Chapter

TrkA Signalling and Parkinson's Dementia

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Abstract

Cognitive impairment and dementia are the most frequently occurring non-motor symptoms in Parkinson's disease (PD), yet these symptoms are mostly overlooked and are not diagnosed and treated exceptionally like the cardinal motor symptoms in clinical practice. It is only in the late twentieth century that dementia has been recognized as a major clinical manifestation in PD. The possible mechanisms that cause dementia are complex with different patterns of cognitive behavior that disrupt the patient's quality of life. It is preeminently considered that the cholinergic denervation in the basal forebrain region mediates dementia in PD. So far, dopamine-based therapy is the key objective in the treatment of PD and the nonmotor symptoms are mostly neglected. Interestingly, the loss of Tyrosine kinase receptor-A (TrkA) signaling in basal forebrain results in neuronal atrophy, which precedes cholinergic denervation and cognitive impairment. Nerve Growth Factor (NGF) binds to TrkA receptors, inducing a cascade of events like PI-3Kinase/Akt and MAPK signaling pathways that render cholinergic degeneration and upregulate the choline acetyltransferase activity and neuronal differentiation. Hence, TrkA receptor activation by small molecules might attenuate the dementia symptoms associated with PD, and may be targeted as a novel treatment strategy along with regular clinical agents.

Keywords: dementia, Trk receptors, nonmotor symptoms, cholinergic neurotransmission, neuroprotective signaling pathways

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder with a wide range of clinical symptoms. Even though PD is traditionally considered a disorder of movement, mounting number of evidences emerged that concerned the connection of dementia and other nonmotor symptoms (NMS) that occurs from the early stages of PD and contradicted the statement of *James Parkinson's (1817) "the senses and intellects being uninjured"* [1, 2]. But more attention was paid to dementia and cognitive impairment associated with PD only from the levodopa era and most researchers agreed that dementia (particularly organic dementia) occurs more frequently in patients suffering from PD [1, 3].

Jeffery Cummings reported a mean dementia prevalence of 40% in his review of 27 studies that included 4336 patients with PD [4]. In spite of these studies being crucially considered, most studies represented patients with unselected PD population based on patients being referred to neurology clinics and some studies did not specify the exclusion of patients who were already diagnosed with

Dementia with Lewy Bodies (DLB) [4, 5]. Another systematic review of 13 studies employing strict methodological inclusion and exclusions screened 1767 patients, out of which 554 were diagnosed with dementia, reflecting a prevalence of 31.3%. This review also proclaimed the prevalence of dementia in general population that included PD patients, and revealed that 3–4% of dementia in patients was due to dementia associated with PD, which sums to a total of 0.3–0.5% among the overall general population aged 65 years and above [5, 6]. Most of the studies evaluating the incidence of dementia associated with PD are based on longitudinal study of community-based cohorts, from which the prevalence of PD has been estimated [5]. Some studies revealed incident rates of 95 [7] and 112 [8] in 1000 patient years, revealing that approximately 10% of the patients diagnosed with PD are at a higher risk category and develop dementia within 10 years [5, 7, 8]. In 2008, Hely and team reported the data from their 20-year follow-up multi-center longitudinal study, which demonstrated that up to 80% of patients with PD will develop dementia over a 20-year period and this finding implies that most patients with PD will eventually develop dementia if they live long enough [9].

Dementia currently is considered the most significant nonmotor symptom (NMS) in PD due to its crucial contribution towards the morbidity and mortality of the disease and has also evinced remarkable clinical consequences to the patients in terms of disability, increased risk of psychosis, and reduced Quality of Life (QoL) [5]. Recent advancements in treatment have increased patient survival, which has in turn increased the incidence and prevalence of dementia in PD population. Although a slight cognitive deficit is sometimes noticed in the initial stages of PD, overt dementia and cognitive impairment manifest more commonly in the later stages when the patient's age advances [2]. The prevalence of dementia and cognitive impairment remains controversial and eminently depends upon the study population and on the diagnostic tools and methods used. Various studies estimating the frequency of dementia and cognitive impairment in PD have used a variety of methods and study designs that may alter study outcomes [2, 5].

The cholinergic neurons are projected in three major areas in the brain: brainstem [10], striatum [11], and the basal forebrain (BF) region [12]. The cholinergic projection in the brainstem extends to the thalamus and it functions in risk aversion [11, 13, 14], while the cholinergic interneurons in the striatum play a key role in the regulation of dopamine secretion [11]. The basal forebrain cholinergic neurotransmission system principally originates in the medial septum, vertical limb of the diagonal band (MS/VDB), and the nucleus basalis, which extends to the olfactory bulb, neocortex hippocampus, and amygdala [12, 15]. Basal forebrain cholinergic neurons, especially the ones in the nucleus basalis, are reported to selectively degenerate in certain neurodegenerative disorders and have long been a key focus of research in the determination of the relation between acetylcholine (ACh) and memory [16].

It is widely accepted that the cholinergic neurotransmission system in the basal forebrain region is for normal cognitive function, especially memory and attention. Degeneration of the cholinergic neurotransmission is thought to be responsible for cognitive impairment and dementia associated with neurodegenerative disorders like PD and Alzheimer's disease (AD) [17–19]. Many studies have reported that deficits in cholinergic neurotransmission and signaling are often coupled with neurodegenerative and attentional disorders and impaired cognitive control [20]. While the possible mechanisms resulting in such manifestations are complex and heterogenous and lead to different patterns of cognition and behavior that majorly affects the patient's QoL. The preeminent mechanism through which cholinergic signaling influences cognition is predicted to be direct cholinergic stimulation of

pre- and post-synaptic neuronal receptors. Neuroinflammation is considered to be the hallmark pathology in neurodegenerative disorders like Parkinson's and Alzheimer's and may also contribute to other neurodegenerative disorders [10, 21].

Neurotrophins are proteins that are identified as survival factors of sensory and sympathetic neurons and have been shown to have an imperative control of survival, development, and functioning of neurons in both the peripheral and central nervous system [22]. The neurotrophin family is comprised of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). Pro-neurotrophins bind to and signal via two principal receptor types: $p75^{NTR}$ and TRK receptors. There are three TRK receptors: TRKA, TRKB, and TRKC, and each receptor selectively binds to different neurotrophins: NGF binds to TRKA; BDNF and NT4 bind to TRKB; and NT3 binds to TRKC [23, 24]. TRKB and TRKC are widely found in neuronal populations throughout the central and peripheral nervous system in humans, while the distribution of TRKA is mostly restricted to the basal forebrain cholinergic region, the dorsal root ganglion, and sympathetic neurons [25]. Cholinergic neurons that do not transport NGF are severely shrunken and downregulate the expression of TRKA receptor [26]. Neurotrophins are thought to be a promising therapy for various neurodegenerative disorders including PD and AD. Because of their poor bioavailability and pharmacokinetic properties, make poor drug entities [27]. The major drawback in neurotrophin therapy is reduced passage of peptide hormones across the blood–brain barrier (BBB); peripheral administration of peptide hormones resulted in a slight increase in the intracranial neurotrophin concentration. Hence, considerable efforts are devoted towards the discovery of appropriate small molecule neurotrophin peptidomimetics that can mimic the binding of selective peptides and elicit neuronal regenerative responses like that of neurotrophins [28]. Hence, this chapter points out the risk factors, progression, and possible novel targets for Parkinson's dementia.

2. Risk factors of dementia in PD

Many demographic features, along with cardinal motor and NMS, have been identified as potential risk factors and predictors of dementia in PD [5, 29]. A study outlined that the patient's age was reported to be a common risk factor for dementia in a PD population [30] and some studies also suggested that advanced disease stage, specific subtypes of PD (e.g., akinetic-subtype), and certain NMS like olfactory dysfunction, mild cognitive impairment and mood disorders, rapid eye movement sleep behavior disorder (RBD), and hallucinations are reported to be strong predictors of dementia in PD [29].

2.1 Age

A number of studies have proposed that the patient's age and the age of onset of PD are both associated with a higher risk of dementia, and interestingly, patient's age along with increasing severity of the cardinal motor symptoms and duration of the disease are considered to be the key risk factors of dementia in PD [30, 31]. Based on such proposals, Levy concluded that aging may still play a substantive role in the pathogenesis and progression of the disease and the pathogenic cascades should further account not only for the relative selectivity of the disease process to the substantia nigra pars compacta but also for the widespread involvement of the cholinergic structures in late clinical stages of the disease [32].

2.2 Olfactory dysfunction

Olfactory dysfunction, or hyposmia, is frequently observed in the pre-motor (pre symptomatic stage) phase of the disease, even before dopaminergic denervation is evident and most of the evidence highlights the involvement of cholinergic dysfunction in hyposmia and several other aspects of olfaction [33]. The prevalence of hyposmia in PD patients is reported to be very high with up to 95% being affected [34]. A study conducted in 2012 including PD patients with hyposmia (prevalence ~55%) reported that in contrast to PD patients without hyposmia, PD patients with hyposmia exhibited mild impairment in general cognition, memory, and visuoperceptual functioning. After a follow-up period of 3 years, it was found that the cognitive impairment in the patients with hyposmia was more severe and their scores on Mini-Mental State Examination became significantly worse than compared with that of patients without hyposmia [35].

2.3 Cognition and mood disturbances

Cognitive impairment and dementia are frequent findings in PD patients. Approximately 75% of the PD patients who survive for more than 10 years are expected to develop dementia [36]. Neuropathological studies have shown that cognitive impairment in PD is associated with the cholinergic loss in BF. Reductions of acetylcholine esterase (AChE) activity in frontal cortex are found to be greater in Parkinson's disease dementia (PDD) compared to PD without dementia [37]. Major depression and apathy are commonly reported in PD; although alterations in the monoaminergic systems is thought to result in mood changes, there is evidence that the severity of cortical cholinergic degeneration is strongly associated with the presence of depression and apathy in PD [38]. Depression in PD appears to be associated with cognitive deficits, suggesting a common mechanism, and this hypothesis is justified by the observation that depression is one of the major risk factor for dementia in PD [39, 40].

2.4 Random eye movement sleep behavior disorder (RBD)

RBD is a commonly reported NMS in PD and is mostly reported to precede cognitive impairment and dementia associated with PD [41, 42]. RBD is mainly characterized by disturbed atonia during random eye movement sleep, which results in abnormal motor manifestations [42, 43]. The principal mechanism underlying RBD is considered to be cholinergic dysfunction, which is also assumed to play an imperative role between RBD and increased dementia in PD patients [42–44]. A brain imaging study exposed that cholinergic denervation was strongly associated with RBD, and 33.8% of 80 patients presented with RBD. The patients underwent acetylcholine esterase and dopaminergic dual-tracer PET scanning. The scan reports revealed that patients who presented with RBD and related symptoms exhibited decreased cortical, neocortical, and thalamic cholinergic innervations when compared to PD patients without RBD and related symptoms. This study also summarized that cholinergic denervation can occur in early stages of the disease [43–45].

2.5 Visual hallucinations

Visual hallucinations are generally considered as the main neuropsychiatric feature [46] and it is commonly observed in patients with PD [46, 47], particularly in patients with dementia [48]. A longitudinal study found that visual hallucinations

are associated with higher risk of developing dementia [49]. The relationship between hallucinations and dementia is thought to be related to both Lewy body pathology [50] and cholinergic disturbance [51].

3. Pathophysiology of dementia in PD

The mechanism behind dementia in PD remains uncertain, and a number of neurochemical and neuropathological changes are assumed to be involved. Dementia in PD is thought to be a result of several cortical and subcortical changes, mainly involving the cortical cholinergic deficiency due to neurodegeneration in the nucleus basalis of Meynert (nbM) and the subcortical pathology, including dopaminergic deficiency in the caudate and in mesocortical areas [52]. It is also reported that additional AD-like pathology and the presence of Lewy bodies are likely to furthermore complicate cognitive impairment and dementia [53]. Cognitive impairment in non-demented PD patients is thought to be caused by the depletion of the dopaminergic system in the frontal cortex, which results from degeneration of the mesocortical dopaminergic system mainly projecting from the ventral tegmental area (VTA) [54].

It has also been reported that the loss of neurons in the locus coeruleus along with noradrenergic deficiency in the cortex region may result in dementia in PD. However, this neuropathology was not found or reported in other similar studies. The loss of serotonergic neurons in the dorsal raphe nucleus (DRN) is mainly reported to be associated with depression among PD patients and comparisons between demented and non-demented PD patients did not find differences in neuronal counts in the DRN [55].

In an attempt to establish a connection between dementia in PD and diminished monoaminergic activity in their study, instead identified the association between cholinergic deficiency and dementia and reported that cholinergic deficit is implicated in the neuropathology of dementia in PD and in DLB [52, 56]. It was also reported that more profound and definite cholinergic depletion was found in the nbM region in PD brains when compared to that of AD brains [57]. This hypothesis was further supported by the fact that anti-cholinergics elicited cognitive impairment in PD patients and by the therapeutic benefits of acetylcholine esterase inhibitors in the management of symptoms associated with dementia [58]. Adjacent to these neuropathological changes, important AD-like cortical changes have also been reported and implicated especially due to the abundant expression of AD-neurites in PD patients with dementia, which also correlates with the severity of dementia in PD [58, 59]. Basal forebrain (BF) cholinergic neurons within the nucleus basalis are the major source of cholinergic innervation to the cerebral cortex and play a key role in cognition and attention. In conditions like PD and AD, these cortical projection neurons undergo extensive degeneration, which correlates with clinical severity and disease duration. BF cholinergic neurons require Nerve Growth Factor (NGF) for their survival and biologic activity [60]. NGF mediates its actions on the BF cholinergic neurons via binding to the low-specificity, low-affinity p75NTR and the NGF-specific high-affinity TrkA receptor. Both the receptors are expressed and localized at cholinergic cell bodies and at nerve terminals [61]. The embryonic development of the BF cholinergic neurons is highly dependent on the expression of NGF and TrkA expression. Aging causes mammalian NGF expression and release to diminish to basal levels; however, the trophic dependence of cholinergic neurons on NGF remains critical even in the mature and fully differentiated CNS [61, 62].

4. TrkA receptor activation, a new target in Parkinson's dementia

Neurotrophins are proteins that were initially identified as survival factors of sensory and sympathetic neurons, and since have been shown to have an imperative control of survival, development, and functioning of neurons in both the peripheral and central nervous system [22]. The neurotrophin family comprises nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). These neurotrophins are formerly synthesized in the endoplasmic reticulum as pre-proproteins, and cleavage of the signal peptide of pre-proproteins converts these into pro-neurotrophins. In the *trans*-Golgi network and in secretory vesicles, pro-neurotrophins dimerize and are proteolytically processed by proprotein convertase subtilisin kexin (PCSK) enzymes to their mature forms prior to their release from the cell. Pro-neurotrophins binds to and signals via two principal receptors: $p75^{NTR}$ and the TRK receptors. $p75^{NTR}$, a tumor necrosis factor (TNF) receptor family member that unselectively binds all of the neurotrophins and lacks known intrinsic enzymatic activity but recruits signaling adaptors and modulates molecular signaling via TRK receptors [25]. Depending on the expression of TRK receptors and other intercellular signaling adaptors, $p75^{NTR}$ induced effects vary a wide range including neuronal cell survival, regulation, proliferation, and inhibition of neurite growth and is also known to regulate various proteins and pathways like phosphoinositide 3-kinase (PI3K)–AKT pathway, nuclear factor-κB, (NF-κB), and mitogen-activated protein kinase (MAPK) [63–65].

There are three TRK receptors: TRKA, TRKB, and TRKC, and each neurotrophin selectively binds to different receptors: NGF binds to TRKA; BDNF and NT4 bind to TRKB; and NT3 binds to TRKC [23, 24]. In addition, there is heterologous binding, with NT3 and NT4 both provoking some activation of TRKA, and NT3 prompting some activation of TRKB [23, 25]. TRKB and TRKC are widely found in neuronal populations throughout the central and peripheral nervous system in humans, while the distribution of TRKA is mostly restricted to basal forebrain cholinergic region, the dorsal root ganglion, and in sympathetic neurons [25]. TRK signaling occurs

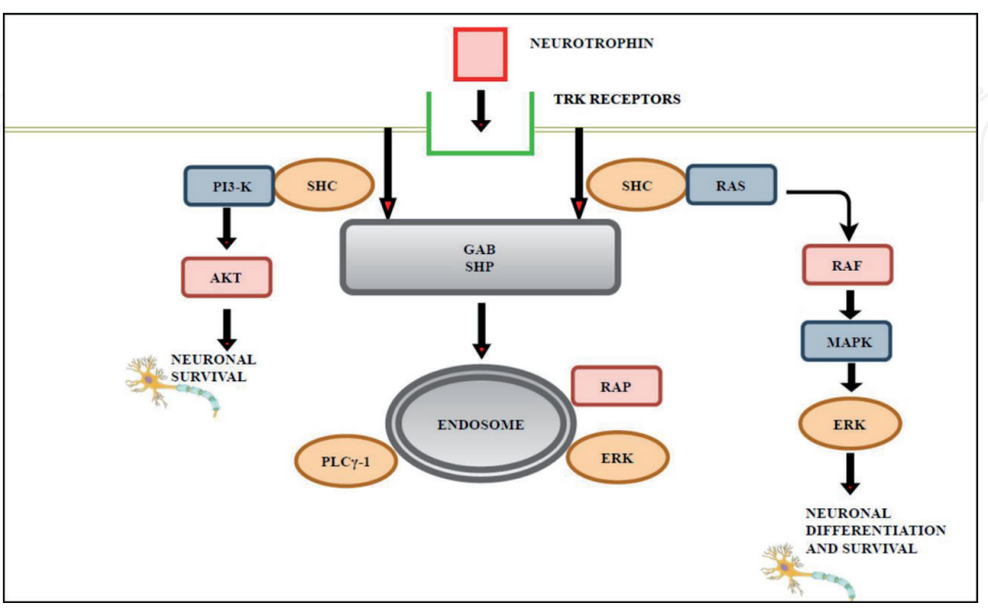


Figure 1.
Neurotrophin-TRK receptors signaling pathway. Neurotrophins binds and activates the TRK receptors, which phosphorylates the SCH domain that in turn triggers the RAS mediated MAPK signaling pathway and PI3-K/AKT pro-survival pathway that results in neuronal differentiation and survival.

Receptors	Preferred ligand	Non-preferred ligand	Non-ligand
TRKA	NGF	NT3, NT4	BDNF
TRKB	BDNF	NT3	NGF
TRKC	NT3	None	NGF, BDNF AND NT4
$P75^{NTR}$	NGF, BDNF, NT3, AND NT4	None	None

Table 1.
Binding specificity of neurotrophins.

principally through three tyrosine kinase–mediated pathways: MAPK–ERK (extracellular signal-regulated kinase) pathway, PI3K–AKT pathway, and phospholipase Cγ-1 (PLCγ-1)–PKC pathway (summarized in **Figure 1**). The effects elicited via these signaling pathways predominantly endorse cell survival and differentiation [24]. Even though these neurotrophin receptors possess overlapping expression patterns and functions, certain neural mechanisms, such as NGF-TRKA mediated basal forebrain cholinergic upregulation [66], BDNF–TRKB mediated improved synaptic plasticity, and NT3-TRKC mediated survival of peripheral proprioceptive neurons, are receptor specific. The receptor-ligand specificity is mentioned in **Table 1**.

4.1 TrkA neuroprotective signaling pathways

NGF was discovered as a molecule that promoted survival and differentiation of sensory and sympathetic neurons. Cellular responses to NGF are elicited through binding and activation of TRKA receptor [67]. Major pathways activated include Ras stimulated MAPK/ERK protein kinase pathway, PI3-K stimulation of AKT, and PLCγ1-dependent generation of IP3 and diacylglycerol (DAG) that results in mobilization of calcium stores and activation of Ca^{2+} and DAG-regulated protein kinases [22]. These signaling pathways prevent apoptotic cell death and promote cellular differentiation, axon regulation, and choline acetyl transferase (ChAT) upregulation [68]. NGF mediated neuroprotective signaling most likely depends on PI3K/Akt in PC12 cells, cerebellar cortex, sympathetic, sensory and motor neurons [69]. The neuroprotective pathways induced by TrkA receptor is summarized in **Figure 1**.

4.1.1 RAS signaling pathway

Reticular Activating System (RAS) regulates neuronal differentiation and also promotes neuronal survival, through either the PI3K or the mitogen-activated protein kinase (MAPK)/Extracellular-Signal-Regulated Kinase (ERK) pathways. In PC12 cells, different adaptors appear to facilitate transient versus prolonged activation of ERK signaling. In each case, phosphorylation of Y490 initiates the recruitment of an adaptor protein, initiating a cascade of signaling events [69]. Shc recruitment and phosphorylation in turn results in recruitment to the membrane complex of the adaptor Grb-2 and the Ras exchange factor son of Sevenless (SOS), thereby stimulating transient activation of Ras. Ras in turn activates PI3K, p38 MAPK/MAPK-activating protein kinase 2 pathway, and the c-Raf/ERK pathway [70].

4.1.2 PI3K signaling pathway

Trk receptors can activate PI3K at least via two distinct pathways, depending upon the neuronal subpopulations. In many neurons, Ras-dependent activation of PI3K is the most important pathway through which neurotrophins promote cell

survival. In some cells, however, PI3K is also activated by three adaptor proteins, Shc, Grb-2, and Gab-1 [71]. Shc binding with phosphorylated Y490 results in recruitment of Grb-2. Phosphorylated Grb-2 provides a docking site for Gab-1, which is bound by PI3K [72]. In some neurons, the insulin receptor substrate (IRS)-1 is also phosphorylated by neurotrophins that recruit and activate the PI3K signaling pathway [73]. In addition to providing a linker for activation of PI3K, Gab-1 also nucleates formation of a complex including the protein phosphatase Shp-2, [74] which enhances activation of the Ras/ERK signaling pathway [72, 75].

4.1.3 PLC-γ1 signaling pathway

Phosphorylated Y785 on TrkA and similarly placed residues on other Trk receptors recruit the PLC-γ1 signaling pathway. The Trk kinase then phosphorylates and activates PLC-γ1, which acts to hydrolyze phosphatidylinositides to generate diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP3). IP3 induces the release of calcium ion (Ca^{2+}) stores, thereby increasing the levels of cytoplasmic Ca^{2+}, which in turn activates many pathways controlled by Ca^{2+}. It has been shown that NGF activates DAG-regulated protein kinase and protein kinase C (PKC)-δ, which is required for ERK cascade activation and neurite outgrowth [76]. PKC-δ appears to act between Raf and MAPK/ERK in this signaling cascade.

5. TrkA activation and cholinergic regeneration

TrkA gene expression is under positive feedback from NGF signaling, and this pathway may be disturbed by reduced retrograde transport of cortical NGF to nbM cholinergic consumer neurons [77]. In sustenance of this hypothesis, NGF levels are stable [78] or increased [79] in the cortex, whereas the levels of NGF are decreased in nbM [77]. Notably, defective retrograde transport of NGF within cholinergic projection neurons was reported in a transgenic mouse model of Down syndrome [80]. In aged rats, these neurons exhibited a pronounced reduction in NGF retrograde transport, TrkA protein expression, and severe atrophy. Defective NGF retrograde transport may therefore underlie the reductions in nbM TrkA gene and protein expression observed in single nbM neurons, leading to eventual reduction of TrkA protein in cortical projection sites [81] and further trepidations in NGF signaling within the nbM. This putative "off trk" cycle of deficient NGF signaling may contribute to the selective degeneration of cholinergic nbM neurons and deficits in cortical cholinergic tone [60, 82]. Several lines of evidence support the role of NGF in the survival of cholinergic neurons in the BF brain region. *In vitro* studies, using dissociated rat nbM cultures [83, 84] or organotypic nbM slices, [85] revealed that NGF treatment prevented the cholinergic neurodegeneration that was observed in untreated preparations. These results are similar to those demonstrating that infusion of NGF can prevent septal cholinergic neuron death following septo-hippocampal axotomy [86].

Finally, transgenic mice that express anti-NGF antibodies in adulthood display an age-dependent loss of CBF neurons [87]. These reciprocal correlations between reduced cortical TrkA and elevated pro-NGF levels with MMSE scores recommend that cholinotrophic aberrations play a significant role in cognitive impairment and may underlie the subsequent demise of nbM cholinergic neurons and extensive cholinergic deficits seen in the late stages of neurodegenerative disorders. Similar studies in nonhuman primates showed that recombinant human NGF reverses both age-related and lesion-induced cholinergic neuronal

degeneration and promotes cholinergic neurite sprouting [88, 89]. In addition, exogenous NGF rescues age-related and cholinergic lesion-induced spatial memory deficits in rodents [90, 91]. Thus, restoration of NGF signaling may demonstrate efficacious for the prevention of cognitive deficits resulting from nbM dysfunction.

6. TrkA ligands

Initially, the approach for the development of ligands targeting the neurotrophin receptors was to create small synthetic peptides with amino acid residues corresponding to various domains of neurotrophins and to assess those small molecules for their ability to mimic or inhibit the neurotrophic functions of the neurotrophins. The discovery of synthetic peptic ligands that correspond to specific neurotrophic domains with agonist and antagonist activities enacted the vital proof that small molecules, including those that bind monomerically to the Trk receptors are able to modulate the receptor functions and also provide a useful basis for the discovery of new non-peptide small molecules [25].

6.1 Gambogic amide

Combinatorial compound library screening identified several TrkA activators, including asterriquinone (1H5) and mono-indolyl-quinone (E5E). These compounds activated the receptor possibly by binding to an intercellular site that promoted PC12 cell survival at micromolecular concentrations [92]. Another screening study identified Gambogic amide (MW 628), which prevented the death of TrkA expressing cell line [68]. It was also found that the gambogic amide binds to the intracellular juxta-membrane domain of the receptor instead of the extracellular ligand binding region, which suggests that gambogic amide results in allosteric activation of the receptor [93]. Gambogic amide was reported to activate TrkA and its downstream signaling pathways and promoted the survival of cells that were reported to express TrkA [68]. However, additional studies are required to establish the degree of specificity for the receptor.

6.2 Amitriptyline

The antidepressant amitriptyline was found to bind with the extracellular domains of the TrkA and TrkB receptors(?) and induce their activation, which promoted heterodimerization that does not occur with NGF or BDNF, which suggests that amitriptyline induces alternative signaling outcomes [94]. Amitriptyline was shown to prevent the apoptosis of cultured hippocampal cells and stimulate neurite outgrowth in PC12 cells. *In vivo* studies reported that amitriptyline abridged kainic acid–triggered neuronal cell death. Studies in inducible TrkA-null mice demonstrated the key role of TrkA in mediating the effects of amitriptyline. However, given the broad spectrum of mechanisms affected by amitriptyline, the issue of target specificity needs to be cautiously considered [94].

6.3 MT2

Several small peptidomimetics were also found to interact with the immunoglobulin-like domain of the TrkA receptor [95]. Interestingly, MT2 exhibits a dominant effect on the survival of PC12 cells, similar to neurotrophin NGF. It was

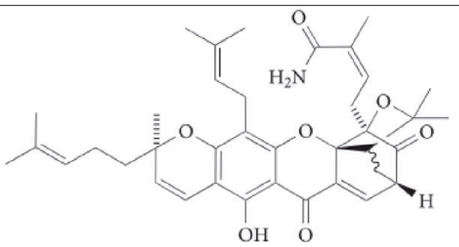
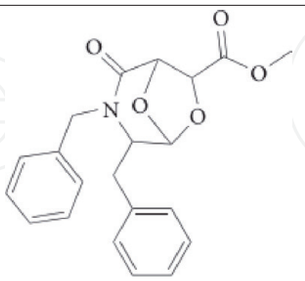
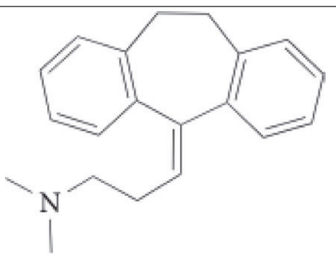
Compound	Structure	*In-vitro* activity
Gambogic amide		Inhibits the death of hippocampal neurons
MT2		Inhibits the death of hippocampal neurons
Amitriptyline (binds to both TRKA and TRKB receptors)		Inhibits the death of hippocampal neurons

Table 2.
Ligands targeting TrkA receptor.

also found that the compound was less capable of inducing TrkA phosphorylation. Additional analysis showed that MT2 and NGF stimulated TrkA-Tyr490 phosphorylation to a similar degree, where MT2 induced significantly less phosphorylation at Tyr674, Tyr675, and Tyr785, which insinuates that there is differential activation of signaling between the compound MT2 and NGF [95]. Whether this idiosyncratic signaling pattern provides any therapeutic compensations or disadvantages relative to NGF relics undetermined.

Taken together, these studies demonstrate that capable small molecules can be created or identified that activate TrkA receptors, in some cases through non-ligand receptor sites. A remaining challenge in most cases is demonstrating the degree of receptor specificity towards TrkA. TrkA receptor agonists and their *in vitro* activity are given below in **Table 2**.

6.4 Limitations of small molecule ligands

Although small molecule activators of the neurotrophin receptors have numerous advantages over native neurotrophins, there are potential limitations that should be considered during their development.

6.4.1 Inadequate receptor specificity

- These molecules bind only to a limited number of motifs present in the protein interaction regions, which leads to identical epitopes occurring in another protein interface, which could produce off-target effects.

- Protein interfaces largely cover several interaction hotspots comprising groups of amino acid residues. The structures and chemical constituencies of these

hotspots are not unique, but their combination in a three-dimensional structure produces a larger interaction region with the potential for high degrees of specificity.

6.4.2 Continuous dosing requirement

- Unlike nucleic acids and other proteins, that are permanently transduced with viral vectors, small molecules cannot be readily produced endogenously and consequently, continued exogenous administration is likely required to maintain their therapeutic efficacy.

6.4.3 Neurotrophin receptor mediated side effects

- Even decidedly specific small molecules may produce abnormal signaling patterns through neurotrophin receptors via detouring the homeostatic mechanisms (for example, proteolysis and endocytosis), which would normally limit the extent of receptor activation.

- These considerations, along with the potential for broad tissue exposure, recommend that some small molecules may have the tendency to elicit on-target side effects like pain, epilepsy, promotion of neoplasia, or hypertension in neural and non-neuronal tissues.

7. Conclusion

The cholinergic system is widely affected in PD, with widespread denervation that contributes to a number of clinical features associated with PD, especially cognitive impairment, abnormal olfaction, and mood disturbances. Multisystem neurodegeneration may play an imperative role in the etiology of nonmotor as well as motor symptoms in PD. While nigrostriatal dopaminergic denervation occurs in all PD patients, there are PD patients with additional degeneration of non-dopaminergic systems (especially the cholinergic system), which significantly impacts the patient quality of life. NGF promotes survival and differentiation of sensory and sympathetic neurons and the cellular responses are elicited via binding and activation of TrkA receptors. BF cholinergic neurons are highly dependent on NGF, which mediates actions on the BF cholinergic neurons via NGF-specific high-affinity TrkA receptors. Cholinergic neurons that do not transport NGF are severely shrunken and downregulate the expression of TrkA receptors. Hence, restoration of NGF signaling may prove efficacious for the prevention of cognitive deficits resulting from nbM dysfunction in PD. The development of small-molecule neurotrophin receptor ligands has only recently begun and only a few ligands have been created and characterized. Nevertheless, observations in *in vitro* and *in vivo* studies using prototype compounds have indicated various vital mechanistic principles that could be used for the future expansion of such similar compounds. These include the discovery that small molecules might achieve patterns of signaling and biological end points that are distinct from those induced by the native neurotrophins. Additionally, 'monovalent' small molecules are capable of activating TRK receptors or modulating $P75^{NTR}$. These capabilities, along with the fundamental roles of neurotrophin receptors in several neurological disorders, will encourage the development and broad application of many more ligands. Moreover, several of the recently described compounds have favorable pharmacological features demonstrating that they could be advanced to clinical studies. However, the possible boundaries of small-molecule

modulation of neurotrophin receptors should be taken into consideration, and it will be crucial to better characterize *in vivo* target binding and establish the pharmacodynamic properties of these compounds. Though, as neurotrophin receptor signaling mechanisms and pathways are better understood, it may be possible to design small molecules to achieve tailored signaling profiles, which could lead to the development of 'disease specific designer ligands'.

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References

[1] Marttila RJ, Rinne UK. Dementia in Parkinson's disease. Acta Neurologica Scandinavica. 1976;**54**(5):431-441

[2] Hanagasi HA, Tufekcioglu Z, Emre M. Dementia in Parkinson's disease. Journal of the Neurological Sciences. 2017;**374**(2016):26-31. Available from: http://dx.doi.org/10.1016/j.jns.2017.01.012

[3] Gaspar P, Gray F. Dementia in idiopathic Parkinson's disease—A neuropathological study of 32 cases. Acta Neuropathologica. 1984;**64**(1):43-52

[4] Cummings JL. Intellectual impairment in Parkinson's disease: Clinical, pathologic, and biochemical correlates. Journal of Geriatric Psychiatry and Neurology. 1988;**1**(1):24-36

[5] Aarsland D, Kurz MW. The epidemiology of dementia associated with Parkinson disease. Journal of the Neurological Sciences. 2010;**289**(1-2):18-22. Available from: http://dx.doi.org/10.1016/j.jns.2009.08.034

[6] Aarsland D, Zaccai J, Brayne C. A systematic review of prevalence studies of dementia in Parkinson's disease. Movement Disorders. 2005;**20**(10):1255-1263

[7] Aarsland D, Andersen K, Larsen JP, Lolk A, Nielsen H, Kragh-Sørensen P. Risk of dementia in Parkinson's disease: A community-based, prospective study. Neurology [Internet]. 2001;**56**(6):730-736. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L32240091%5Cnhttp://wt3cf4et2l.search.serialssolutions.com?sid=EMBASE&issn=00283878&id=doi:&atitle=Risk+of+dementia+in+Parkinson%27s+disease%3A+A+community-based%2C+prospective+study

[8] Goymer P. The frequency and associated risk factors for dementia in patients with Parkinson's disease karen. Nature Reviews. Cancer. 2006;**6**(6):695-701

[9] Hely MA, Reid WGJ, Adena MA, Halliday GM, Morris JGL. The sydney multicenter study of Parkinson's disease: The inevitability of dementia at 20 years. Movement Disorders. 2008;**23**(6):837-844

[10] Martinez-Gonzalez C, Bolam JP, Mena-Segovia J. Topographical organization of the pedunculopontine nucleus. Frontiers in Neuroanatomy. 2011;**5**(April):1-10

[11] Mesulam M-M, Mufson EJ, Levey AI, Wainer BH. Cholinergic innervation of cortex by the basal forebrain: Cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (Substantia innominata), and hypothalamus in the rhesus monkey. The Journal of Comparative Neurology. 1983;**214**(2):170-197

[12] Perez-Lloret S, Barrantes FJ. Deficits in cholinergic neurotransmission and their clinical correlates in Parkinson's disease. npj Parkinson's Disease. 2016;**2**(1). Available from: http://dx.doi.org/10.1038/npjparkd.2016.1

[13] Ye M, Hayar A, Strotman B, Garcia-Rill E. Cholinergic modulation of fast inhibitory and excitatory transmission to pedunculopontine thalamic projecting neurons. Journal of Neurophysiology. 2010;**103**(5):2417-2432

[14] Goldberg JA, Reynolds JNJ. Spontaneous firing and evoked pauses in the tonically active cholinergic interneurons of the striatum.

Neuroscience [Internet]. 2011;**198**:27-43. Available from: http://dx.doi.org/10.1016/j.neuroscience.2011.08.067

[15] Woolf NJ. Cholinergic systems in mammalian brain and spinal cord nancy. Neurobiology. 1977;**37**:285-295

[16] Himmelheber AM, Sarter M, Bruno JP. Increases in cortical acetylcholine release during sustained attention performance in rats. Cognitive Brain Research. 2000;**9**(3):313-325

[17] Ballinger E, Ananth M, Talmage DA, Role L. Basal forebrain cholinergic circuits and signaling in cognition and cognitive decline. Physiology & Behavior. 2018;**176**(1):139-148

[18] Bartus RT, Dean RL 3rd, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science. 1982;**217**:408-417

[19] Zhagn L, Li Z. Alzheimer and the discovery of Alzheimer's disease. Zhonghua Yi Shi Za Zhi. 2014;**44**(5):288-290

[20] Ben Achour S, Pascual O. Glia: The many ways to modulate synaptic plasticity. Neurochemistry International. 2010;**57**(4):440-445. Available from: http://dx.doi.org/10.1016/j.neuint.2010.02.013

[21] Müller MLTM, Albin RL, Kotagal V, Koeppe RA, Scott PJH, Frey KA, et al. Thalamic cholinergic innervation and postural sensory integration function in Parkinson's disease. Brain. 2013;**136**(11):3282-3289

[22] Reichardt LF. Neurotrophin-regulated signalling pathways. Philosophical Transactions of the Royal Society B: Biological Sciences. 2006;**361**(1473):1545-1564

[23] Barbacid M. Structural and Functional properties of the TRK family of neurotrophin receptors. Annals of the New York Academy of Sciences. 1995;**766**(1):442-458

[24] Huang EJ, Reichardt LF. Trk receptors: Roles in neuronal signal transduction. Annual Review of Biochemistry. 2003;**72**:609-642

[25] Longo FM, Massa SM. Small-molecule modulation of neurotrophin receptors: A strategy for the treatment of neurological disease. Nature Reviews Drug Discovery. 2013;**12**(7):507-525. Available from: http://dx.doi.org/10.1038/nrd4024

[26] Mufson EJ. SEC and SDG. Cholinergic System. Pharmacology for Dental and Allied Health Sciences. 2017. 49-49 p.

[27] Fred JF. Risk in CNS drug discovery: Focus on treatment of Alzheimer's disease. BMC Neuroscience. 2008;**9**(SUPPL. 3):1-8

[28] Miller G. Drug targeting: Breaking down barriers. Science (80-). 2002;**297**(5584):1116-1118

[29] Xu Y, Yang J, Shang H. Meta-analysis of risk factors for Parkinson's disease dementia. Translational Neurodegeneration. 2016;**5**(1):1-8. Available from: http://dx.doi.org/10.1186/s40035-016-0058-0

[30] Levy G, Schupf N, Tang MX, Cote LJ, Louis ED, Mejia H, et al. Combined effect of age and severity on the risk of dementia in Parkinson's disease. Annals of Neurology. 2002;**51**(6):722-729

[31] Aarsland D, Kvaløy JT, Andersen K, Larsen JP, Tang MX, Lolk A, et al. The effect of age of onset of PD on risk of dementia. Journal of Neurology. 2007;**254**(1):38-45

[32] Levy G. The relationship of Parkinson disease with aging. Archives of Neurology. 2007;**64**(9):1242-1246

[33] Bohnen NI, Müller MLTM. In vivo neurochemical imaging of olfactory dysfunction in Parkinson's Disease. Journal of Neural Transmission [Internet]. 2014;**23**(1):571-576. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

[34] Haehner A, Boesveldt S, Berendse HW, Mackay-Sim A, Fleischmann J, Silburn PA, et al. Prevalence of smell loss in Parkinson's disease—A multicenter study. Parkinsonism & Related Disorders. 2009;**15**(7):490-494. Available from: http://dx.doi.org/10.1016/j.parkreldis.2008.12.005

[35] Baba T, Kikuchi A, Hirayama K, Nishio Y, Hosokai Y, Kanno S, et al. Severe olfactory dysfunction is a prodromal symptom of dementia associated with Parkinson's disease: A 3 year longitudinal study. Brain. 2012;**135**(1):161-169

[36] Ruberg M, Ploska A, Javoy-agid F, Agid Y. Muscarinic binding and choline acetyltransferase activity in Parkinsonian subjects with reference to dementia. Brain Research. 1982;**232**:129-139

[37] Kulisevsky J, Pagonbarraga J, Pascual-Sedano B, García-Sánchez C, Gironell A. Prevalence and correlates of neuropsychiatric symptoms in Parkinson's disease without dementia. Movement Disorders. 2008;**23**(13):1889-1896

[38] Fields JA, Tr AI, Stalp LD, Koller WC, Paolo AM. Neuropsychological impairment in Parkinson's disease with and without depression. Archives of Neurology. 1995;**52**:1164-1169

[39] Cicerone KD. Depression, intellectual impairment, and parkinson disease: To the Editor. Neurology. 1982;**32**(4):455-458

[40] Grover S, Somaiya M, Kumar S, Avasthi A. Psychiatric aspects of Parkinson's disease. Journal of Neuroscience in Rural Practice. 2015;**6**(1):65-76

[41] Marion MH, Qurashi M, Marshall G, Foster O. Is REM sleep Behaviour Disorder (RBD) a risk factor of dementia in idiopathic Parkinson's disease? Journal of Neurology. 2008;**255**(2):192-196

[42] Vendette M, Gagnon JF, Décary A, Massicotte-Marquez J, Postuma RB, Doyon J, et al. REM sleep behavior disorder predicts cognitive impairment in Parkinson disease without dementia. Neurology. 2007;**69**(19):1843-1849

[43] Postuma RB, Gagnon JF, Montplaisir J. Cognition in REM sleep behavior disorder—A window into preclinical dementia? Sleep Medicine. 2008;**9**(4):341-342

[44] Kotagal V, Albin RL, Müller MLTM, Koeppe RA, Chervin RD, Frey KA, et al. Symptoms of rapid eye movement sleep behavior disorder are associated with cholinergic denervation in Parkinson disease. Annals of Neurology. 2012;**71**(4):560-568

[45] Postuma RB, Gagnon JF, Montplaisir JY. REM sleep behavior disorder: From dreams to neurodegeneration. Neurobiology of Disease. 2012;**46**(3):553-558. Available from: http://dx.doi.org/10.1016/j.nbd.2011.10.003

[46] Williams DR, Warren JD, Lees AJ. Using the presence of visual hallucinations to differentiate Parkinson's disease from atypical parkinsonism. Journal of Neurology, Neurosurgery, and Psychiatry. 2008;**79**(6):652-655

[47] Fénelon G, Mahieux F, Huon R, Ziégler M. Hallucinations in Parkinson's disease. Prevalence, phenomenology

and risk factors. Brain. 2000;**123**(4):733-745

[48] Aarsland D, Brønnick K, Ehrt U, De Deyn PP, Tekin S, Emre M, et al. Neuropsychiatric symptoms in patients with Parkinson's disease and dementia: Frequency, profile and associated care giver stress. Journal of Neurology, Neurosurgery, and Psychiatry. 2007;**78**(1):36-42

[49] Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sørensen P. Prevalence and characteristics of dementia in Parkinson disease: An 8-year prospective study. Archives of Neurology. 2003;**60**(3):387-392

[50] Harding AJ, Broe GA, Halliday GM. Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe. Brain. 2002;**125**(2):391-403

[51] Perry EK, Kerwin J, Perry RH, Blessed G, Fairbairn AF. Visual hallucinations and the cholinergic system in dementia. Letters. 1989;**1**(1):88

[52] Bosboom JLW, Stoffers D, Wolters EC. Cognitive dysfunction and dementia in Parkinson's disease. Journal of Neural Transmission. 2004;**111**(10-11):1303-1315

[53] Wolters EC, Francot CMJE. Mental dysfunction in Parkinson ' s disease. Parkinsonism & Related Disorders. 1998;**4**:107-112

[54] Lange KW, Robbins TW, Marsden CD, James M, Owen AM, Paup GM. Psychopharmacology L-Dopa withdrawal in Parkinson ' s disease selectively impairs cognitive performance in tests sensitive to frontal lobe dysfunction. Psychopharmacology. 1992;**107**:394-404

[55] Scatton B, Javoy-Agid F, Rouquier L, Dubois B, Agid Y. Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. Brain Research. 1983;**275**(2):321-328

[56] Perry EK, Mckeith I, Thompson P, Edwardson JA, Ince P, Blessed G. Topography, extent, and clinical relevance of neurochemical deficits in dementia of Lewy body type, Parkinson' s disease, and Alzheimer' s disease. Annals of the New York Academy of Sciences. 1990:197-202

[57] Perry EK, Curtis M, Dick DJ, Candy JM, Atack JR, Bloxham CA, et al. Cholinergic correlates of cognitive impairment in Parkinson's disease: Comparisons with Alzheimer' s disease. Journal of Neurology, Neurosurgery, and Psychiatry. 1985;**48**:413-421

[58] Pillon B, Dubois B, Duchesne N, Masson H. Acute and long-term administration of anticholinergics in Parkinson's disease: Specific effects on the subcortico-frontal syndrome. Brain and Cognition. 1999;**313**:289-313

[59] Harding AJ, Halliday GM. Cortical Lewy body pathology in the diagnosis of dementia. Acta Neuropathologica. 2001;**102**:355-363

[60] Counts SE, Mufson EJ. The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. Journal of Neuropathology and Experimental Neurology. 2005;**64**(4):263-272

[61] Gibbs RB, Pfaff DW. In situ hybridization detection of trka mRNA in brain: Distribution, colocalization with p75NGFR and up-regulation by nerve growth factor. The Journal of Comparative Neurology. 1994;**341**(3):324-339

[62] Holtzman DM, Li Y, Parada LF, Kinsman S, Chen CK, Valletta JS, et al. p140trk mRNA marks NGF-responsive forebrain neurons: Evidence that trk gene expression is induced by NGF. Neuron. 1992;**9**(3):465-478

[63] Roux PP, Bhakar AL, Kennedy TE, Barker PA. The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent Pathway. The Journal of Biological Chemistry. 2001;**276**(25):23097-23104

[64] Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhäuser N, Böhm-Matthaei R, Baeuerle PA, et al. Selective activation of NF-κB by nerve growth factor through the neurotrophin receptor p75. Science (80-). 1996;**272**(5261):542-545

[65] Volonte C, Angelastro JM, Greene LA. Association of protein kinases ERK1 and ERK2 with p75 nerve growth factor receptors. The Journal of Biological Chemistry. 1993;**268**(28):21410-21415

[66] Sofroniew MV, Howe WC Mobley CL. Nerve growth factor signaling, neuroprotection, and neural repair. Annual Review of Neuroscience. 2001;**24**(5):1217-1281

[67] Bothwell M. Functional interactions of neurotrophins and neurotrophin receptors. Annual Review of Neuroscience. 1995;**18**:223-253

[68] Jang SW, Okada M, Sayeed I, Xiao G, Stein D, Jin P, et al. Gambogic amide, a selective agonist for TrkA receptor that possesses robust neurotrophic activity, prevents neuronal cell death. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(41):16329-16334

[69] Nguyen N, Lee SB, Lee YS, Lee KH, Ahn JY. Neuroprotection by NGF and BDNF against neurotoxin-exerted apoptotic death in neural stem cells are mediated through TRK receptors, activating PI3-kinase and MAPK pathways. Neurochemical Research. 2009;**34**(5):942-951

[70] Xing J, David D, MEG G. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science (80-). 1996;**273**(August):959-963

[71] Vaillant AR, Mazzoni I, Tudan C, Boudreau M, Kaplan DR, Miller FD. 3-Kinase – Akt pathway to synergistically regulate neuronal survival. Cell. 1999;**146**(5):955-966

[72] Holgado-Madruga M, Moscatello DK, Emlet DR, Dieterich R, Wong AJ. Grb2-associated binder-1 mediates phosphatidylinositol 3-kinase activation and the promotion of cell survival by nerve growth factor. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**(23):12419-12424

[73] Yamada M, Ohnishi H, Sano SI, Nakatani A, Ikeuchi T, Hatanaka H. Insulin receptor substrate (IRS)-1 and IRS-2 are tyrosine-phosphorylated and associated with phosphatidylinositol 3-kinase in response to brain-derived neurotrophic factor in cultured cerebral cortical neurons. The Journal of Biological Chemistry. 1997;**272**(48):30334-30339

[74] Shi Z-Q, Yu D-H, Park M, Marshall M, Feng G-S. Molecular mechanism for the Shp-2 tyrosine phosphatase function in promoting growth factor stimulation of Erk activity. Molecular and Cellular Biology. 2000;**20**(5):1526-1536

[75] Yamada M, Ohnishi H, Sano SI, Araki T, Nakatani A, Ikeuchi T, et al. Brain-derived neurotrophic factor stimulates interactions of Shp2 with phosphatidylinositol 3-kinase and Grb2 in cultured cerebral cortical neurons. Journal of Neurochemistry. 1999;**73**(1):41-49

[76] Corbit KC, Soh J-W, Yoshida K, Eves EM, Weinstein IB, Rosner MR. Different protein kinase C isoforms

determine growth factor specificity in neuronal cells. Molecular and Cellular Biology. 2000;**20**(15):5392-5403

[77] Scott SA, Mufson EJ, Weingartner JA, Skau KA, Crutcher KA. Nerve growth factor in Alzheimer's disease: Increased levels throughout the brain coupled with declines in nucleus basalis. The Journal of Neuroscience. 1995;**15**(9):6213-6221

[78] Allen SJ, MacGowan SH, Treanor JJS, Feeney R, Wilcock GK, Dawbarn D. Normal β-NGF content in Alzheimer's disease cerebral cortex and hippocampus. Neuroscience Letters. 1991;**131**(1):135-139

[79] Crutcher KA, Scott SA, Liang S, Everson WV, Weingartner J. Detection of NGF-like activity in human brain tissue: Increased levels in Alzheimer's disease. The Journal of Neuroscience. 1993;**13**(6):2540-2550

[80] Cooper JD, Salehi A, Delcroix JD, Howe CL, Belichenko PV, Chua-Couzens J, et al. Failed retrograde transport of NGF in a mouse model of Down's syndrome: Reversal of cholinergic neurodegenerative phenotypes following NGF infusion. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(18):10439-10444

[81] De Lacalle S, Cooper JD, Svendsen CN, Dunnett SB, Sofroniew MV. Reduced retrograde labelling with fluorescent tracer accompanies neuronal atrophy of basal forebrain cholinergic neurons in aged rats. Neuroscience. 1996;**75**(1):19-27

[82] Chu Y, Cochran EJ, Bennett DA, Mufson EJ, Kordower JH. Down-regulation of trkA mRNA within nucleus basalis neurons in individuals with mild cognitive impairment and Alzheimer's disease. The Journal of Comparative Neurology. 2001;**437**(3):296-307

[83] Hartikka J, Hefti F. Comparison of nerve growth factor's effects on development of septum, striatum, and nucleus basalis cholinergic neurons in vitro. Journal of Neuroscience Research. 1988;**21**(2-4):352-364

[84] Hatanaka H, Nihonmatsu I, Tsukui H. Nerve growth factor promotes survival of cultured magnocellular cholinergic neurons from nucleus basalis of Meynert in postnatal rats. Neuroscience Letters. 1988;**90**(1-2):63-68

[85] Humpel C, Weis C. Nerve growth factor and cholinergic CNS neurons studied in organotypic brain slices. 1990

[86] Williams LR, Varon S, Peterson GM, Wictorin K, Fischer W, Bjorklund A, et al. Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. Proceedings of the National Academy of Sciences of the United States of America. 1986;**83**(23):9231-9235

[87] Capsoni S, Ugolini G, Comparini A, Ruberti F, Berardi N, Cattaneo A. Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(12):6826-6831

[88] Burgos I, Claudio Cuello A, Liberini P, Pioro E, Masliah E. NGF-mediated synaptic sprouting in the cerebral cortex of lesioned primate brain. Brain Research. 1995;**692**(1-2):154-160

[89] Kordower JH, Winn SR, Liu YT, Mufson EJ, Sladek JR, Hammang JP, et al. The aged monkey basal forebrain: Rescue and sprouting of axotomized basal forebrain neurons after grafts of encapsulated cells secreting human nerve growth factor. Proceedings of the National Academy of Sciences of the

United States of America. 1994;**91**(23):10898-10902

[90] Fischer W, Wictorin K, Björklund A, Williams LR, Varon S, Gage FH. Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. Nature. 1987;**329**(6134):65-68

[91] Bäckman C, Rose GM, Hoffer BJ, Henry MA, Bartus RT, Friden P, et al. Systemic administration of a nerve growth factor conjugate reverses age-related cognitive dysfunction and prevents cholinergic neuron atrophy. The Journal of Neuroscience. 1996;**16**(17):5437-5442

[92] Lin B, Pirrung MC, Deng L, Li Z, Liu Y, Webster NJG. Neuroprotection by small molecule activators of the nerve growth factor receptor. The Journal of Pharmacology and Experimental Therapeutics. 2007;**322**(1):59-69

[93] Zhang B, Salituro G, Szalkowski D, Li Z, Zhang Y, Royo I, et al. Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. Science (80-). 1999;**284**(5416):974-977

[94] Tang X, Jang S, Okada M, Chan C, Feng Y, Liu Y, et al. The antidepressant amitriptyline is a TrkA and TrkB receptor agonist that promotes TrkA/TrkB heterodimerization and has potent neurotrophic activity. Chemistry & Biology. 2010;**16**(6):644-656

[95] Scarpi D, Cirelli D, Matrone C, Castronovo G, Rosini P, Occhiato EG, et al. Low molecular weight, non-peptidic agonists of TrkA receptor with NGF-mimetic activity. Cell Death and Disease. 2012;**3**(7):e339-e313. Available from: http://dx.doi.org/10.1038/cddis.2012.80