Homage to Rita Levi-Montalcini

MOLECULAR MECHANISMS OF ALZHEIMER’S DISEASE: NGF MODULATION OF APP PROCESSING

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Abstract

The therapeutic focus of the scientific community in Alzheimer’s disease (AD) has been moving in the last years from attempting late rescue against cholinergic degeneration and amyloid beta plaque clearance to (i) the discovery of blood or cerebrospinal fluid markers for early diagnosis, and (ii) early therapeutic intervention with modulators of amyloid precursor protein (APP) processing. It is currently accepted idea that subtle synaptic alterations determine the first neuronal dysfunctions and cognitive deterioration, progressing overtime into neuronal degeneration in the sporadic and late onset form AD (LOAD), the most diffuse one (90% of AD cases). Synaptic loss occurs long before the appearance of a frank neuronal degeneration in LOAD. The perturbation of the nerve growth factor (NGF) signalling system in brain neurodegenerative disease like AD and Down’s syndrome was for long time considered to be a pathological event, subsequent to amyloid-driven disruption of NGF retrograde transport from the cortex and hippocampus to the cell bodies of basal forebrain cholinergic neurons. Nowadays, an increasing amount of data indicate that the observed dysregulation of NGF-TrkA (tyrosine kinase A) and proNGF-p75NTR signalling systems is a good candidate for being the primus movens in the neuropathology of sporadic AD. Accordingly, an amyloid-independent strong correlation of cognitive deficits with reduction of NGF and TrkA, and accumulation of proNGF has been recently observed in mild cognitive impairment and its progression to AD. This review highlights the current knowledge about NGF and early events occurring in LOAD. The involvement of muscarinic acetylcholine receptors, cholesterol, sirtuin1, insulin-like growth factor-1, and sunday driver protein in APP processing are also discussed. The studies reported here confirm a multitasking ability of NGF in slowing down, through both distinct and overlapping mechanisms, the amyloidogenic processing of APP in neurons of the central nervous system.

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Key words: proNGF, p75NTR, TrkA, ADAM10/17, SIRT1, NRIF, cholesterol, IGF1, syD protein
Introduction
Alzheimer’s disease (AD), the most common cause of dementia in the elderly, is a progressive neurodegenerative disorder characterized by impaired memory and cognition. There are two forms of AD: (i) the genetic early onset familial AD (FAD), with mutations in the amyloid precursor protein (APP) presenilin (PS1 and PS2) genes, and (ii) the age-related, sporadic and late onset AD (LOAD), representing 90 % of AD cases (1). FAD and LOAD are characterized by increased levels of APP, and the presence of neuritic amyloid plaques, cerebrovascular amyloidosis and neurofibrillary tangles.

APP is a transmembrane protein, and its expression is ubiquitous (2), although neuronal tissues express a tissue-specific isoform, the APP695 (human APP numbering) (3). APP is thought to be a pathogenic factor in AD, as a result of the proteolytic processing of the protein, and the generation of neurotoxic carboxyl-terminal (C-terminal) derived peptides (4). APP cleavage occurs at the membrane by beta secretase 1 (BACE1), or by a disintegrin and metalloproteinase 10 and 17 (ADAM10, ADAM17), the neuronal alpha secretases. As a result the shedding of the large APP ectodomain occurs and the extracellular soluble APP derivatives are released (sAPPbeta and sAPPalpha, respectively), and membrane-tethered C-terminal fragments (CTFs) are generated (CTFbeta or CTFalpha, respectively). The CTFs are subsequently cleaved by gamma-secretase to generate either amyloid beta (Abeta) and the APP intracellular domain (AICD) (from APP-CTFbeta), or a 3 kD product (p3, from APP-CTFalpha). CTFbeta derived fragments (Abeta and AICD) and the CTFbeta itself are considered to be the toxic species in AD (5).

Despite recent progress in AD research, the treatment of AD remains a major challenge. The knowledge of the triggering events leading to the selective degeneration of basal forebrain cholinergic neurons (BFCN), a characteristic of AD brains, is far to be exhaustive.

Neurotrophic factors have attracted the attention for neuroprotection in neurological disorders (6,7). In this regard, nerve growth factor (NGF) has generated a great interest as a potential target for the treatment of AD (8-11). This is based on the observation of the concomitant degeneration of BFCN, reduced availability of NGF and its high-affinity receptor, the tyrosine kinase A (TrkA), and increased APP levels in the AD post mortem forebrain.

Increased APP levels have been found also in other age related and neurodegenerative pathology of the central nervous system (CNS), like Down's syndrome (DS) (12) and traumatic brain injury (TBI) (13,14). DS patients present a disrupted NGF trafficking and develop a form of dementia similar to AD (15). An age-related decrease of NGF-like immunoreactivity and a parallel increase of APP (16) have been reported also in the basal forebrain of senescence-accelerated mice 10 (SAMP 10), a valuable mouse model for brain ageing (17).

Numerous evidences from both animal and human studies indicate NGF may be useful in reversing, stopping, or attenuating the cognitive deficit associated with AD, DS and TBI (10,12,18-20). A phase I clinical trial based on ex vivo NGF gene delivery by implantation of fibroblast engineered to express human NGF in the forebrain of AD patients showed no long-term adverse effects and a promising degree of efficacy as evaluated by Mini-Mental Status Examination (MMSE), PET scan and brain autopsies (21). This study didn’t include a placebo control group, thus warranting additional analyses. A Phase II clinical study of Ceregene’s CERE-110, a gene therapy product designed to selectively deliver NGF to the Nucleus Basalis of Meynert for the treatment of early and mean phase AD, is currently underway by the Alzheimer’s Disease Cooperative Study (ADCS; http://www.adcs.org).

Since AD is a pathology of BFCN and NGF is a tropic and trophic factor for BFCN, it has been hypothesized that NGF might exert neuroprotective effects in AD. However, it is also possible that the AD cognitive deficits are acetylcholine independent and that the effect of NGF does not simply rely on restoration of cholinergic neurotransmission and BFCN survival. A recent study (22) demonstrated that not all the BFCN strictly depend on NGF-TrkA for their differentiation and/or survival. In this study, transgenic mice with ngf or trkA conditional gene ablation in the CNS showed a developmental reduction of only 35-40% of BFCN expressing choline acetyltransferase enzyme (ChAT). Moreover, young and middle aged mice showed normal behaviour in a number of septo-hippocampal, cortical and para-hippocampal tasks (22), questioning about relevance of
NGF-dependent BFCN for AD-targeted cognition. The ongoing analysis on aged NGF and TrkA CNS knockout (KO) mice will allow a better understanding of NGF and cholinergic dependency of cognitive functions in the healthy and AD forebrain. An increasing amount of data let us speculate that NGF relevance in AD involves early brain changes other than the well-known cholinergic dysfunction in AD. Studies on cholinergic NGF and TrkA expressions in MCI and in AD brain indicated that the early NGF-TrkA system alteration correlates with cognitive deficits and occurs before the appearance of any cholinergic damage (23,24). Though it is not definitively assessed whether the NGF deficits is a primary perturbation in AD or a secondary effect of the Abeta disrupted axonal transport, recent evidences offer a new perspective on NGF in AD pathogenesis.

The present review updates the current knowledge of cellular and molecular mechanisms of NGF-modulated APP processing, underlying its anti-amyloidogenic effect in healthy, aged and AD brain: a central issue in the discovery of novel therapy for this devastating disease.

Muscarinic acetylcholine receptors 1 (MR1) and non-amyloidogenic APP processing

The activation of muscarinic acetylcholine receptor 1 (MR1) has been shown to stimulate non-amyloidogenic APP processing in cultured cells and brain slices (25, 26). Also the in vivo stimulation of muscarinic tone on cholinergic targets of the BFCN by the administration of MR1 agonist resulted in increased sAPPAlpha levels (27) and decreased Abeta levels in the cerebrospinal fluid (CSF) of AD patients (28,29). Conversely, MR1 inhibitors favour the amyloidogenic route in the Tg2576, transgenic AD mouse model (30). Studies with MR1-KO mice indicated that MR1 is sufficient to modulate alpha cleavage of APP and sAPPAlpha release (31). Muscarinic tone is affected also in the AD11 mouse model of AD, expressing neutralizing levels of anti-NGF antibody. The fact that the nicotine-dependent enhancement of synaptic efficacy failed at the CA1 synapses of AD11 (32) probably reflects an NGF-dependent deficit at the cholinergic targets rather than a lack of cholinergic neurotransmitters. Although further studies are needed to specifically address the role of NGF on MR1-dependent anti-amyloidogenic APP processing, the available data support the idea of NGF as anti-amyloidogenic neurotrophin of the basal forebrain.

The mature NGF versus proNGF signalling pathways in APP metabolism

Neurotrophins are synthesised as preproforms, subsequently cleaved by furin and procaspases to generate mature neurotrophins. The precursor and mature forms of the neurotrophines are thought to activate distinct and sometimes opposite effects on APP metabolism in the CNS (33). For instance, the mature form of NGF (mNGF) signals through the specific receptor TrkA (34) and the panneurotrophic receptor p75NTR (35), to induce MAPK/ERK survival pathways and neuronal differentiation (36). The unprocessed proneurotrophin peptide (proNGF) has a minimal affinity for TrkA, being a preferential high-affinity ligand for the p75NTR-sorilin signalling complex, and leading to JNK/SAPK activation and neuronal death (37,38).

The proNGF-p75NTR-sortilin trimeric complex drives a pro-apoptotic pathway and induces an age-dependent ceramide production, BACE activation, and beta fragments production in cultured hippocampal neurons (39). A prevailing proNGF signalling may further accelerate neuronal damage and worsen brain pathology in AD, because of its ability to downregulate mNGF-TrkA driven survival signals, as it has been shown in vitro, both in PC12 (40) and in brain neurons (41). In vivo, proNGF levels augment in AD tissues, where it localizes to the Abeta plaques (42). The accumulation of proNGF has been found to induce cholinergic degeneration following a pharmacologically induced failure in NGF maturation (43) and to cause learning and memory deficits in transgenic mice expressing a furin cleavage-resistant form of proNGF (proNGF transgenic mice) (44).

Epileptic activity is associated to AD, and AD carries a significant increased risk of seizures, observed in 10-22% of LOAD patients. Interestingly, proNGF transgenic mice display spontaneous epileptic-like discharges (44), and epileptic seizures, on the other hand, triggers a significant increase in proNGF and p75NTR expression in hippocampal neurons (45,46). Although it is not clear whether AD and seizures are one the epiphenomenon of the other or two distinct diseases sharing some initial mechanisms, these data further indicate the proNGF-p75NTR system as a molecular substrate for the early events taking place in LOAD (46).

A shift from mRNATrkA to mRNAP75NTR transcription occurring as a result of brain ageing and insulin-like growth factor 1 (IGF1) signalling, and leading to reduced TrkA and increase p75NTR-ceramide signalling pathways was first hypothesized by Puglielli (47,48). Of notice, the expression of IGF1 and the IGF receptor (IGF1R), classically associated to diabetes and metabolic diseases, has been recently shown to modulate lifespan, age-related amyloidogenesis and LOAD-like pathology (49-51). These observations, claiming AD as a brain type diabetes, or “type 3 diabetes” (52), link the TrkA-to-p75NTR signalling switch with the IGF1-affected neuronal metabolism in brain aging and...
age-related AD. In agreement with this, cognitive impairment is associated with decreased levels of TrkA and doubling of p75<sup>NTR</sup> levels in the brain of aged rats (53) and in early AD patients (54).

Given the opposite functions of mNGF and proNGF in the mature brain, and the pro-amyloidogenic effect of a prevailing proNGF-p75<sup>NTR</sup> signalling in ageing and AD, novel proNGF-based approaches could represent a very promising route for LOAD treatment. Experimental studies have been successfully attempted in order to minimize proNGF levels, p75<sup>NTR</sup> signalling or rescue mNGF/proNGF ratio in several AD animal models. Among these, (i) the administration of mNGF (55), (ii) the selective inhibition of proNGF/p75 signalling (56), (iii) the use of cerebrolysin (CBL) in the Tg2576 AD mouse model and in AD patients (57), and (iv) the treatment of APP/PS1 transgenic mice with the green tea derived compound epigallocatechin-3-gallate (EGCG) (58).

Interestingly, a recent paper showed lack of correlation of Abeta with both the severity and progression of cognitive deficits in MCI and early AD, while a strong correlation was found with increased proNGF, and decreased NGF and TrkA levels, leading to the hypothesis that an initial and amyloid independent perturbation of the neurotrophic signalling can occur in sporadic dementia (59).

**p75<sup>NTR</sup> signalling, NRIF and the cholesterol connection**

Numerous epidemiological studies suggest that cholesterol plays a significant role in the development of AD. Patients with AD, administered with cholesterol synthesis inhibitors, collectively termed statins, have reduced incidence of the disease (60). Indeed, the identification of a genetic variant of the apolipoprotein E (APOE), a cholesterol transporter, as a major genetic risk factor for AD is also consistent with a role for cholesterol in the pathogenesis of AD (61). In the same vein, deficiency of ATP-binding cassette transporter A1 (ABCA1), which regulates cholesterol efflux, increased Abeta deposition in different AD models (62).

Furthermore, animal studies have shown that a high fat/high cholesterol diet exacerbated the signs of the disease in a transgenic mouse model of AD (63), while treatment with inhibitors of cholesterol synthesis was protective (64,65). These findings are supported by several biochemical data reporting that the APP processing is directly affected by cellular cholesterol content (66,67).

Cholesterol, the main component of caveolae and lipid rafts, influences the activity of the enzymes involved in the metabolism of the APP, by promoting beta and gamma cleavage of APP (68), and generating the toxic CTF beta and Abeta toxic species (69). Moreover, increased membrane cholesterol might render mature hippocampal neurons more susceptible to Abeta-induced tau toxicity (70) and cause the memory impairments and cognitive decline characteristic of AD (71). Recent advances in the neurobiology of neurotrophins suggest p75<sup>NTR</sup> signalling as the one responsible for the increased cholesterol content in neurons. In fact, p75<sup>NTR</sup> regulates the expression of several cholesterologenic enzymes in both Neuro2a cells and primary neuronal cultures (72).

The mechanisms of the p75<sup>NTR</sup>-dependent regulation of cholesterol synthesis seems to involve a 94 kD zinc finger protein of the Kruppel family, called neurotrophin receptor interacting factor (NRIF) (73), which may function as an intracellular interactor of p75<sup>NTR</sup>, thus mediating ceramide and BACE1 increase as well as p53-dependent apoptosis in neuronal cells (74,75). NRIF has also been shown to be an essential activator of cholesterol biosynthetic genes. In fact, NRIF reduction resulted in reduced expression of sterol-sensing domain protein SCAP, followed by a decrease in mRNA levels of SRE-motif containing genes (HMGCR, FASN, SREBP2, S1P, and SQS1) in both in vitro and in vivo paradigms of NRIF depletion (73). The exact molecular events leading to p75<sup>NTR</sup>-NRIF driven cholesterol synthesis have not been described yet, while the mechanisms of the p75<sup>NTR</sup>-NRIF mediated apoptosis have been well characterized. In the p75<sup>NTR</sup>-NRIF driven apoptotic cascade, the release of the p75 intracellular domain (ICD) by ADAM-17 (76), through regulated intramembrane proteolysis (RIP), is necessary for NRIF polyubiquitination by the E3 ligase TRAF6 (77), and subsequent nuclear translocation and target gene regulation (78).

The study of NRIF expression and cholesterol biosynthesis in vivo in animal models with unbalanced proNGF-p75<sup>NTR</sup> versus mNGF-TrkA signalling, like proNGF transgenic mice, and TrkA CNS-KO mice could give new highlights on the molecular events of cholesterol-dependent amyloidogenesis in adult CNS neurons. Further studies are needed to address the question of the potential involvement of the p75<sup>NTR</sup>-NRIF complex in the regulation of neuronal cholesterol homeostasis. However, the hypothesized p75<sup>NTR</sup>-NRIF-cholesterol cascade is likely to be very important for neuronal membrane homeostasis and synaptic function in brain ageing and AD (79).

**NGF and the longevity:**

**the anti-amyloidogenic protein sirtuin1**

Silent information regulator (sir) genes encode for epigenetic regulatory proteins, collectively designated sirtuins (SIRT).
SIRT1 is the best characterized enzyme of the sirtuin family of nicotinamide adenine dinucleotide NAD+-dependent protein deacetylases, which include 7 members (SIRT1–7). Sir2 protein found in the yeast is most likely one of the key proteins in mediating the calorific restriction (CR)-dependent lifespan extension in yeast as well as in invertebrates (80). Because sir2 is conserved from prokaryotes to mammals, the human SIRT1 has gained considerable attention for its impact on mammalian physiology, and for defining novel therapeutic targets for treating diseases associated with aging, like AD, and in extending human lifespan.

Although most of the sirtuins, and SIRT1 in particular, are highly expressed in the brain (81), at present very little is known about their roles and targets in the nervous system. Most studies of SIRT1 in the mammalian brain have focused on its role in neuroprotection. SIRT1 appears to inhibit axonal degeneration, a process that often precedes neuronal death in neurodegenerative diseases (82). As for the APP metabolism, SIRT1 was found to protect neurons by downregulating the amyloidogenic Rho kinase (ROCK1) expression in neurons (83,84). In vitro, SIRT1 was able to suppress Abeta production and cognitive decline in the p25 transgenic mice (85) and in the double APPsw/PS1de9 transgenic mice, by inducing ADAM10 expression (86).

As a confirmation of SIRT1 relevance to normal cognitive functions in the normal mammalian brain, SIRT1 has been shown to sustain brain metabolism (87), neurogenesis (88), as well as learning and memory in the adult brain (89). On the opposite, the decline in serum concentration of SIRT1 correlates with both tau pathology (90) and severity of cognitive deficits in patients with MCI and AD, as well as in aged healthy individuals (91).

Together, these findings call for increased examination of SIRT1 and for novel potent SIRT1 activators as protective strategy in neuronal ageing and AD-like neurodegeneration (80). Intriguingly, NGF is a potential SIRT1 activator in the mammalian neurons in vitro and in vivo. NGF signalling through the cAMP responsive-element binding (CREB) has been shown to activate SIRT1 transcription in both pheochromocytoma cell line (PC12) and primary neurons (92). Moreover, NGF has been shown to induce a well known SIRT1 substrate, the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha), thus promoting mitochondrial biogenesis in PC12 and primary neurons (93). The decrease of PGC-1 alpha mRNA expression was found to significantly correlate with the progression of AD (94), and the reconstitution of exogenous PGC-1alpha expression in Tg2576 neurons attenuated ROCK1 driven amyloidogenesis (94–90).

Overall, NGF could exert its anti-amyloidogenic effect through SIRT1-dependent increase of both ADAM10 transcription on one side and of PGC1alpha (inhibiting ROCK1 mediated beta processing) on the other side: a valid rationale for therapeutic targeting of NGF and/or NGF downstream molecules in AD neurodegeneration.

NGF and APP metabolism: a direct link?

Several lines of evidence point to a direct regulatory effect of NGF on APP metabolism and processing. The p75NTR (but not the TrkA) signalling is able to increase APP translation rates in PC12 cells (95), while TrkA signalling promotes the anti-amyloidogenic APP processing, favouring the release of sAPPalpha (96). Contrary, the interruption of NGF signalling by NGF withdrawal leads to a strong increase in total APP levels and Abeta production in PC12 and primary neurons (97,98). Moreover, the stable expression of a neutralizing anti NGF antibody in AD11 transgenic mice caused an age dependent increase in Abeta, leading to both short and long term plasticity defects in vivo (99–101).

In the APP molecule, the APP C-terminal has a critical role for developmental functions of APP (102) and in regulating APP processing (103), possibly through the modulation of the APP interacting proteins binding its intracellular tail (104). Several residues are involved (S675, Y682, T686, and Y687), but the most relevant in the adult brain functions are the tyrosine 682 (Y682) in the Y682NPTY motif and the upstream threonine 668 (T668, numbering of APP695) (104). The binding of interacting proteins to APP depend on the pattern of APP phosphorylation at the cytosolic tail. Several APP interactors have been found to bind the Y682 when phosphorylated (shc, grb2/7, src) and others when not phosphorylated (Fe65, Numb, JIP1) (104). Phosphorylated Y682 (pY682) and, thus, the specific APP interactors binding to pY682, have been associated with altered APP processing in vitro, in vivo and in AD (103,105).

More than a decade ago D’Adamio found very high levels of tyrosine phosphorylated APP and TrkA in cells overexpressing constitutively active form of the Abl tyrosine kinase (106). He also demonstrated that (i) activated TrkA is necessary to achieve APP tyrosine phosphorylation, given that the dominant negative TrkA mutant form lacking the kinase domain is unable to phosphorylate APP, and (ii) TrkA overexpression modulate APP processing decreasing the amount of AICD produced (107). Furthermore, TrkA is capable not only of modulating the phosphorylation pattern of APP, but it also directly binds to APP when phosphorylated at Y682. The lack of the Y682 docking
site, in mice bearing a tyrosine to glycine point mutation (APP<sup>YG</sup> mice), abolishes APP interaction with TrkA, affects their cellular distribution (108), and impairs TrkA signalling, cholinergic functions and cognition (109).

As described above, the APP C-terminal can be also phosphorylated at the threonine residue T668, upstream the Y<sup>682</sup>NPTY motif (APP<sup>pT668</sup>). T668 phosphorylation is likely to be involved in the intracellular sorting and trafficking of APP, which in turn impacts on the APP amyloidogenic cleavage (110). In fact, APP<sup>pT668</sup> levels are known to (i) be elevated in dystrophic neuritis and amyloid plaques of AD brain (110), (ii) favour APP/BACE interaction and the consequent beta cleavage of APP (110), and (iii) lead to Aβ and tau accumulation in AD and in Tg2576 mice (111). Notably, IGF1 has been shown to modulate APP processing by reducing the levels of APP<sup>pT668</sup> in human neuronal cell lines (112). Since T668 is the best characterized residue of the APP cytosolic tail, strongly implicated in amyloidogenic processing, production of the toxic beta CTFs and neuronal degeneration in AD, the potential role of NGF in the described events could be of great therapeutic interest for AD and age-related neurodegeneration.

Taken together, the effect of NGF-TrkA on Y682 phosphorylation and APP processing indicate a very tight connection between NGF-TrkA signalling system and APP molecule and suggest a complex scenario bridging the NGF-driven pattern of APP phosphorylation with a phosho-driven specific APP interactome of great potential interest for neurodegeneration in the CNS.

**NGF, APP and the sunday driver protein:** stuck along the axon

The endocytic internalization of the ligand-receptor complex and the axonal trafficking of neurotrophin receptors suffer perturbations in a number of neurodegenerative diseases, including the NGF-TrkA system retrograde transport in the BFCN affected by AD. In particular, NGF requires an intact axonal cytoskeleton to transduce its signalling potentials (113). Coherently, NGF receptor TrkA has been found to interact with a key cytoskeleton protein, the sunday driver (SYD) protein (114). SYD2, a recently characterized member of the SYD family, is another name for the c-jun kinase (JNK) interacting protein 3 (JIP3). SYD2-JIP3 is a neuronal-enriched scaffolding protein for JNK: it binds both to JNK3 and APP, favouring APP phosphorylation, leading to amyloidogenesis, and activating the apoptotic cascade. Thus, SYD2/JIP3 protein can be speculated to be the missing link between perturbations of cytoskeletal trafficking of key neurotrophin receptor, like TrkA, amyloidogenic APP phosphorylation and the JNK dependent apoptotic signalling cascade (114). It is unknown whether the impaired NGF-TrkA signalling cascade or the cytoskeletal machinery defects come first in AD neurodegeneration. The possibility that an initial neurotrophic unbalance may in turn affect APP/SYD2-JIP3 transport and signalling, thus mediating apoptosis via a switch from the (inhibited) RAS/MAPK-Pi3K-signaling toward the (activated) SYD2-JIP3/JNK3/p38MAPK amyloidogenic-apoptotic pathway cannot be excluded and deserves further studies.

The deeper understanding of the NGF signalling defects and cytoskeleton dysfunction in AD will hopefully allow more targeted and effective interference with amyloidogenic-apoptotic events occurring in MCI and early AD (115,116).

**Conclusion**

Though brain ageing is universally considered to be a major risk factor for developing LOAD, nowadays there is a lack of knowledge on molecular pathway(s) responsible for the shifting from normal brain aging toward AD neurodegeneration. On the other hand, normal ageing doesn’t necessarily evolve into AD. Genetic abnormalities, environmental factors (exposome), physical illnesses, and cardiometabolic diseases (50-52,117,118) and/or a combination of them could act as a second “hit”, breaking brain homeostatic responses and leading to progressive neuronal damage and final cell loss.

The evidences herein reported prompt the idea of NGF exerting pleiotrophic actions through multiple substrates and pathways and down-regulating the amyloidogenic APP processing (Fig. 1), thus opening a new perspective in the understanding of neuronal pathology in age-related LOAD. After decades of scientific hypotheses centered on acetylcholine, Aβ and tau, today we may re-think to the significance of NGF alterations in AD and consider the age-driven perturbation of NGF-TrkA and proNGF-p75<sup>NT</sup>R signalling systems in the CNS neurons as a promising first “hit” in LOAD.

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**Figure 1.** A schematic representation of the anti-amyloidogenic mNGF-TrkA versus the amyloidogenic proNGF-p75NTR signalling pathways. A prevalent TrkA, and ERK/MAPK activation sustain normal metabolism and synaptic plasticity, while the increase of p75NTR and JNK/SAPK signalling pathways induced by age/stress lead to neurodegeneration and apoptosis in neurons of the central nervous system.

**SELECTED LIST OF ABBREVIATIONS**

- AD: Alzheimer’s disease
- ADAM: a disintegrin and metalloproteinatease
- AICD: APP intracellular domain
- APP: amyloid precursor protein
- BACE1: beta secretase 1
- BFCN: basal forebrain cholinergic neurons
- CNS: central nervous system
- CTF: carboxyl-terminal fragment
- KO: knock-out
- LOAD: late onset Alzheimer’s disease
- MCI: mild cognitive impairment
- mNGF: mature NGF
- MR1: muscarinic acetylcholine receptor 1
- NGF: nerve growth factor
- NRIF: neurotrophin receptor interacting factor
- p75NTR: p75 neurotrophic receptor
- proNGF: precursor NGF
- sAPP: secreted APP
- SIRT1: sirtuin 1
- SYD: sunday driver
- TrkA: tyrosine kinase A

**AGEING, ILLNESS, ENVIRONMENTAL OR METABOLIC DISTURBANCES**

- Alpha processing
  - Neuronal plasticity
- Beta processing
  - Apoptosis
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