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Nerve Growth Factor and Alzheimer's Disease: New Facts for an Old Hypothesis

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Abstract Understanding sporadic Alzheimer's disease (AD) onset and progression requires an explanation of what triggers the common core of abnormal processing of the amyloid precursor protein and tau processing. In the quest for upstream drivers of sporadic, late-onset AD neurodegeneration, nerve growth factor (NGF) has a central role. Initially connected to AD on a purely correlative basis, because of its neurotrophic actions on basal forebrain cholinergic neurons, two independent lines of research, reviewed in this article, place alterations of NGF processing and signaling at the center stage of a new mechanism, leading to the activation of amyloidogenesis and tau processing. Thus, experimental studies on NGF deficit induced neurodegeneration in transgenic mice, as well as the mechanistic studies on the anti-amyloidogenic actions of NGF/TrkA signaling in primary neuronal cultures demonstrated a novel causal link between neurotrophic signaling deficits and Alzheimer's neurodegeneration. Around these results, a new NGF hypothesis can be built, with neurotrophic deficits of various types representing an upstream driver of the core AD triad pathology. According to the new NGF hypothesis for AD, therapies aimed at reestablishing a correct homeostatic balance between ligands (and receptors) of the NGF pathway appear to have a clear and strong rationale, not just as long-term cholinergic neuroprotection, but also as a truly disease-modifying approach.

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The Debate Around the Mechanisms Leading to Alzheimer's Disease

Progressive synaptic and neuronal loss in Alzheimer's disease (AD) leads to cognitive decline. No cure and no early diagnosis are presently available for AD. An early and predominant loss of basal forebrain cholinergic neurons (BFCNs) is the major functional basis for the cognitive impairment in AD, laying the grounds for the "cholinergic hypothesis" of AD [1]. At the neuropathological level, amyloid plaques and neurofibrillary tangles are the histological hallmarks, made from Aβ peptide, a cleaved product of the amyloid precursor protein (APP) and from hyperphosphorylated Tau, a microtubule binding protein, respectively. Despite intensive research in the past two decades, no generally accepted mechanism has yet been formulated causally linking the AD triad (cholinergic deficit, amyloid Aβ, and Tau pathologies) into one unified conceptual scheme. Following the discovery that mutations in genes encoding APP and Tau cause dementing illnesses, the cholinergic hypothesis was abandoned, and the Alzheimer's field was dominated by disputes over whether Aβ or Tau abnormalities represented an upstream pivotal pathogenic cause driving the disease (the Baptist vs. Taoist confrontation).

Genetic studies of rare monogenic forms of the disease (early-onset Alzheimer's disease; EOAD), which recapitulate the neuropathological and clinical profile of sporadic, late-onset Alzheimer's disease (LOAD), provided the main driving force in the debate on AD mechanisms. Mutations in APP [2] and in presinilin PS-1 and PS-2 genes [3–5], determining an increased Aβ processing, were found to underlie EOAD. In contrast, mutations in the gene encoding Tau, although found to cause Tau hyperphosphorylation,

lead to frontotemporal dementia [6], which is histologically distinct from AD and has no amyloid plaques. These findings reinforced the case for a serial model of causality in AD, with elevation of Aβ as the prime pathogenic driver of AD, the "amyloid hypothesis" [7, 8] (Fig. 1). Because EOAD and LOAD phenocopy each other clinically (except for the age of onset) and histologically, the amyloid hypothesis, although based on EOAD genetic data, was de facto extended to underlie LOAD as well. Indeed, bridging the knowledge of the molecular mechanisms of EOAD to the etiology of sporadic LOAD is a great challenge to unraveling AD mechanisms.

The Amyloid Hypothesis Failed to Deliver Effective Therapeutic Treatments

Largely because of the strong genetic data, the amyloid hypothesis has been the driving force in guiding pharmaceutical efforts toward new treatments. Accordingly, pharmacological agents reducing brain Aβ levels should act as effective drugs for AD. However, it is noteworthy that, to date, the results of many clinical studies testing new amyloid-lowering treatments failed to deliver the expected results and have been largely disappointing. Among the different anti-Aβ agents tested in LOAD patients, Aβ immunization is the most innovative approach [9]. Anti-Aβ antibodies are either elicited by active immunization or directly delivered as human recombinant antibodies (passive immunization). Despite preclinical studies in mouse models showing effective clearing of Aβ from the brain, Aβ42 immunization clinical trials (as well as those with other anti-Aβ pharmacological treatments) failed to show any clinical efficacy whatsoever. One study for all [10], documenting the long-term effects of active Aβ42 immunization, illustrates well the case. The clinical trial was halted because of lethal meningoencephalitis in a small number of subjects, but the study followed a group of patients free of the drug's negative side effects. The immunized patients showed no significant effect of immunization on any clinical measure over a 6-year period. On the other hand, immunized patients, with high titers of anti-Aβ antibodies, had a significant reduction of brain Aβ, while Tau pathology was heavily disseminated across the cortex. Thus, effectively clearing amyloid plaques from the patients' brains had no effect whatsoever on their cognitive decline. A range of possible explanations was put forward to explain these incontrovertible negative results [10, 11]. Since then, a number of other clinical trials, targeting the Aβ pathway in different ways have failed to provide positive results (reviewed and discussed in 12). The amyloid hypothesis could be defended by assuming that Aβ acts as an initial "trigger" of downstream events, so that, once initiated, neurodegeneration might progress even if Aβ levels are reduced. One could also assume that the truly neurotoxic forms of Aβ, such as oligomers, might not have been reduced in that clinical setting. Thus, the adjourned version of the amyloid hypothesis puts the accent on "early" and on "Aβ oligomers" (critically discussed and reviewed in [13]) and considers synapses the earliest target for these "more toxic" Aβ species [14]. Also, when interpreting the negative outcome of the immunotherapeutic strategy targeting βA, one needs to keep into account the recently discovered physiological trophic role of βA (see below, "**The Triple State Hypothesis"**). Nevertheless, the unequivocally negative results of the clinical studies targeting Aβ in different ways call for a reevaluation of the whole amyloid hypothesis, notwithstanding the compelling genetic evidence in its favor [12] and call for a paradigm shift [15] and for alternative models of causality, linking Aβ and Tau pathology to an upstream common molecular or cellular driver [16]. Therefore, there is great interest to take into consideration the "nongenetic elevators of Aβ," and in the following, we shall present data from our groups and from the literature that led us, some time ago, to propose neurotrophic deficits as a common upstream mechanism linking cholinergic, Aβ, and Tau abnormalities into one comprehensive "multiple pathway" mechanism of neurodegeneration (Fig. 1). From this framework, any therapy aimed at reestablishing a correct balance between ligands (and receptors) of the nerve growth factor (NGF) pathway appears to have a clear and strong rationale.

Nerve Growth Factor and Alzheimer's Disease: An Old Connection

NGF [17] has been classically connected to AD [18], on a purely correlative basis, because of the selective vulnerability of BFCNs in AD (cholinergic connection) and of the retrograde transport of NGF in these neurons (retrograde transport connection). BFCNs provide major projections to the cerebral cortex and the hippocampus, subserving cognitive functions and memory. NGF is BFCN's principal target-derived neurotrophic factor, protecting them from various insults and from their aging dependent atrophy. Accordingly, a decreased trophic support, due to a reduced amount of NGF available to BFCNs, could contribute to the cholinergic cell loss observed in AD. NGF is produced in the cortex and hippocampus and retrogradely transported to BFCN. NGF expression is not altered in AD. Several findings collectively support the view that a diminished retrograde transport of NGF can determine a reduced neurotrophic support to BFCNs. A reduced capacity of TrkA-dependent retrograde transport of NGF may lead to the loss of BFCNs observed in early AD [19, 20] and explains why, in AD postmortem

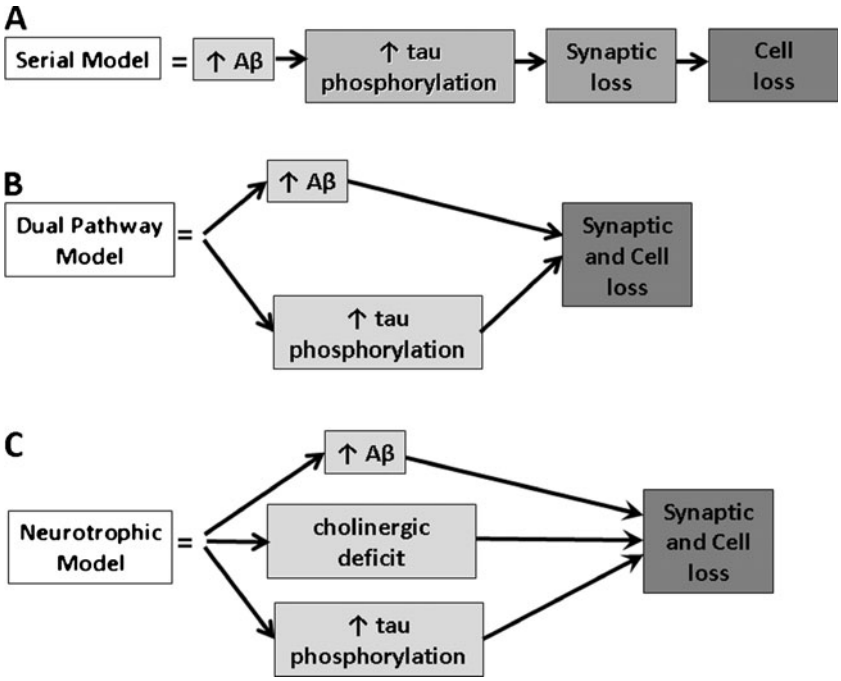

Fig. 1 Outline of the serial amyloid hypothesis (***a***), in its classic version, of the dual pathway model (***b***) and of the NGF hypothesis as an upstream driver for AD neurodegeneration

brains, NGF and proNGF proteins are increased in the cortex and hippocampus and are diminished in basal forebrain [21].

Cytoskeletal transport dysfunctions, and a reduced axonal transport of NGF, represent a common link between NGF trophic deficits, cholinergic dysfunction, and neurodegeneration [22, 23], so that neurons in AD cannot take full advantage of NGF, since both APP and Tau are involved in axonal transport. These conclusions are supported and documented by in vitro experiments reported in sections "Linking Directly NGF Deficits to the Activation of the Amyloidogenic Cascade" and "The Paradoxical, Functional Switch of the TrkA Receptor". In partial trisomy 16 Ts65Dn mice, increased APP expression is directly linked to reduced retrograde transport of NGF in BFCN, resulting in cholinergic neuronal degeneration, which can be reversed by exogenous NGF delivery to BFCN cell bodies [22]. However, according to this view, a reduced neurotrophic support would be downstream of APP (or Tau) alterations.

The Emerging Complexity of proNGF/NGF Equilibrium

Other mechanisms, besides neuronal transport defects, could account for a reduced neurotrophic support to BFCN in AD. The emerging complexity of the proNGF/NGF system adds another element to this picture.

NGF is translated as a pre-pro-protein, which is cleaved by furin in the trans-Golgi network to yield mature NGF (reviewed in [24]). proNGF can also be released as such and cleaved extracellularly by plasmin and matrix metalloprotease-7 [25]. ProNGF was initially thought to be a chaperone to assists folding and secretion of mature NGF, but proNGF and NGF turned out to have distinct biological activities [26]: proNGF has a higher affinity for p75NTR and a lower one for TrkA compared to mature NGF and induces p75NTR dependent apoptosis. ProNGF can also induce TrkA-dependent neuronal survival, although less effectively than NGF [27].The first structural study on proNGF provides a structural interpretation to the distinct receptor binding profile of proNGF versus NGF [28].

The prodomain of NGF interacts with sortilin, a neuronal type-1 VPS10-domain receptor, a co-receptor with p75NTR for proNGF [29]. Sortilin, together with the VPS10-containing protein sorLA, binds the retromer complex in neurons. Converging evidence, including genetic data, implicates the retromer sorting pathway in LOAD (reviewed in 16). The levels of proNGF and its co-receptor sortilin increase in mild cognitive impairment and early AD brains [19, 30], paralleling the progressive decline in TrkA receptors. A diminished conversion of proNGF to mature NGF and an increased NGF degradation in AD brains were recently reported [31].

Thus, the biological effects of proNGF versus NGF influence the balance between cell death and cell survival [32, 33], and an imbalance in this complex ligand/receptor system has been correlatively linked to AD neurodegeneration, although no causally direct proof in vivo has been available until recently (see below).

The multiple correlative links between NGF and AD, described above, do not, however, provide evidence for a cause–effect mechanism leading to AD neurodegeneration

and do not explain what activates the aberrant processing of APP and of Tau in sporadic AD.

In the past decade two independent lines of evidence have converged on the common conclusion that deficits or alterations in the signaling and processing of NGF represent potential upstream drivers for Alzheimer's neurodegeneration: results obtained with transgenic mice expressing anti-NGF antibodies and results obtained from primary neuronal cultures deprived of NGF. Subsequent sections will summarize both lines of research.

Anti-NGF AD11 Mice as a Comprehensive Model for Sporadic Alzheimer's Disease

The first demonstration that NGF deficits could have consequences beyond a direct interference with the cholinergic system came from studies in the anti-NGF AD11 mouse model [34, 35]. These mice express a recombinant, highly specific anti-NGF antibody in the adult brain and allow studying the effects of a chronic neutralization of NGF in the adult brain after normal development. Quite unexpectedly, these mice progressively develop a comprehensive AD-like neurodegeneration, more severe than the expected cholinergic deficit per se, with functional and behavioral impairments, encompassing several features of human AD. The progressive impairment of working memory, revealed by a number of behavioral tasks [36, 37] is accompanied by synaptic plasticity deficits in the cortex [38] and the hippocampus [39]. At the neuropathological level, besides the expected deficit in BFCNs, AD11 mice show an intracellular and extracellular accumulation of β-amyloid in the hippocampus and a progressive neuronal expression of hyperphosphorylated and truncated Tau [40]. The time progression of phenotypic alterations in AD11 mice is summarized in Fig. 2.

The neurodegeneration in AD11 mice is NGF-dependent since it can be fully reverted by NGF administration [37, 41], demonstrating that NGF sequestration by antibodies results in Alzheimer's neurodegeneration. Cholinergic drugs fail to substantially revert neurodegeneration in AD11 mice, confirming that the cholinergic neurotransmission deficit is not a primary event of the neurodegeneration cascade in AD11. Breeding AD11 mice under environmental enrichment (EE) conditions rescues their memory and neuropathological deficits [36]. EE is a complex stimulus, and its effectiveness in rescuing AD11 neurodegeneration confirms this neurodegeneration to be a multifactorial process.

In summary, AD11 mice display a comprehensive and progressive neurodegeneration reminiscent of LOAD (Fig. 2). Unlike other transgenic AD models, and similarly to LOAD, in AD11 mice, beta-amyloid pathology arises from endogenous APP, in the absence of a mutation in APP/APP processing genes. The phenotype of AD11 mice has uncovered a new mechanism whereby neurotrophic deficits are an upstream driver, causally linked to altered APP processing and Tau pathology, in addition to determining a cholinergic deficit [42, 43].

"Too Little NGF—Too Much ProNGF"

How can we explain this neurodegenerative process, in mechanistic terms? What have we learned from this mouse model that could be translated to mechanisms operating in human LOAD? The NGF binding properties of anti-NGF mAbαD11, expressed in the brain of AD11 mice, provided a first clue [44]. It turns out that the anti-NGF mAbαD11, expressed in AD11 brains, binds NGF with a three order of magnitude higher affinity than that for proNGF (KD=10–12 and 10–9 M for NGF and proNGF, respectively), with binding to NGF being virtually irreversible.

The preferential binding of mAbαD11 to mature NGF, with respect to proNGF, would determine, under limiting concentrations in the mouse brain, an experimentally induced functional imbalance between NGF and proNGF by irreversibly "sequestering" mature NGF while leaving proNGF free to act in the functional "absence" of mature NGF. proNGF would activate the proneurodegeneration,

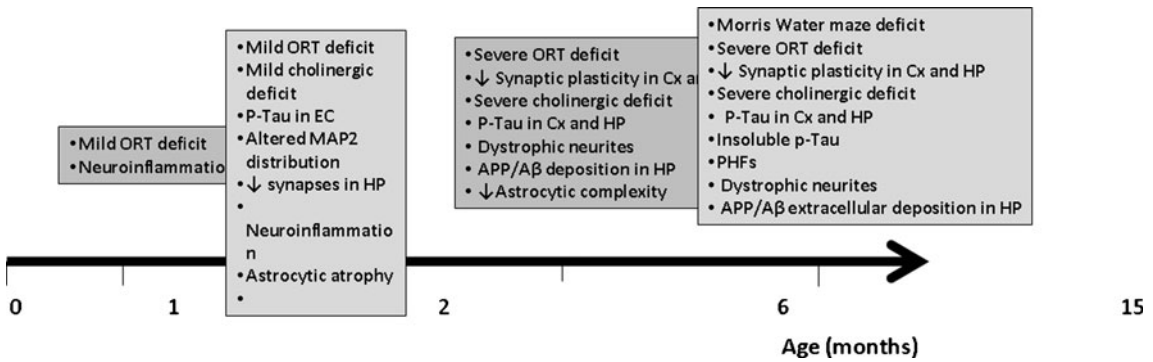

Fig. 2 Time progression of phenotypic alterations in anti-NGF AD11 mice

proamyloidogenic pathways, interacting with sortilin and p75NTR receptors [42]. A first test of this mechanism [45] comes from studies in which AD11 mice have been crossed to p75NTR knock-out mice (p75NTR –/–). The resulting offspring (AD12 mice) shows a complete reversion of the Aβ phenotype, demonstrating that amyloidogenesis in the AD11 model involves proNGF/p75 signaling.

Accordingly, the main determinant of neurodegeneration in anti-NGF mice would be the selective neutralization of NGF versus proNGF by an antibody in the brain: "too little NGF, too much proNGF" [43] (see Fig. 3). In a more recent study, transgenic mice expressing a furin-uncleavable form of proNGF in the postnatal brain were derived. As a result, proNGF mice express not only high levels of proNGF, but also higher levels than normal of mature NGF. Surprisingly, these mice develop a striking cholinergic deficit phenotype, accompanied by formation of Amyloid beta oligomers and severe learning and memory behavioral deficits (Tiveron et al., submitted for publication). This provides a missing link for a proNGF/NGF centered vicious cycle, integrating data on Amyloid beta inducing dysmetabolism of proNGF [25, 31] and on proNGF increase in AD brains [30].

Synaptic Remodeling: Alterations in the Excitatory Versus Inhibitory Balance

Mounting evidence suggests that AD is a synaptic failure and begins with subtle alterations of hippocampal synaptic efficacy prior to frank neuronal degeneration [14]. In AD11 mice, recent evidence demonstrated a major early synaptic remodeling involving functional changes in the excitatory versus inhibitory drive in the hippocampus. GABAergic signaling shifts to an "immature" state, from hyperpolarizing to depolarizing, due to an alteration of chloride homeostasis and a downregulation of chloride transporter KCC2 expression [46]. This major reorganization of the GABAergic circuitry within the AD11 hippocampal network may represent a homeostatic response to counterbalance neurodegeneration, or it may itself contribute to the progressive neurodegeneration. It is interesting to note that the state of permanent depolarization is a condition to keep alive cerebellar granule cells as discussed in "Linking Directly NGF Deficits to the Activation of the Amyloidogenic Cascade". The developmental shift of GABA from depolarizing to hyerpolarizing is known to exert a critical control on the refinement of synaptic connections. The observed abnormal excitatory to inhibitory imbalance in

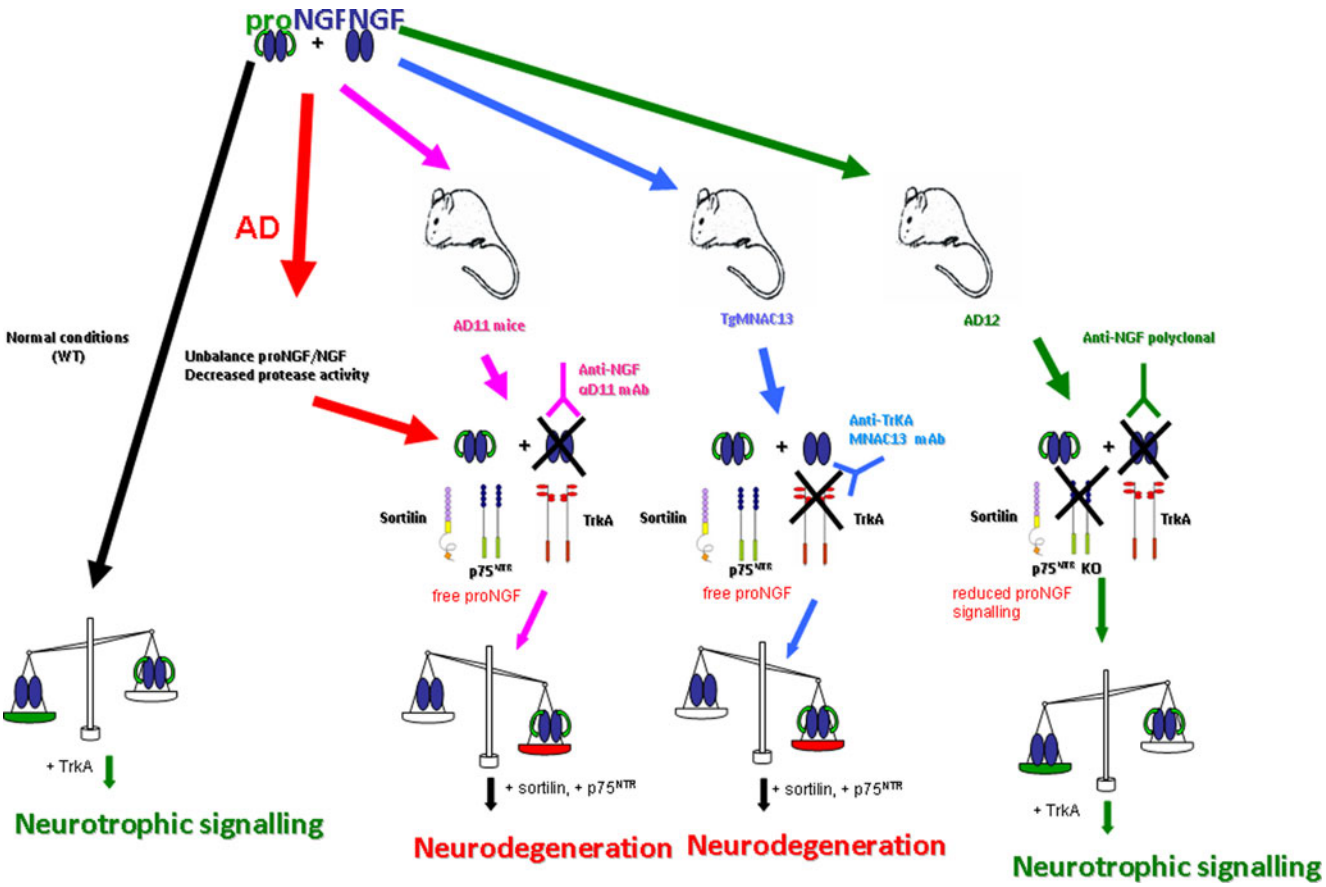

Fig. 3 The proNGF/NGF imbalance mechanism for neurodegeneration and its experimental evidence

the AD11 hippocampus, at 6 months of age, when intracellular Aβ peptide is first detected (Fig. 2), shows that synaptic remodeling and network alterations represent a major event in early neurodegeneration that could contribute to profoundly altering hippocampal circuits.

The Earliest Events in the Progression of Anti-NGF-Induced Neurodegeneration

What are the earliest events involved in the neurodegeneration mechanism, following the initial expression of anti-NGF antibodies in AD11 brains? Global changes of gene expression were investigated, by microarray mRNA analysis, in the AD11 brain, during the earliest phases of incipient neurodegeneration [47]. Postnatal days P30 to P60 are a "presymptomatic" phase of the progressive neurodegeneration.

Surprisingly, wide changes in gene expression profiles occur even at P30, when no overt neuropathology is evident. The most significant differentially regulated mRNA families are [47]: inflammation and immune response, Wnt signaling, and synaptic neurotransmission. In the first cluster, mRNAs encoding for complement factor proteins, major histocompatibility complex, autophagic proteins, and cytokines/chemokines, show the largest differential expression. This parallels the involvement of inflammatory molecules in early stages of AD [48]. Proteins of the innate immune system, such as complement proteins, have a "nonimmune" role in the physiological, developmentally regulated synapse elimination, a mechanism possibly reactivated in neurodegenerative diseases [49]. The early overexpression of complement cascade factors in the AD11 mouse model could contribute, very early on, to the synaptic deficits in this model, a prediction presently being tested.

The early massive modulation of Wnt pathway mRNAs is also noteworthy, in light of the independent, growing evidence showing the Wnt signaling pathway to be at the center of a dual pathway driving Abeta and Tau pathology in AD, also linked to APOE isoforms, GSK-3/Tau hyperphosphorylation, and the retromer sorting pathway [16]. This gene expression study shows that an early event in AD11 neurodegeneration is represented by a striking overall "immunotrophic," neurotrophic, and synaptic imbalance [47], broader than what would be expected on the basis of the simple, direct NGF/cholinergic connection.

What does this teach us? We believe the answer is in the specific mode of NGF neutralization, with anti-NGF antibodies in the brain. We propose that in AD11 mice, the disease-causing event(s) is a dual mechanism, whereby a direct selective neutralization of NGF versus proNG is combined with a response to the expression of an antibody in the brain, that might induce a neuroinflammatory and immunotrophic imbalance [50].

How could this mechanism relate to LOAD? In a human AD setting, the two postulated pathological processes, NGF deficit(s) and presence of antibodies in the brain, need not be physically linked into one anti-NGF molecule, as in AD11 mice, and may or may not be causally linked. Thus, one could envisage that, in (a subset of) sporadic AD, the NGF system might be altered by one of several possible reasons (signaling alterations, processing imbalance, axonal transport defects, etc.) [22, 23, 42, 43, 50], while for related or unrelated causes, systemic antibodies might diffuse into the brain across a defective blood–brain barrier [51], and determine neuroinflammation and immunotrophic imbalance. More generally, the AD11 mouse model could be considered an animal model for neurodegeneration, rather than an AD-specific model, highlighting molecular aspects that may occurr in several neurodegenerative processes beyond AD. This would further strengthen the direct molecular link between NGF deprivation and AD key features.

Linking Directly NGF Deficits to the Activation of the Amyloidogenic Cascade

The AD11 model establishes direct links between alterations in NGF signaling and aberrant APP/Tau processing (Fig. 4). Further independent evidence supporting this concept comes from recent studies in cultured neurons, which allow dissecting the precise mechanisms whereby deficits in NGF signaling determine the activation of the amyloidogenic cascade. These studies also uncover a different and novel role for NGF induced TrkA signaling, in concert with APP.

These studies were not, initially, carried out on NGF target neurons but on cultured cerebellar granule neurons (CGC). They will be summarized because they are instrumental to those directly connected with NGF.

CGC have the peculiar property of better surviving under depolarizing concentrations (25 mM) of KCl. If the concentration of this cation is lowered to 5 mM, a rapid process of apoptosis is activated [52]. This finding, incidentally, was indicating that CGC are in a better shape when grown under a continuous state of activity, possibly because depolarization mimics the in vivo mossy fibers deafferentation induced by axotomy [53] and suggest that this migth be, at least for certain neuronal populations, the best way for preserving their state of health and security. Apoptosis following repolarization was reversible up to 4–8 h after treatment with high concentration of KCl, forskolin, and IGF-1 [52]. Neuronal loss was characterized by de novo RNA/protein synthesis and coordinated gene expression [54, 55] nuclear condensation and fragmentation, decrease of intracellular free calcium levels, and by an imbalance of the physiological APP metabolism toward the amyloidogenic noxious counterpart. The amount of alfa-APP released in the

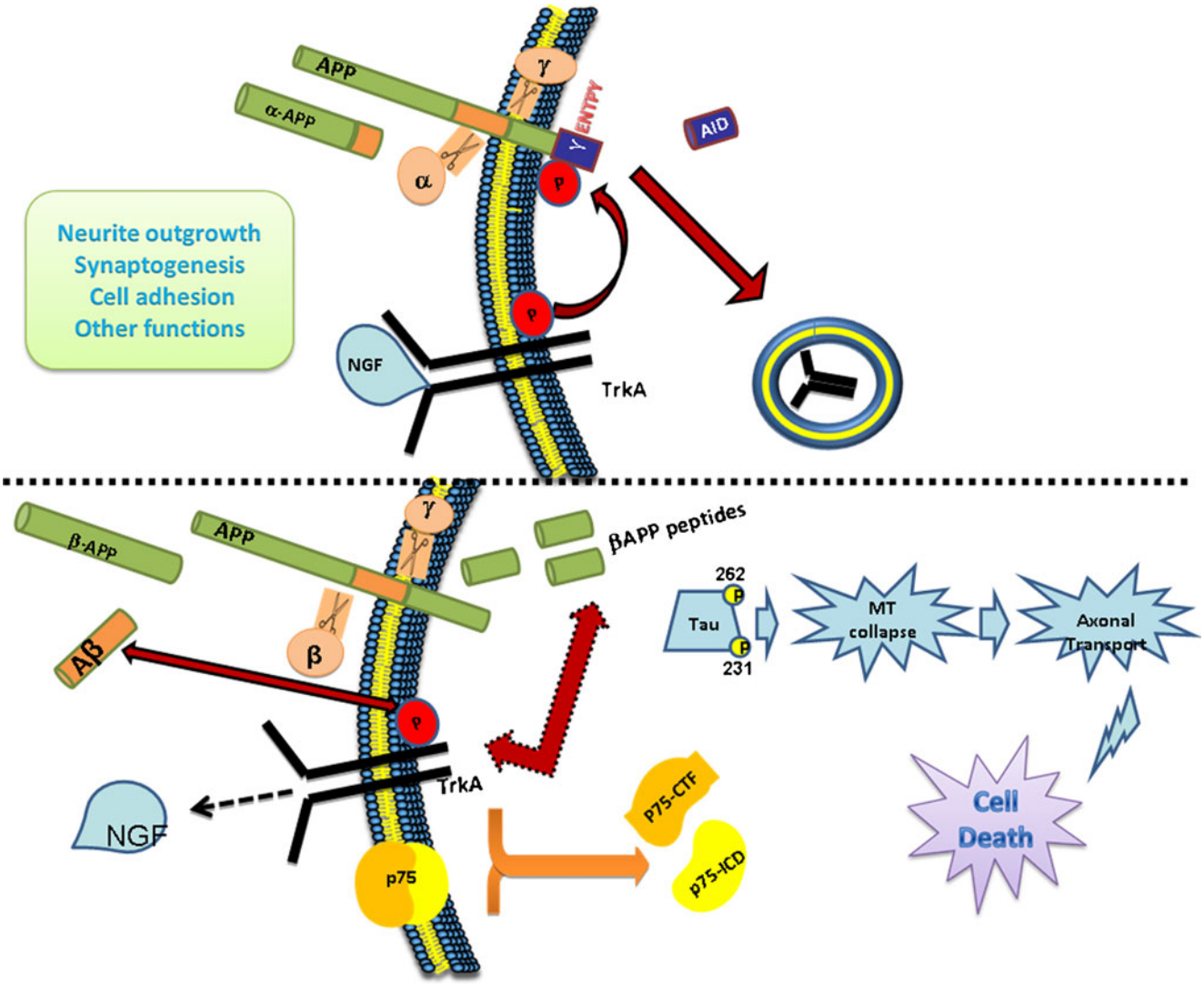

Fig. 4 NGF-mediated APP processing. In the *upper part* of the figure, NGF-mediated processing of APP is depicted. NGF binds TrkA receptor and induces APP phosphorylation on its tyrosine residues located on YENTPY/C-terminal domain. Such interaction may give rise to APP processing by alfa secretase with consequent production alfa-APP and AID peptides in extra- and intracellular compartment(s), respectively. Both alfa-APP and AID peptides may exert their trophic function by inducing neurite outgrowth, synaptogenesis cell adhesion, and other activities reported in literature. According to this view, alfa-APP may act as a sort of operational "messenger" of NGF multiple functions. In turn, APP may affect NGF signaling by modulating TrkA availability on membrane surface and trafficking (see also [91]). The *lower part* of the figure reports the events occurring when NGF signaling is impaired or blocked. Under this condition, APP is cleaved by beta and gamma secretases leading to intra- and extracellular accumulation of beta-APP/Ab peptide(s). In turn, beta-APP/Ab peptide(s) induce an unexpected NGF-independent TrkA phosphorylation and an increase in p75 processing. Both these events are accompanied by other posttraslational events dealing with tau protein and eventually leading to cell death as also depicted in details in Fig. 5

conditioned apoptotic medium was significantly decreased, while β-APP inversely increased, in the absence of any significant change of intracellular level of full-length APP. The overproduced extracellular 4-kDa Aβ peptides (A1-40; A1-42) aggregated into oligomeric and high-molecular weight species of different size and morphology [56].

Contextually, tau protein post-translational modifications—such as site-specific change of its phosphorylation state and caspase(s) and calpain-I-mediated cleavage—occurred, with a consequent microtubule disassembly in dying neurons [57]. Furthermore, if one of such truncated tau forms was introduced into the same healthy neurons by adenovirus-mediated transduction, it evoked a massive and rapid cell death involving extrasynaptic NR2B-subunit-containing NMDARs, dephosphorylation of cAMP-response-element binding protein, and sustained and delayed activation of extracellular-regulated kinases1/2 and calpain-I [58, 59].

In addition, the pro-apoptotic shifting to a low-potassium medium induced a marked failure of mitochondrial oxidative

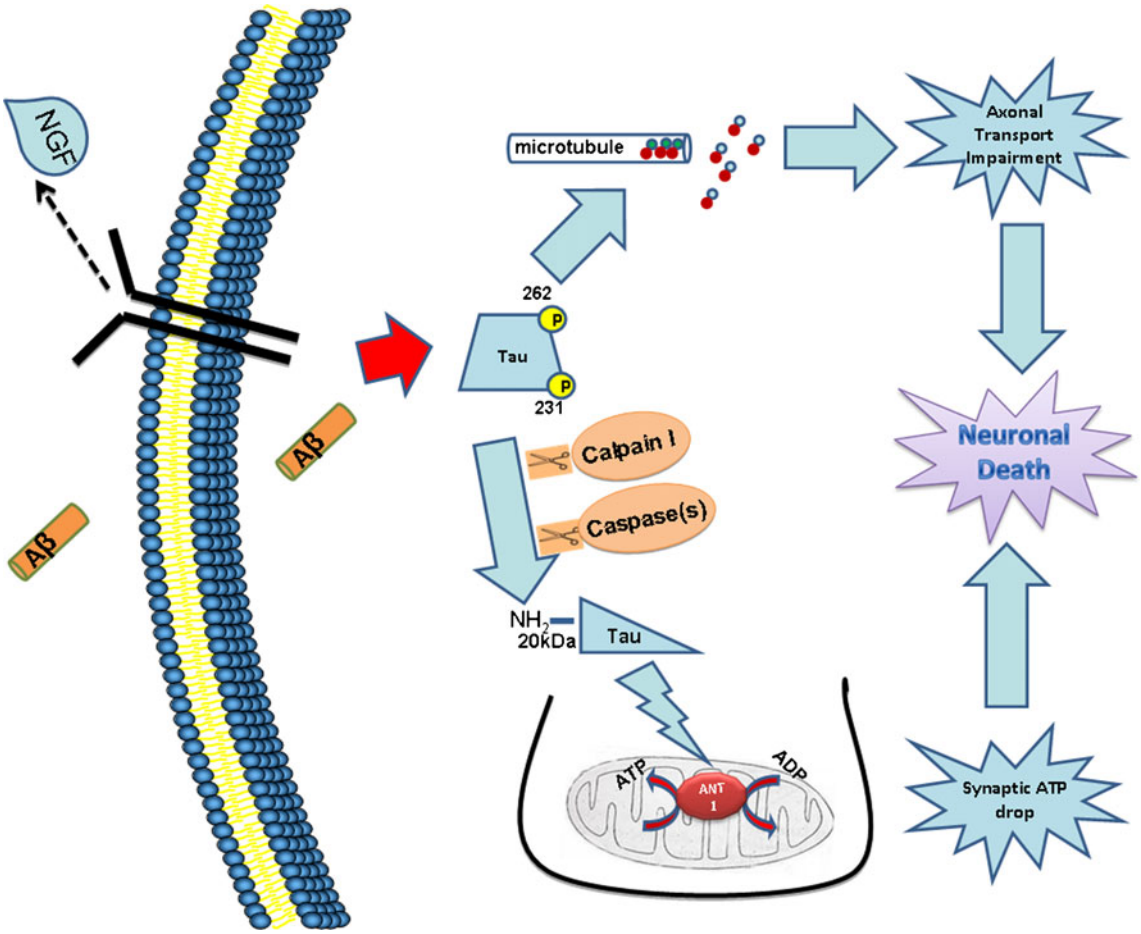

Fig. 5 Tau processing following NGF withdrawal from target neurons. Lack of NGF(N) supply to TrkA(R) is rapidly followed, as drawn in Fig. 4, by increased production and intra and extracellular accumulation of Ab 1-40 and 1-42. Ab. These peptides, In turn, and with mechanism(s) to be elucidated and partially described in text, induce tau hyperphosphorylation at AD pathognomonic sites such as Ser 262 and Thr 231. Tau is subsequently cleaved by calpain1 and caspase3/6 giving rise to a NH_2 fragment of 22 kDa which, when overexpressed in neurons causes an extrasynaptic, NMDA/NR2B-mediated toxicity. Higher levels of the 22 kDa fragment is preferentially found in association with Ab oligomers in synaptic mitochondria of AD human autoptic specimens. Both the 22 kDa fragment and Ab oligomers act synergistically in impairing in vitro oxidative phosphorylation at the ANT1-mediated ADP/ATP exchange. Microtubules disassembly due to tau detachment and axonal transport impairment, and ATP reduced supply at synaptic mitochondria collectively lead to neuronal death

phosphorylation accompanied by a dramatic intracellular ATP drop and by an increased production of reactive oxidative species [60–62]. A functional impairment of proteasomes, causing a progressive accumulation of ubiquitinated-unfolded proteins in the cytoplasm, and an early perturbation of autophagic-lysosomal structures were also revealed in apoptotic neurons [63, 64].

Interestingly, co-culture experiments showed that an Aβ antibody (4 G8) partially inhibited the "transfer" of apoptotic process to healthy, but separated neighboring neurons. This finding suggested that the Aβ pool released by apoptotic neurons diffused to the healthy cells, creating an autocrine and paracrine toxic loop [65]. As reported below, amyloidogenesis, tau altered processing, and onset of an autocrine toxic loop were found also in NGF-deprived neuronal cultures.

NGF and the Amyloid Cascade in PC12 Cells

The experimental paradigm of CGC, however, opened some caveat and criticisms. At that time, the general hypothesis of a link between apoptotic events, amyloidogenesis, and abnormal tau processing was generally discarded, on the issue that no apoptotic signs were usually detectable in specimens of cerebella from AD patients. Thus, the attention was turned to NGF and its target neurons since this factor represented the first trophic protein identified and probably the most convincing and impressive one endowed with an antiapoptotic activity. Furthermore, the in vivo studies mentioned above, in anti-NGF mice, which were at the same time under way, suggested an intriguing connection.

Thanks to the seminal work by Greene and Tischler [66] introducing the PC12 system, the experiments on NGF and

its target neurons could be performed under strictly controlled conditions and with the availability of large populations of cells rather than the scarce amount of neurons obtainable from embryonic dorsal root ganglia. NGF-differentiated PC12 cells became the object of studies aimed at assessing the hypothesis linking NGF deprivation, apoptotic activation, and amyloidogenesis.

It was found that a release and accumulation of secreted Abeta peptide—with prevalence of Aβ1-42 over Aβ1-40 peptides—occurred during the progressive death of NGF-deprived differentiated PC12 cells. The released pool of Abeta peptides isolated by ultracentrifugation was also found to form aggregates of different molecular size which were partially SDS-resistant and moderately soluble in formic acid (70 %), a condition generally employed to dissolve Ab-positive aggregates extracted in vivo from human senile plaques of AD subjects [67]. The secreted Aβ peptides seemed also to affect healthy neurons, in a similar fashion to that previously found in CGC, prompting the set up of experimental conditions to answer the following question: if Aβ peptides are the upstream trigger—acting as molecular "killer" of healthy neurons, could inhibition of their production reduce or block the timing/extent of cell death occurring after NGF deprivation? It was found that the downregulation of amyloidogenic APP processing with selective beta and gamma secretase(s) inhibitors or with the 4 G8 antibody (Aβ residues 17–24) significantly reduced the apoptotic death of NGF-deprived PC12 neurons. These results seemed not only to provide the first clear evidence linking apoptosis and amyloidogenic APP metabolism but also provided new insights into the causal and temporal sequence of events occurring during neuronal death induced by the lack of trophic supply.

NGF-Deprived Hippocampal Neurons: The Amyloid Cascade and Tau Involvement

The actual link between NGF deprivation and amyloidogenesis was further corroborated in NGF-deprived primary hippocampal cultures, a neuronal population directly affected in human AD patients, which underlies the anatomo-pathological and clinical signs of such neurodegenerative dementia [68].

In a first set of experiments, an attempt was made to confirm data previously obtained in NGF-differentiated PC12 cells. Thus, also in NGF-deprived hippocampal neurons, it was observed that: (1) the released Abeta peptides created a neurotoxic loop since the extent of ensuing cell death was much greater than that expected on the basis of the potential number of NGF-responsive target neurons previously reported; (2) beta and gamma secretase inhibitors and Aβ antibodies (4 G8), largely prevented not only the Aβ intra- and extracellular production but also neuronal death; and (3) partial silencing the mRNA coding for APP correspondingly reduced the extent of cell death [69]. The oligomeric nature of the βA peptides formed in hippocampal neurons after NGF withdrawal (in particular, the production of the synaptotoxic βA soluble dimers and trimers) has not been investigated in detail and warrants further analyses.

As for the involvment of other neurotrophins, a preliminary set of experiments showed that also BDNF withdrawal was followed by activation of the amyloidogenic pathway, although this finding deserves further studies.

Tau Protein as a Primary Target of Overproduced Aβ

The impressive involvement of Aβ in the chain of events following NGF withdrawal raised the obvious question: what is the role, if any, of tau protein in such events? The temporal interplay between Aβ overproduction and tau protein post-translational modifications (see also Fig. 5) was also investigated and established that two specific amino-acidic sites (Ser 262 and Thr 231)—pathognomonic of abnormal AD-like tau protein detected in aggregated insoluble intracellular NFTs—were rapidly (3–6 h) and transiently (<12 h) hyperphosphorylated in hippocampal neurons upon NGF withdrawal. The early, specific, and temporally-restricted phosphorylation state changes of tau were: (1) temporally related to a decrease of the ratio of secreted aAPPs/bAPPs; (2) caused by a deregulation of Akt-GSK3b signaling, known to be in vivo correlated with the onset of AD pathology; (3) mimicked by externally added synthetic Ab1-42 peptides but not by reverse-sequence peptide Ab 42-1; (4) sensitive to LiCl treatment; and, more importantly, (5) significantly reduced upon treatment with Ab antibodies or with β- and γ-secretase(s) inhibitors [70]. The increased pool of hyperphosphorylated tau, which is unable to bind microtubules, subsequently detached from them, as shown by the reduction in the intracellular level of acetyl (stable)-tubulin and by a concomitant increase of its a-tyrosinylated (instable) form. Contextually, a progressive disassembly of the cytoskeleton network occurred, and the microtubule tracks-based axonal transport was acutely impaired, as demonstrated from observing that mitochondria accumulated in the perykarion and were no longer transported along axonal processes [70].

The tau-dependent loss of microtubule integrity and axonal neuritic beading associated with jamming of mitochondria were also partially rescued by the treatment with Ab antibody and GSK3b inhibitors. In addition, calpain-I and caspase-3 protease(s) were only late upregulated, and pharmacological inhibition of such apoptotic proteases not only partially protected hippocampal neurons from death following the NGF removal but also did not significantly interfere with GSK3b-mediated site-specific tau hyperphosphorylation.

This finding suggests that the delayed caspase-3/calpain-I pathway does not regulate GSK3b-dependent changes of tau

phosphorylation state in agreement with previous studies reporting that, in differentiated PC12 induced to cell death by NGF deprivation, the GSK3b kinase inhibition only exerted a small effect on the caspase(s) pathway.

An early tau hyperphosphorylation, with a reduction of its microtubule binding affinity has been found in NGF-deprived differentiated PC12 cells [71]. In addition, although other research groups have reported that Abeta antibodies can reduce tau hyperphosphorylation in vitro and in vivo [72, 73], the functional relationship between endogenous overproduced Ab, tau hyperphosphorylation/cleavage, and apoptotic signaling in the same neuronal model has not been investigated before. As mentioned before, similar NGF-dependent modifications of site-specific tau phosphorylation have been found in the AD11 animal model in correlation with the temporal appearance of Aβ peptide species [40].

Furthermore, a caspase(s)-mediated truncation of N-terminal tau domain, which possibly interacts with dynactin/dynein motor complex [74], also occurs in vitro and in vivo on NGF signaling interruption. GSK3b-mediated tau phosphorylation is associated with a proper anterograde organelle transport [75] in NGF-dependent differentiated PC12, and synthetic Aβ peptides cause a GSK3b-mediated impairment of mitochondrial transport in hippocampal cultured neurons. Finally, a mislocalization of pThr 231-tau protein and GSK3b in basal forebrain cholinergic neurons of aged rats is causally linked to an in vivo defective retrograde axonal transport [76, 77]. Together, these studies show that NGF deprivation may affect its own retrograde transport due to microtubule default, which, in turn, appears in these cultures as a downstream event of Aβ overproduction and intracellular accumulation.

Subsequent studies on the action of the tau 20–22 kDa NH_2 proteolytic fragment have shown that it specifically impairs mitochondrial phosphorylation at the ATP/ANT (ANT) trasporter site in test tube evaluations [60]. Furthermore, by Western blotting, immunofluorescence, and TEM analysis, it was found that this peptide is largely enriched in human mitochondria from cryopreserved synaptosomes of AD brains and that its amount in terminal fields correlates with the pathological synaptic changes and with the organelle functional impairment [70]. This NH2-truncated tau form is also found in other non AD human tauopathies, while its presence in AD patients is linked to Aβ multimeric species and likely to pathology severity, suggesting that the mitochondrial NH2-derived tau peptide distribution may exacerbate the synapses degeneration occurring in tauopathies, including AD.

The Paradoxical, Functional Switch of the TrkA Receptor

TrkA belongs to the large family of tyrosine kinases transmembrane receptors sharing, as a common denominator, the property of trans-autophosphorylating on binding to their cognate ligand. Receptor engagement with its specific neurotrophin changes the inactive conformation into an active state, which triggers and activates the appropriate signal transduction pathway(s) by recruiting intracellular adaptor proteins, effectors, or both. The active conformation regains its inactive, dephosphorylated state within minutes after the 16 extracellular concentration of ligand decreases to a value favoring the free unbound state [78, 79]. It was therefore an unexpected finding that, 24 h after NGF withdrawal, TrkA regained its phosphorylated state (pY490) in the apoptotic hippocampal primary neurons contextually to: (1) an Akt dephosphorylation, (2) an upregulation of phospholipase-C (PLC) pathway; and (3) the progression of neuronal death [80]. This paradoxical, NGF-independent delayed TrkA phosphorylation could be assumed either as a side-effect of death-inducing changes evoked by NGF withdrawal or as an intracellular signaling linked to Aβ increase. Subsequent experiments confirmed this latter hypothesis. Incubation of NGF-deprived neurons with two different TrkA antagonists, namely K252 and CEP-2563, generally used to inhibit TrkA autophosphorylation, largely protected hippocampal cultures from apoptotic death behaving, under this condition, as sort of agonists rather than antagonists. Furthermore, an identical anomalous TrkA post-translational modification was evoked in neurons by exogenously added synthetic Aβ peptide, suggesting a causal relationship between NGF withdrawal, amyloidogenic APP metabolism, and NGF-independent TrkA phosphorylation. Similarly, partial TrkA mRNA silencing paradoxically favored neuronal survival, suggesting that the NGF receptor may switch from pro-survival to pro-apoptotic action in the absence of its specific ligand. In addition, pharmacological inhibition of Cdk and Src kinases, known to trigger amyloidogenesis by affecting APP phosphorylation and processing [81–84], reduced the TrkA and PLC phosphorylation levels and rescued neuronal cultures from death. Relevantly, in p75-silenced neurons, TrkA did not appear phosphorylated following NGF removal, and the interruption of the amyloidogenic pathway was eliminated [80]. Furthermore, at early times following NGF removal, p75 and particularly CTF (its C-terminal fragment produced by alpha-secretase cleavage [85]) bound Aβ peptides and the 28 kDa presenilin1 (PS1) fragment (one of the components of gamma–secretase complex [86]) and led to apoptotic death. These findings further support data previously reported about a p75 role as an early mediator of the toxic Aβ effect [87] and support the working hypothesis of a possible imbalance in secretase(s) activity as a possible cause triggering the amyloidogenic pathway activation [88].

Together, these results also seem to open a new paradigm in the field of NGF intracellular signal transduction since the same TrkA transmembrane receptor may switch from pro-survival to pro-apoptotic action in the presence or absence

of its physiological cognate ligand. In addition, the evidence that NGF receptors, directly or indirectly, interact with Ab peptide(s) and PS1 in apoptotic neurons and that analogous effects are induced by synthetic Ab exogenously added peptide [80, 89] corroborates the ongoing hypothesis of a cellular interplay between the NGF receptors (TrkA and p75) and the amyloidogenic APP processing machinery [90].

This conclusion is further supported by more recent findings underlying a tight link between TrkA and APP. It has been shown that a residue in the APP intracellular region, Y682, is indispensable for the essential functions of APP. Studies testing whether the NGF/TrkA signaling pathway is a physiological regulator of APP phosphorylation demonstrated that NGF induces tyrosine phosphorylation of APP, that this protein interacts with TrkA and the interaction requires Y682. It was also found that APP-Y682 regulates activation of the NGF/TrkA signaling pathway in vivo, the subcellular distribution of TrkA, and the sensitivity of neurons to the trophic action of NGF [91]. These studies suggest that NGF and APP functions are strictly interconnected, probably via some sort of direct interaction, and that the NGF/TrkA signaling pathway could indeed be causally involved in AD pathogenesis (see Fig. 4).

Thus, one of the consequences of trophic factor deprivation (or other NGF signaling and processing alterations) of BFCN in vivo could be a local action on their synaptic axonal terminals. NGF can therefore be considered as an anti-amyloidogenic factor that normally keeps the amyloidogenic pathway under control. Whenever a normal neurotrophic supply of NGF to target neurons is interrupted or altered, the amyloidogenic pathway is activated and a negative feedback, toxic loop is activated also involving, in a tight chain of events, tau, MTs stability, and axonal trasport.

In this context, it is worth noting that also BDNF appears to keep the amyloidogenic pathway under control since its withdrawal from target neurons is accompanied by overproduction of Aβ [69], suggesting that the concept of NGF trophic action as an anti-amyloidogenic action could be extended to other neurotrophins as well.

The Triple State Hypothesis

Studies addressing the bidirectional TrkA–APP interactions in cultured neurons were necessarily carried out in an "all or none" setting in vitro, whereby the neurotrophic factor is either continuously present or totally absent. On the other hand, the NGF bioavailability in vivo is spatially and temporally controlled [79]. Also, the ratio of NGF to proNGF can be variable and subject to fine regulation, influencing the outcome of a given level of mature NGF [42, 50]. It is therefore reasonable to postulate that the outcome of the TrkA–APP interaction in vivo might include a third type of state, depending on the actual in vivo bioavaliability and kinetics of NGF receptor interactions and or on the actual NGF to proNGF ratios. The existence of a third type of TrkA/APP state, depending on NGF transient concentration, processing or maturation, site of release or intracellular location, is suggested by the following question: could minute amounts of Aβ be produced during such a dynamic, rapidly reversible TrkA–NGF interaction and exert some physioiogical role? Indeed, several studies provide evidence that low concentrations (in the nanomolar to picomolar range) of Aβ peptide(s) exert positive actions, such as a neurotrophic role in cell culture [92, 93], modulation of hippocampal synaptic plasticity and of memory [94, 95], regulation of lipid homeostasis [96], control of synaptic neurotransmission [97], or protection of mature neurons against excitotoxic death [98]. Therefore, we propose that depending on the local bioavailability of NGF in vivo and/or on the actual ratio between NGF and proNGF, and on the resulting mode and kinetics of NGF receptor interactions (location, duration, and type of co-receptor engagement), a specific APP pathway (non-amyloidogenic, transiently and reversibly amyloidogenic, or irreversibly amyloidogenic) might be preferentially activated, thus generating a functional gradient of APP-derived fragments. According to this view, AD could involve a progressive shift from a regulated triple state of ligand/receptor/APP interactions to a state of permanent amyloidogenic APP processing due to a reduced supply or defective signaling of NGF or to an altered NGF to proNGF processing ratio.

The Trophic Action as an Anti-amyloidogenic Activity

On a more general note, we can consider the anti-amyloidogenic action of NGF as a trophic effect. The term trophic is widely used to refer to a generalized, pro-survival action exerted on target cells by extracellular messengers of differing chemical nature such as vitamins, hormones, or growth factors. Although this term is widely used to indicate a generalized "nutritional" action mediated through several metabolic pathways, more detailed and in-depth experiments have indicated that this term is often too broad or ambiguous.

NGF, for instance, first came to light for its vigorous, extraordinary nerve growth-promoting activity on chick embryo sensory ganglia. Later, it was also reported that sympathetic ganglia degenerated and massively died when deprived of NGF with a novel and ingenious manipulation, defined as immunosympathectomy [99]. Thus, the nerve growth-promoting action found in sensory ganglia was extended to a significantly and pronounced "trophic" vital effect. Subsequent studies—performed on in vitro cultured sympathetic neurons—demonstrated that these cells, when deprived of NGF, die due to the activation of programmed

cell death. These findings provided a more specific definition of the broader term "trophic" [100].

Thus, on the basis of the studies presented in this review, an even more specific and complete definition of the anti-apoptotic action exerted by NGF—and most probably by other neurotrophins—consists in keeping under control the amyloidogenic processing of APP, a crucial trigger in AD pathogenesis. According to this view, the pro-survival, NGF-mediated activity on its target neurons appears to consist of an inhibitory effect on amyloid Aβ peptide(s) overproduction.

Nerve Growth Factor and Alzheimer's Disease: The "New" Story

The experimental studies on NGF deficit-induced neurodegeneration in transgenic mice as well as the mechanistic studies on the anti-amyloidogenic actions of NGF/TrkA signaling in primary neuronal cultures demonstrated a novel causal link between neurotrophic signaling deficits and Alzheimer's neurodegeneration. Around these results, a new NGF hypothesis could be built, with neurotrophic deficits of various types representing an upstream driver of core AD triad pathology (see Fig. 1). Thus, AD neurodegeneration would arise from alterations of the homeostatic equilibrium of the NGF system leading, through a series of interconnected loops, to the activation of local and global neurodegeneration processes, ultimately determining the central core of AD hallmarks.

These neurotrophic deficits would be "located" upstream of the "amyloid cascade," as currently described, but would be part of a negative feedback loop that involves several feedback steps from the downstream process itself (e.g., links between APP, Tau, and axonal transport). Also, the cellular targets for NGF/proNGF actions, in this negative loop, could be more widespread than envisaged so far (Fig. 3).

Essential elements of this mechanism, besides the "classical" NGF/TrkA/p75NTR system, are the proNGF/sortilin pathway and immune and inflammatory effectors acting on microglia and synapses, progressively broadening the cellular basis of the pathology.

Intercellular relay mechanisms for local neurodegeneration events determine cell-to-cell, or synapse-to-synapse, spread of local neurodegeneration and the broad cell involvement observed. A crucial issue related to our new hypothesis relates to the question whether NGF deprivation acts directly, and locally, on cholinergic synaptic terminals and BFCN function, thereby triggering the amyloid cascade that eventually spreads to other cell types, or whether NGF deprivation renders BFC neurons more vulnerable to concomitant and independently generated βA production. Future studies will clarify this important issue.

The "new" NGF hypothesis for LOAD (see Fig. 1) has significant therapeutical implications. Any therapy aimed at reestablishing the correct balance between ligands (and receptors) of the NGF pathway appears to have a clear and strong rationale, as being truly able to interfere directly with a neurodegeneration mechanism involved in the disease process.

Nerve Growth Factor-Based Therapies for Alzheimer's Disease: Taking Pain Out of NGF

In this framework, the first therapeutic choice would be to use NGF itself as a drug. Clinical application of NGF requires solving two major problems: effective CNS delivery and limitation of adverse effects (most notably, pain). It is indeed a challenge to deliver NGF into the brain in a safe and efficient manner. First, NGF does not readily cross the blood–brain barrier. A second major issue is represented by the pronociceptive actions of NGF.

Thus, NGF is a key pain mediator, controlling both the neural and the inflammatory components of pain [101]. The capacity of NGF to cause pain has been demonstrated in humans in the course of pilot clinical trials in AD patients [102], as well as during clinical trials undertaken to explore the potential use of NGF in peripheral polyneuropathies [96]. This has severely limited, in previous clinical trials, the dosage administrable to patients, jeopardizing the efficacy of the treatment [103].

The clinical application of NGF in AD is therefore limited by a double constraint of achieving a pharmacologically adequate concentration in target brain areas while preventing its adverse pain effects. For this reason, clinical trials evaluating NGF for AD have used invasive approaches, such as neurosurgery for the implant of autologous fibroblasts, engineered to secrete NGF, directly in the brain [104], the direct stereotactic delivery into the brain of adeno-associated viral vectors encoding human NGF, or the chronic neurosurgical implant into the brain of biopolymer capsules filled with NGFproducing cells.

These invasive clinical approaches of NGF gene/cell therapy provide an independent validation of the therapeutic potential of NGF in AD, and their outcome will provide insights into the safety, efficacy, and liabilities of NGF therapies. However, the approach is highly impractical for its extension to large numbers of AD patients. Therefore, a safe route for an effective, noninvasive delivery of NGF to the brain is required.

We have shown that the intranasal route allows a noninvasive, safe, and pharmacologically effective delivery of NGF to the brain [37, 41, 105]. NGF intranasal delivery represents an effective compromise to meet the required therapeutic window for NGF, leading to NGF buildup in

target brain areas while minimizing its biodistribution to nontargeted districts where it induces pain (CSF and bloodstream).

The ideal NGF molecule for an NGF-based therapy should be traceable against endogenous NGF, to facilitate optimal dosing, and should have a reduced ability to activate nociceptive pathways while retaining identical neurotrophic activities. Thus, we designed a modified human NGF (hNGFP61S), "tagged" with a single distinctive residue, that can be easily traced against endogenous NGF and has a potency and bioactivity identical to hNGF [106]. hNGFP61S constitutes a backbone whereby additional desirable functions, such as antinociceptive properties, could be further engineered into the therapeutic NGF molecule.

Is it possible to take pain out of NGF, engineering an NGF mutein having neurotrophic properties identical to hNGF but lacking its nociceptive pain-inducing activity? Toward this aim, genetic data on a rare human syndrome, hereditary sensory and autonomic neuropathy, type V (HSAN V), were a source of inspiration. Two rare forms of human congenital insensitivity to pain are due to mutations in genes related to NGF signaling: hereditary sensory and autonomic neuropathy, type IV (HSAN IV), is due to mutations in the gene for TrkA [107, 108], while HSAN type V is associated with a mutation (R100W) in the NGF gene [109]. Both HSAN IV and V are characterized by profound loss of pain sensitivity and perception, accompanied, in HSAN IV, by severe mental retardation and learning problems. On the other hand, HSAN V patients show no mental retardation and have most neurological functions intact. The HSAN V mutation NGFR100W separates, from a clinical point of view, the neurodevelopmental effects of NGF from those involved in the activation of peripheral pain pathways after development. This mutation could form the rational basis for the design of a "painless" NGF variant that, while displaying a full neurotrophic activity, shows a reduced nociceptive activity. We therefore studied mechanistic aspects of the R100W mutation [110, 111]. Receptor binding measurements, with NGF recombinant proteins, demonstrated that while the affinity of NGF R100 mutants for the TrkA receptor was substantially unchanged, the binding for p75NTR was disrupted (1.5–2.2 nM for hNGF against 125–200 nM for hNGF R100 [110]. The neurotrophic potency of hNGF R100 mutants was indistinguishable from that of wild type hNGF.

Interestingly, mutants hNGFR100 and hNGFP61S/R100 were equally effective as hNGF and hNGFP61S in activating, through TrkA, downstream shc and Akt pathways but failed to activate PLC-1γ. This selective TrkA signaling failure is noteworthy since the PLC-1γ pathway has been implicated in TrkA-mediated sensitization of sensory nociceptors [112, 113]. Thus, HSAN V-related hNGFR100 mutants have a decreased binding to the p75NTR receptor and an altered signaling of pronociceptive pathways. In vivo studies in pain and neurodegeneration models have confirmed the therapeutic potential of these painless NGF molecules, which display full neurotrophic activity, in culture and in neurodegeneration models, and show a reduced pain-related signaling capability [111, 114]. The painless NGF molecule has the potential for being developed into a disease-modifying noninvasive therapy for Alzheimer's Disease.

Conclusions: In Vivo and In Vitro Studies as Parallel, Progressively Convergent Pathways

The studies that we have presented in this review were originally conceived and carried out on the basis of two distinct, apparently nonconnected hypotheses: (1) a direct link between NGF and the onset of AD and (2) the possible, primary involvement of apoptotic events in the onset of this disease. Thus, the former studies were conducted on NGF target neurons by resorting to an ad hoc devised animal model of NGF deprivation via expression of NGF neutralizing antibodies. The involvement of apoptotic events as a direct trigger of the amiloidogenic pathway were initially carried out on cerebellar granule neurons, since previous studies had presented this in vitro cultured neurons as most suitable for testing this possible causal connection. Subsequent studies, often carried out by joining experimental efforts of our research groups, progressively converged on the same topic and conclusions: the direct link between in vivo and in vitro NGF deprivation, apoptotic events, and onset of an AD-like syndrome, characterized by molecular, histological, and behavioral signs. In this scenario, NGF discontinued, or imbalanced, supply was the trigger, amyloid excessive production was the effector, main character of the plot and apoptotic events, eventually leading to cell death, were the contour players of the show. Thus, on one hand, it was demonstrated that NGF deprivation, by a selective inhibition of mature NGF with respect to proNGF, induced AD-like syndrome in the mouse brain. On the other side, apoptosis occurring after in vitro NGF deprivation came to light as a late consequence of the upstream activation of amyloidogenesis and tau aberrant metabolism by the NGF deficit trigger. The convergent path presently appearing in this scenario is that a trophic interruption of a factor such as NGF (but other trophic substances, such as BDNF, could be envisaged and should be investigated further) leads to amyloidogenesis, tau altered processing, and a subsequent set of events, eventually ending in the degeneration and death of neuronal populations which play a crucial role in cognitive functions. On the basis of the findings presented in this review, we conclude that altering the homeostasis and equilibrium of NGF processing and signaling in target

neurons represents an upstream driver, even if possibly not the only one, of all the cellular and molecular central hallmarks of AD. In line with these results, polymorphisms in the genes for NGF or NGF receptors should be considered [115] and investigated as potential risk factors for sporadic AD.

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