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Brain cholinergic vulnerability: Relevance to behavior and disease

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Abstract

The major populations of cholinergic neurons in the brain include two ''projection'' systems, located in the pontine reticular formation and in the basal forebrain. These two complexes comprise, in part, the anatomical substrates for the ''ascending reticular activating system'' (ARAS). The pontine cholinergic system relays its rostral influences mainly through thalamic intralaminar nuclei, but it also connects to the basal forebrain and provides a minor innervation of cortex. The basal forebrain cholinergic complex (BFCC) projects directly to cortex and hippocampus, and has a minor connection with the thalamus. Recent data reveal that a parallel system of basal forebrain GABAergic projection neurons innervates cortex/hippocampus in a way that seems to complement the BFCC. Generally, the picture developed from more than 50 years of research is consistent with a ''global'' influence of these two ascending cholinergic projections on cortical and hippocampal regions. Seemingly, the BFCC acts in tandem or in parallel with the pontine cholinergic projection to activate the electro-encephalogram, increase cerebral blood flow, regulate sleep–wake cycling, and modulate cognitive function. There are quite a number and variety of human brain conditions, notably including Alzheimer's disease, in which degeneration of basal forebrain cholinergic neurons has been documented. Whether the corticopetal GABA system is affected by disease has not been established. Studies of degeneration of the pontine projection are limited, but the available data suggest that it is relatively preserved in Alzheimer's disease. Hypotheses of BFCC degeneration include growth factor deprivation, intracellular calcium dysfunction, amyloid excess, inflammation, and mitochondrial abnormalities/oxidative stress. But, despite considerable research conducted over several decades, the exact mechanisms underlying brain cholinergic vulnerability in human disease remain unclear.

1. Brain cholinergic degeneration in human disease

The basal forebrain cholinergic complex (BFCC) includes several thousand neurons located within the medial septum, diagonal band, substantia innominata, and nucleus basalis of Meynert. The cholinergic neurons lying in this complex degenerate in Alzheimer's disease (AD), Parkinson's disease (PD), Down syndrome, the Parkinsonism–dementia complex, progressive supranuclear palsy, Jakob–Creutzfeldt disease, Korsakoff's syndrome, olivopontocerebellar atrophy, dementia pugilistica, and (possibly) Pick's disease (references cited in [1]). Cholinergic loss is also observed after chronic ethanol intake [2] and basal forebrain degeneration is implicated in traumatic brain injury [3]. In AD, the cholinergic degeneration is much more profound in the early-onset form [4]. Only two of these diseases (AD and Down syndrome) involve amyloid deposition to a greater extent than that seen in normal aging.

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2. Is basal forebrain-derived acetylcholine important for cognition?

During the past 25 years many experiments were directed at testing whether the BFCC is involved in aspects of cognition, including learning and memory (for recent reviews, see [5–7]). Earlier animal behavioral studies using electrolytic or excitotoxic ablation methods had been interpreted as largely substantiating the view that basal forebrain cholinergic systems play important roles in ''learning and memory''. The caveat that seems to be intrinsic to such studies is that, since non-cholinergic populations were affected by these manipulations, a ''pure'' cholinergic lesion was not attained. Lesioning with the anti-NGF immunotoxin method [8] appears to be more selective and behavioral testing of animals lesioned this way has produced results that some view as eliminating a major role for cholinergic systems in learning and memory (e.g., [9]), although further studies document a role for attention (reviewed in [7]). On the other hand, some recent efforts appear to support the traditional view of a key role for acetylcholine in cognitive function [10–17]; some of

this work was performed using the immunolesioning method [15,18,19]. In view of the physiological evidence supporting powerful modulatory actions of acetylcholine on cortical/hippocampal function, perhaps we should not conclude too quickly that this transmitter is only a minor contributor to ''learning and memory''.

Workers employing the immunotoxin lesioning method have noted the difficulty in attaining deficits in spatial tasks unless the doses used are high enough to kill cholinergic neurons throughout the BFCC [20–22]. Since NGF-receptors are not exclusively expressed by brain cholinergic neurons, intraventricular administration of the immunotoxin probably causes destruction of both cholinergic and non-cholinergic populations. The direct injection of the toxin into the BFCC is presumably more selective but, because of the distributed nature of rodent cholinergic neurons, effects are limited to only a part of the BFCC. The basal forebrain cholinergic neurons projecting to the amygdala are not eliminated with this lesioning method [23]. Cholinergic influences on the amygdala, which connects directly to cortex, and reciprocal influences of the amygdala on the BFCC projections to cortex, appear to be essential in some forms of learning [24–28] and in the BFCC-mediated activation of the cortical electrocortiocogram [29]. Either the BFCC projection to the amygdala or cholinergic neurons intrinsic to the amygdala [30] could survive to function in animals after lesioning other portions of the BFCC with the immunotoxin.

When using AchE histochemistry or immunostaining for choline acetyltransferase to assess the effects of denervation, the immunotoxin method can appear to completely remove targeted BFCC neurons. However, as Gold has noted [6], apparently complete removal of MS/DB cholinergic neurons reduces the level of hippocampal acetylcholine release only down to ~40%. Compensatory reactions were invoked for possibly explaining this. But, it is also possible that some of this residual acetylcholine is supplied by cholinergic neurons intrinsic to the hippocampus [31,32]. Similarly, cholinergic neurons intrinsic to the cortex could supply adequate amounts of acetylcholine after a lesion of the BFCC to support behavioral tasks that do not put too much demand on the circuits. The anatomy of cholinergic fibers in the cortex and hippocampus suggests that many of the transmitter's effects may be mediated by ''volume transmission'' (reviewed in [33]). That is, acetylcholine released from either synapses or non-synaptic structures like varicosities may diffuse some distance before activating cholinergic receptors, which may be either synaptic or extrasynaptic. If, after an immunotoxin injection, a reservoir of acetylcholine remains available from cortical/hippocampal interneurons, this supply may provide sufficient levels of the transmitter to keep these circuits ''primed''. Consistent with this idea are results from studies of muscarinic receptor down-regulation in response to chronic administration of an acetylcholinesterase inhibitor, in animals with or without basal forebrain lesions [34]. With ''complete'' removal of cortical cholinergic innervation from the basal forebrain using an excitotoxin (removal of about 70% of the cortical ChAT activity), the cortical receptors were still downregulated in response to chronic blockade of acetylcholinesterase. This cannot occur without acetylcholine availability and the conclusion was that intrinsic cortical cholinergic neurons were the source of that acetylcholine. One way to reveal the shift of demand from extrinsic to intrinsic acetylcholine sources in the cortex, and the involvement of any reserve, would be to use a muscarinic receptor antagonist in basal forebrain lesioned animals (e.g. [35]). The level of difficulty of the behavioral task being used is also a somewhat overlooked factor [7].

It has been known for some time that other ascending subcortical neurotransmitter systems may interact with or work in parallel with acetylcholine in modulation of cortical circuitry (e.g., norepinephrine and serotonin; for review see [36]). It is now being increasingly realized that the physiology of the BFCC is probably also linked with another subcortical population, the basal forebrain GABAergic projection neurons, which intermingle with the cholinergic neurons [37–41]. There are probably complementary influences of these two transmitters on cortical function that cannot be separated in any simple way. The basal forebrain GABA corticopetal component has to date been far less studied than the cholinergic complex, but this inhibitory transmitter must certainly influence the cortical GABA interneurons to which they connect, and these undoubtedly are integrated into circuits [42]. It could very well be that the earlier ablation studies of the basal forebrain region actually gave a more relevant picture of importance of this dual transmitter projection system in behavior. When comparing immunolesioned animals with subjects with excitotoxic lesions to the basal forebrain, a dissociation between GABAergic and cholinergic neuron loss can be achieved and correlated with differential effects on behavior [43]. There is a close relationship between the basal forebrain and cortical activity (for reviews see [44,45]). Direct stimulation of the basal forebrain elicits striking effects on the cortical electro-encephalogram, increases cerebral blood flow, and enhances responsiveness to sensory input [46–51]. BFCC stimulation can promote the acquisition of a learning task [17]. With such a manipulation it seems likely that both cholinergic and GABAergic projection systems would be activated. Whether the GABA projection degenerates in the AD brain is unknown, and this would seem to be an important issue to resolve if the neurotransmitter bases for alterations in cognitive decline in this disease are to be fully understood. The GABA terminals lie in the same regions as those of the cholinergic projection and are presumably exposed to the cortical pathology to the same extent as cholinergic terminals.

3. Does amyloid cause cholinergic degeneration?

The major hypotheses of mechanisms of cholinergic neurodegeneration in AD that have been pursued include: (1) excitotoxicity; (2) growth factor deprivation; (3) oxidative stress; (4) inflammation; (5) mitochondrial dysfunction; and (6) amyloid toxicity. Of course, each hypothesis can incorporate a fairly complex array of sub-mechanisms (e.g., gliosis, nitric oxide excess, calcium dysregulation), and each mechanism may border, overlap, or intersect with one or more of the others. Earlier studies largely dealt with the possible involvement of aberrant activation of ionotropic glutamate receptors and/or some kind of dysfunction in the supply or reception of neurotrophic support. Probably, the most prevalent current view is that the BFCC neurons die from toxicity to their cortical/hippocampal afferents caused by the accumulation of toxic insoluble amyloid within senile plaques in their terminal regions. This so-called ''amyloid hypothesis'' has been invoked to explain AD pathophysiology hundreds of times in the scientific and lay literature, and some of its supporting evidence is fairly persuasive. But, the basic fact of the matter is that the establishment of a direct link between cortical amyloid pathology and BFCC degeneration has not been established. One of the difficulties with the original hypothesis is that cognitive deficits in AD do not strongly correlate with the senile plaques themselves; there are often better correlations with the number of neurofibrillary tangles [52–54] or the extent to which synapses are lost [55,56]. More recently, the amyloid hypothesis has been morphed into other versions, including the ''soluble Aβ oligomer toxicity'' hypothesis, the ''free radical or oxidative-stress generating Aβ'' hypothesis, the ''glial/neuroinflammatory Aβ'' hypothesis, the ''Aβ-metal chelation'' hypothesis, and other varieties.

In typical studies of experimental amyloid toxicity, micromolar levels of Aβ or its analogues are administered to cells in culture or injected into the brain. Since the levels of the peptide produced naturally in vivo are probably much lower, the physiological relevance of many published findings with Aβ administration are somewhat unclear. A counter-argument that could be invoked is that toxic concentrations of amyloid peptide might obtain in the vicinity of generative loci or around plaques. Certainly, some histochemical studies of the senile plaque are consistent with localized pathological effects, and this is usually attributed to the presence of amyloid deposits. But, whether this is due to the insoluble plaque, the production of Aβ, or as a result of some secondary response (like microglial activation), is uncertain.

Transgenic models would seem to be the definitive approach to proving that amyloid excess is neurotoxic. Indeed, studies of cognitive function and neurodegeneration are now being conducted with transgenic mouse models in which one or more mutant AD genes are overexpressed and abundant plaques are found, or at least some amount of human Aβ peptide is present. These AD-related features of the transgenic brain seem to increase in intensity or level during maturation and aging of the animal. The mouse models also replicate some other features of the AD brain, including signs of gliosis [57–60] and increased oxidative stress [61,62]. With respect to cholinergic degeneration, however, these models have not proven to be very satisfying in demonstrating that ''amyloid excess'' per se elicits loss of the BFCC. While cholinergic fibers can be found in association with cortical plaques in these animals [63–65], whether the fiber density is decreased, increased, or rearranged is not entirely clear. The levels of presynaptic biochemical cholinergic markers appear to be unchanged or moderately altered in the cortex or hippocampus, as compared to non-transgenic mice [64–66]. More problematically, several studies suggest that basal forebrain cholinergic neuron loss (decreased neuron numbers) does not occur in these models [64,66–69], while one report indicates that loss does occur [70]. There is one study suggesting an *increase* in the number of cholinergic neurons [71]. It would seem that simply increasing the level of Aβ is not enough to cause cholinergic loss like that seen in AD, although some of the mouse data do indicate effects on the topology or extent of cholinergic fibers [64,68,69,71–73]. Assuming the observations are correct, either the simple view that Aβ excess causes neurodegeneration is wrong or there is something deficient in the model. For example, the mouse brain may be missing some key factor or process that the human brain needs for Aβ to exert the type of toxicity seen in AD. Perhaps, decades of life may be required for Aβ-related neurodegeneration to occur. In effect, this implicates multifactorial mechanisms.

In a study of the muscarinic and nicotinic receptor densities in an amyloid precursor protein/presenilin (APP/PS) dual-transgenic mouse model it was concluded that cholinergic receptors were not altered [64]. This suggests that the receptors did not develop denervation supersensitivity. In contrast to the APP, PS, or APP/PS models, mice expressing a combination of human Aβ and alpha-synuclein, a gene involved in Parkinson's Disease [74], and mice with a ''knockout'' of nerve growth factor [75], exhibit prominent loses of cholinergic neurons. The latter group reports that the anti-NGF model also exhibits cortical neuronal loss, amyloid deposition, and neurofibrillary tangles [75–77].

More recently, a number of workers have been examining whether intracellular Aβ or Aβ oligomers, rather than plaques, might initiate the pathology or cause cognitive deficits [78–80]. For example, the study by McGaugh, LaFerla, and colleagues in a ''triple-transgenic'' mouse model has shown that cognitive impairment is related to intraneuronal Aβ levels [80]. This model also reproduces the AD-type neurofibrillary tangles, suggesting that perhaps this model has achieved a connection between amyloid excess and another important element in AD pathology. There are two reports that intracellular Aβ42

elicits neurodegeneration [81,82], while another study indicates that Aβ production is required for neuronal viability [83]. It seems possible that immunotherapeutic approaches to lowering Aβ levels might be detrimental [84,85], and there has been discussion about whether the amyloid hypothesis of neurotoxicity is an oversimplification (e.g., [86]).

At this point a reasonable view of the amyloid hypothesis is that of agnosticism, at least with respect to BFCC degeneration. On the one hand, considerable evidence suggests that neurotoxic effects of the Aβ peptide can be easily produced and that humans possessing AD-related mutations or phenotypes of several kinds are predisposed to AD. Additionally, there have been many demonstrations that direct application of Aβ peptides can alter neuronal function and that transgenic mice expressing high levels of Aβ have altered behavior, even memory dysfuction. On the other hand, mechanistic links between amyloid cortical pathology and BFCC degeneration have not been established. Moreover, the ''natural'' functions of APP gene products remain somewhat mysterious. There is evidence that the secreted (sAPP) product may be important in neuron survival [87–89], and a recent study of transgenic F344 rats expressing the ''Swedish'' mutant APP gene have *improved* spatial learning at 6 and 12 months of age; the Aβ levels in these subjects are lower than those found in other transgenic models and the animals do not develop senile plaques [90]. Perhaps these models are revealing some beneficial (natural?) actions of products of the amyloid precursor protein.

4. Vulnerability of cholinergic neurons to oxidative/nitrosative stress

Our studies of cholinergic vulnerability over the last 10 years were stimulated by the discovery that some populations of brain neurons express constitutive nitric oxide synthase (also referred to as ''neuronal NOS'' or nNOS; [91]), an enzyme that can produce toxic levels of the free radical under conditions in which glutamatergic ionotropic receptors are over-stimulated. NADPH-diaphorase activity, which had been histochemically mapped in numerous earlier papers, was shown to indicate the presence of nNOS in the brain [92]. Activation of nNOS during excitoxicity produces neurotoxic levels of NO [93]. One might therefore expect that nNOS-positive (NADPH-diaphorase-positive) neurons would be particularly vulnerable in disease or disease models. To the contrary, there are numerous examples of resistance of these neuronal types [1]. The strongly nNOS-positive LDTN/PPN cholinergic neurons [94,95] seem to be another example, as these neurons are relatively resistant to excitotoxins [96] and survive in AD [97].

The cholinergic neurons in primary cultures of the basal forebrain degenerate when glia are activated [98]. The primary mediator of this degeneration is nitric oxide produced by inducible NOS, but in some circumstances other glia-derived substances might also contribute (e.g., cytokines). Glial activation is a component of ''neuroinflammation'' and brain inflammation is a component of AD neuropathology [99,100]. Experiments in which in vivo administration of lipopolysaccharide was used to activate glia, which causes global oxidative stress [101], have shown that brain cholinergic neurons can degenerate in response to neuroinflammation [102]. Aβ is believed to elicit glial activation in the AD brain [100] and AD transgenic mice reproduce this [57–60]. However, the amount of cholinergic degeneration arising from neuroinflammation evoked by cytokine infusion in AD transgenic mice is not greater than that seen in cytokine-treated non-transgenic mice [103]. These studies seem to support a view that cholinergic degeneration in AD is not directly caused by the neuroinflammation elicited by amyloid excess.

We speculated that the differential expression of the nitric oxide phenotype in cholinergic populations [95] might relate to their differential survival in disease or other pathological conditions (e.g., excitotoxicity). Initially, we hypothesized that neurons with high levels of expression might need to also constitutively express protection systems (e.g., manganese-dependent superoxide dismutase; [104]), but now it is evident that nitric oxide itself has neuroprotective functions [105]. Some of these functions might relate to the ''antioxidant'' nature of the free radical itself, but probably more important are the ways in which nitric oxide can modulate cellular signaling cascades, some of which converge on transcriptional systems. An emerging concept is that nitric oxide modulates cellular signaling pathways largely by covalent modification of protein thiols. The best-documented case is probably that of the small GTPase Ras, which is activated by *S*-nitrosylation [106–108]. Activation of Ras by free radicals is hypothesized to maintain a chronic state of ''survival tone'' in cells [109]. The ''downstream'' targets of activated Ras include Raf-1, the ERK1/2 branch of the MAP kinase family [110], and the PI3K/Akt cascade [108]. Nitric oxide also regulates NF-κB [111]. The ERK1/2, Akt, and NF-κB pathways then can activate downstream cellular survival mechanisms, which include altered transcriptional profiles [111–114]. Evidence for direct modulation of transcription factors by nitrosylation is also accumulating [115].

To test the hypothesis of involvement of nitric oxide in cholinergic survival mechanisms, we studied the effects of nitric oxide donors on primary cholinergic neurons and on immortalized cholinergic cells [116–119]. Probably the most important finding was the demonstration that basal forebrain cholinergic neurons were much more sensitive to nitric oxide excess than were pontine cholinergic neurons [117]. Depletion of glutathione in the cultures made the brainstem population just as vulnerable as the BFCC, while inhibiting the NF-κB cascade increased cholinergic degeneration in response to nitric oxide excess in both brain

regions. These studies were extended in experiments comparing these two populations with striatal cholinergic neurons [119]. Striatal cholinergic neurons do not express nNOS, while BFCC express low levels and pontine cholinergic neurons express high levels. The order of vulnerability of these three populations to nitric oxide excess, and hydrogen peroxide, was: striatum > basal forebrain > pontine cholinergic neurons. Indeed, about 40% of the pontine populations could not be killed even with relatively high concentrations of hydrogen peroxide (commonly used to impose oxidative stress). The differential vulnerability of the basal forebrain and pontine cholinergic neurons to nitrosative stress was still evident in neuron-enriched cultures with very little glia present, which suggests that the regional differences are not related to the supportive or protective functions of astrocytes. Using pharmacological manipulations, the resistance of the pontine cholinergic population was shown to require signaling through NF-κB, PI3K/Akt, and ERK1/2, while basal forebrain cholinergic neurons used only NF-κB and striatal cholinergic neurons did not employ any of these three systems. Using a dual immunostaining approach the pontine cholinergic cells, when treated with a nitric oxide donor, were observed to increase their nuclear levels of phosphorylated Akt, p65, and phosphorylated ERK1/2, indicating that nitric oxide was activating these survival pathways in these cholinergic neurons. The data clearly show, at least in culture, that there are striking variations in the resistance of different brain cholinergic populations to nitric oxide/oxidative stress, and that the most resistant population (pontine) robustly activated three survival pathways and required maintenance of glutathione levels to exhibit this property.

5. Aging and neurodegeneration mechanisms

The common human brain degenerative disorders are part of the human aging phenomenon, i.e., they are age-associated. Aging itself is dynamic: the brain is involved in a continual process of adaptation. In ''successful aging'', cognitive power is maintained even though cellular processes have been modulated to deal with compromises in function, most notably those elicited by oxidative stress. That successful human aging may involve the reorganization of brain functions on a major scale is evident in a study by Grady and colleagues [120]. Cohorts of elderly and young humans were trained to equal performance in a cognitive task and then brain metabolic mapping was used to reveal how different brain regions interacted during the task. Partial least squares analyses and structural equation modeling methods were used to infer the relative employment of connections between specific brain regions that were involved in supporting the task. The main conclusion of the study was that *aged humans employed circuits differently than young subjects* while performing the same task equally well. This suggests that the ''wisdom of aging'' involves actual restructuring of brain circuitry. An example of a molecular view of such restructuring processes was obtained in a microarray study of human brain gene expression over the lifespan [121]. In that study, most quantitated genes were unaltered in level of expression from childhood to very old age, but a small subset of genes that changed levels emerged beginning at about age 40. One group of putative ''plasticity'' genes declined in level, while another group of putative ''survival'' genes were induced. Oxidative stress was hypothesized to cause these changes and this was supported by findings of oxidative damage within the promoters of several of the plasticity genes. This study emphasizes that the brain possesses a reserve of function, which can be depleted as a function of aging, leading to dramatic changes in the deployment of antioxidant and other survival mechanisms. Individual variations in outcome can presumably be explained both by differences in genetic background and differences in the nature or duration of environmental influences. In our studies of the brains of aged Long–Evans rats we have found molecular evidence that oxidative stress-related processes progress at different rates or to different degrees in the individual aged brain. Those subjects that had greater oxidative damage or signs associated with such exhibited lower performance in a spatial learning task [122]. Studies like these emphasize the fact that aging of the brain is highly individualized: cognitive decline is not a necessary consequence because some individuals are either more capable of combating oxidative stress or more able to adapt to it.

6. Some suggestions for research directions

In AD and PD research a good deal of emphasis is being given to understanding the extent to which oxidative stress is involved in neurodegeneration. Oxidative stress is already an established feature of aging and it seems likely that the added pathology present in these diseases (amyloid, glial activation, inflammation, etc.) considerably exacerbates this. The mitochondrion is one prominent organelle in the neuron where this stress could initiate events leading to degeneration. The requirement for ATP generation by mitochondria to support neurotransmission makes the brain's demand for oxygen the greatest of any organ; consequently, the mitochondrion is also the major endogenous source of oxidative stress in neurons. Large projection neurons like those in the BFCC probably require higher levels of mitochondrial activity to support neurotransmission to their extensive terminal fields. The mitochondrion also is the primary point-of-control for a major endogenous apoptotic cascade. Rather than simply being a switch that is activated by upstream events to initiate apoptosis, perhaps the mitochondria play more of a role in the ''decision'' process itself than is generally realized.

There must be complex and robust control systems to dynamically balance mitochondrial support for neurotransmission while integrating and mobilizing a variety of protection mechanisms. A fascinating aspect of mitochondrial biology is the recent emergence of findings that many signaling elements, classically viewed as acting primarily in the cytosol and nucleus, are also contained within mitochondria (reviewed in [123]). These include Akt [124], Raf-1 [125], Ras [126], NF-κB [127], SAPK/JNK [128], p53 [129], APE/Ref1 [130], and CREB [131]. While the specific roles for these signaling entities in mitochondrial functions have not been fully established, most of these signaling systems are known to be involved in cellular survival mechanisms, including management of oxidative stress. Moreover, there is evidence that a NOS enzyme exists in mitochondria [132] and that nitric oxide modulates mitochondrial functions [133–137]. A direction for future research into neuronal vulnerability in aging, and age-associated diseases, might be to investigate whether compromise in mitochondrial functions might extend beyond the performance of the electron transport chain to include signaling abnormalities that prevent or alter the involvement of this organelle in cellular survival signaling. In terms of potential for therapy, added attention should be given to the development and long-term use of antioxidants [138].

7. Conclusions

Whether or not one believes that brain cholinergic neurons are important for learning and memory, a reasonable view is that their loss in several important human diseases is likely to contribute in some way to the cognitive deficits that are manifested. While the biology of brain cholinergic neurons has been addressed probably to a greater degree than any other neurotransmitter phenotype in human disease, with the possible exception of the dopamine system, surprisingly little definitive light has been shed on why they degenerate in the human brain, particularly in AD. A more general, ''universalist'' view about neurodegeneration in AD seems more reasonable to pursue than any single causative factor; i.e., probably multiple mechanisms are involved and probably different vulnerable populations decline for a different combination of mechanisms. But, oxidative stress could be viewed as a linch-pin that brings into convergence multiple age-related stressors. Oxidative stress may even be the final common pathway that executes the neurodegenerative process. If so, then more attention might be profitably given to evaluating how neuronal survival signaling is influenced by age-related changes in mitochondria. The BFCC exhibits a fairly unique combination of features: responses to trophic factors, sensitivity to glial activation, extensive projection topology, relative vulnerability to nitric oxide, and reduced ability to activate some survival pathways. A major cellular point of convergence of stressors that relate to these properties is the mitochondrion.

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