confounded by effects of the micro-environment of neurons. While a major focus of research into selective vulnerability is on neuronal dysfunction, there is also a contribution of the glial compartment in neuronal cell loss [91]. Furthermore this complexity will be further layered on a complexity defined by genetic background and environmental influences.

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