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BDNF in the Aged Brain: Translational Implications for Parkinson's Disease

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Abstract

Brain Derived Neurotrophic Factor (BDNF) is a member of the neurotrophin family of secreted growth factors. BDNF signaling is known to exert both chronic, pro-survival effects related to gene expression and protein synthesis ("canonical signaling"), and acute effects as a modulator of neurotransmission ("non-canonical signaling"). BDNF has received a great deal of attention for its role in neurodegenerative diseases including Huntington's Disease (HD), Alzheimer's Disease (AD), and Parkinson's Disease (PD) and has been extensively reviewed elsewhere in this regard (e.g., [1–6]). However aging-related changes in BDNF function and expression have been studied only rarely, with the majority of studies characterizing changes in structures such as the hippocampus and neocortex. In this review, we attempt to briefly summarize the extent of the existing literature on age-related BDNF changes, and discuss the relevance of these changes as a factor potentially impacting therapeutics in aged parkinsonian subjects.

Keywords

Brain derived neurotrophic factor; Parkinson's disease; Aging; Therapeutics

Introduction

Neurotrophins are important regulators of neuronal survival, development, maintenance, and plasticity. Te mammalian neurotrophin family consists of four proteins: Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT3), and Neurotrophin-4/5 (NT4/5) [7]. Of these, BDNF was the second to be discovered, after the groundbreaking discovery of NGF in the 1950s by Rita Levi-Montalcini, Stanley Cohen, and Viktor Hamburger, for which Levi-Montalcini and Cohen were awarded the Nobel Prize in Physiology or Medicine in 1986 ([8–13], but see [14] for review). In 1982, Yves-Alain Barde and colleagues isolated BDNF for the first time from pig brain [15]. In the 35 years since its discovery, BDNF has been intensively studied, as evidenced by the thousands of publications currently available on this particular neurotrophic factor.

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Despite the plethora of existing BDNF literature, potential aging-induced changes in BDNF expression and signaling have been studied only sparingly. Here, we briefly discuss age-related changes in BDNF and its association with Parkinson's disease (PD), a neurodegenerative disease whose primary risk factor is advanced age. We focus our discussion on putative age-related changes in BDNF-tyrosine receptor kinase B (trkB) signaling dynamics, protein/ messenger RNA (mRNA) expression within the striatum, and implications for therapeutic approaches for the treatment of PD.

BDNF: A Well-Studied Molecule

The BDNF gene: many transcripts, one protein

Te rat *BDNF* gene was first described in 1993 by Timmusk *et al.* [16], whose findings have been expanded in more recent studies ([17–19], see [1] for detailed review). Briefly, the rodent *BDNF* gene contains eight untranslated *5'* exons (exons I–VIII), the majority of which are linked to separate, distinct promoters, and one protein-coding exon (exon IX) [19]. In humans, the *BDNF* gene is even more complex, with 11 distinct exons controlled by nine promoters [20]. Due to multiple promoters, alternative splicing, and polyadenylation, the various BDNF exons produce a large variety of BDNF transcripts, which control tissue-, development-, and stimulus-specific BDNF protein expression [16, 19, 21]. Remarkably, each transcript encodes the same BDNF protein [19, 22]. Of relevance to neurodegenerative disease, the structure of human BDNF is closely related to rat and mouse, and all exons defined in humans are also expressed in mouse and rat, except for human exons VIIB and VIII [23].

Neurotrophin secretion and activation of downstream signaling pathways

All neurotrophins, including BDNF, are initially synthesized as pre-pro-neurotrophin precursors [24]. The pre-mRNA sequence, which gives rise to the signal peptide within the final protein product, directs newly-generated neurotrophins to ribosomes located in the endoplasmic reticulum (ER) where the pre-sequence is cleaved and the initial protein is translated. The resulting pro-neurotrophins accumulate in the trans-golgi network (TGN) and are sorted into secretory vesicles [24]. The pro-sequence is typically cleaved from the final protein product by protein convertases within the TGN or secretory vesicles [24], though uncleaved pro-neurotrophins are also released from cells and are known to activate the p75 neurotrophin receptor ($p75^{NTR}$), initiating apoptosis [25, 26]. However, following the discovery of the Vps10 sortilin family member SorCS2 (Sortilin-related CNS expressed 2) as a co-receptor for p75NTR and trkB [27], it has become apparent that pro-neurotrophins acting *via* the $p75^{NTR}$/sortilin or p75NTR/SorCS2 receptor complex may also regulate signaling pathways that in turn regulate processes such as synaptic activity and pruning, and network reorganization [28]. Furthermore, pro-BDNF is thought to be a key regulator of neuronal circuitry and plasticity, especially in early postnatal periods, as it has been shown in the hippocampus to negatively regulate dendritic complexity, impair long-term potentiation, and enhance long-term depression in the hippocampus [29]. On the other hand, the mature BDNF protein (mBDNF) activates a member of the tyrosine receptor kinase family, trkB, through which it also impacts neuronal morphology and synaptic plasticity

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albeit through mechanisms distinct from pro-BDNF [29], as well as the pro-survival and protein synthesis signaling pathways typically associated with BDNF activity.

BDNF is released from presynaptic terminals *via* two distinct pathways mediated by separate populations of secretory vesicles. When pro-BDNF is cleaved in the TGN, the mature protein is sorted into small secretory granules of the *constitutive* secretion pathway, where it is transported to the cell membrane and released in a stimulus-independent manner [24]. However, when sorted into larger vesicles of the *regulated* secretion pathway, pro-BDNF is cleaved within the vesicles and the mature protein is released in a strictly calcium-dependent manner [24]. Importantly, BDNF is almost exclusively sorted to and released from the regulated pathway. Indeed, Brigadski and colleagues showed that BDNF was preferentially targeted to the regulated pathway in 98% of cultured hippocampal neurons, and that fusing the usually constitutively-released NT-4 to the BDNF pre-pro-sequence more efficiently targeted NT-4 to the regulated pathway [30]. Regulated release of BDNF occurs following neuronal stimulation, for example, after high-frequency electrical stimulation or potassium-induced depolarization. This was demonstrated for the first time in early experiments using cultured hippocampal neurons [31, 32] and confirmed in later studies (e.g., [33–37]). In addition, it is relevant to note that the activity-dependent regulation of BDNF transcription is controlled primarily through exons I and IV of the *BDNF* gene (human *BDNF* exon IV is equivalent to exon III in rat) [25, 38].

Trk receptors are composed of intracellular tyrosine kinase domains and extracellular Immunoglobulin G (IgG) domains that bind ligands. Synaptically released BDNF dimerizes and binds to trkB, which initiates trkB dimerization and autophosphorylation of intracellular tyrosine residues [39]. Subsequently, three major intracellular signaling cascades are known to be activated: the Phospholipase Cγ (PLCγ), Phosphatidylinositol 3-Kinase (PI3K), and Extracellular signal-Regulated Kinase (ERK) pathways (see [39–41] for detailed reviews). These pathways ultimately regulate gene expression and protein translation of their downstream targets. Furthermore, by activating these signaling cascades, BDNF also enhances synaptic transmission and membrane excitability. For example, at glutamatergic synapses such as those formed between cortical afferents to the striatum and striatal medium spiny neurons (MSNs), BDNF-trkB signaling stimulates an increase in the number of docked vesicles within active zones at synapses, and also alters activation kinetics of N-Methyl-D-Aspartate (NMDA) receptors and inhibitory Gamma-Amino Butyric Acid (GABA) receptors in the postsynaptic membrane (see [42] for review). BDNF also regulates actin dynamics and spine remodeling *via* activation of specific kinase-mediated signaling cascades, including TIAM1/Rac1 and ROCK/ LIMK 1 pathways (e.g., [43, 44]). On the other hand, pro-BDNF interaction with p75^{NTR} activates three additional signaling pathways that result in the activation of NF-κB, Jun kinase, or RhoA, which in turn activate pro-survival genes, pro-apoptotic genes, or growth cone motility processes, respectively [41].

BDNF signaling is made more complex by co-receptor binding. For example, the binding of BDNF to trkB can be enhanced by trkB association with the p75^{NTR} receptor [39], and p75^{NTR} association with the sortilin receptors appears to mediate pro-apoptotic actions of p75^{NTR} receptor activation [41]. Interestingly, the Vps10 sortilin family member SorCS2 was recently identified as an additional co-receptor for both trkB and p75NTR, and was

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shown to mediate BDNF-dependent plasticity, and bind to trkB in an activity-dependent manner [27]. It is clear that BDNF transcription, translation, secretion, and signaling are mediated via exquisitely complex mechanisms that produce a variety of different outcomes, from promoting cell apoptosis to controlling spine dynamics and synapse remodeling. This complexity and the divergent functional properties of BDNF present a unique opportunity for BDNF production and/ or signaling processes to become aberrant and accordingly have devastating consequences in association with its dysfunction, a topic of considerable interest in neurodegenerative disease.

BDNF and Parkinson's Disease

Interest in a role for BDNF in PD stems from 1) its documented effects in promoting the survival and function of Substantia Nigra (SN) Dopamine (DA) neurons, and 2) its structural and functional influence on striatal MSNs, the principal target neurons of SN DA afferents. Indeed, more than 20 years of research demonstrates that BDNF is a critical factor for the viability of SN DA neurons. Specifically, BDNF supports the survival of SN neurons *in vitro* and is protective against multiple neurotoxin insults *in vitro* and *in vivo* [45–49]. Further, haplo insufficiency of the BDNF receptor trkB in mice is associated with progressive degeneration of SN DA neurons, and in association with aging, results in excessive accumulation of alpha-synuclein in remaining neurons [50]. Not only is BDNF important for nigral DA neuron survival, it is also important for the maturation and function of these neurons as evidenced by its ability to stimulate motor behavior, electrical activity of SN neurons, and DA turnover in the nigrostriatal system [46, 48, 51].

In the striatum BDNF supports survival of the immature MSNs, promotes maturation of these neurons, and facilitates establishment of striatal connections during brain development [52, 53]. BDNF also is known to play an important role in dendritic spine dynamics and actin remodeling of MSNs in the adult brain, sculpting the structure and function of synapses (e.g., [54–57]). It also is implicated as a critical factor controlling dendritic spine density in many brain regions [40, 46, 58–60], the importance of which is detailed below. Striatal BDNF is primarily derived from cortical afferents that project to the striatum, but also from ascending SN DA afferents that release BDNF in an activity-dependent manner. Upon release, the interaction of BDNF with trkB receptors [40, 44, 61, 62] located on striatal MSNs initiates signaling pathways involved in dendritic spine and synapse dynamics.

In PD, nigral DA neurons that send their afferent axons to the striatum degenerate, leading to detrimental structural changes in the striatum (e.g., decreased spine density, altered synaptic connections, etc., as described in [54–58]) and both motor and non-motor symptoms. To date, there is strong evidence linking BDNF to PD pathology in the SN in individuals with PD. For instance, postmortem analysis of PD brains reveals a significant decrease in BDNF mRNA and protein in neurons of the SN pars compacta [59, 60] and serum levels of BDNF correlate with the severity of motor symptoms [61], findings that could suggest a link between decreased BDNF and SN DA neuron death in PD. In addition, there is a compelling attribute of altered BDNF signaling that may pose a potential risk factor in the aged parkinsonian population in response to a variety of therapeutics. Specifically, a common Single Nucleotide Variant (SNV) in the *BDNF* gene that encodes the protein BDNF is

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present in the human population (rs6265; Val66Met) [62, 63]. In this SNV, there is a Methionine (Met) substitution for Valine (Val) at codon 66 (Val66Met). The Met allele of the *BDNF* SNV rs6265 has a prevalence of 40.6% in the general population (Major/Minor or Val/Met = 35.4%, Minor/Minor or Met/Met = 5.2%, allelic frequency assuming Hardy-Weinberg) [64]. Both the heterozygous major allele (Val/ Met) and homozygous minor allele (Met/Met) of the *BDNF* SNV result in decreased activity-dependent release of BDNF by disruption of packaging into secretory vesicles, whereas constitutive levels of BDNF remain unaffected [65]. Although there are multiple SNVs in the *BDNF* gene, rs6265 is relatively unique in that this SNV is in the BDNF coding region, has a direct and well-studied impact on BDNF protein function, and is relatively prevalent within the human population. As discussed above, the majority of BDNF in the adult brain is released from neurons *via* the regulated secretory pathway; therefore, the impact of the *BDNF* SNV rs6265 leads to a significant decrease in available BDNF [65] in approximately 40% of the human population. While the rs6265 SNV does not appear to impact the clinical features of PD [66] and seems unlikely to play a major role in PD pathogenesis ([67–69]; however see [70]), it remains controversial whether subjects with this *BDNF* SNV risk allele are more susceptible to induction of levodopa-induced dyskinesias [71, 72]. The full nature and extent of the influence of the rs6265 SNV on PD therapeutics, the potential decrease in BDNF signaling in aged parkinsonian brain, and/or the interaction of these factors remains uncertain. However, we contend that these factors warrant further investigation based on the discussion presented in the remainder of this review.

BDNF, Aging, and the Parkinsonian Striatum

Aging is the primary risk factor for PD, yet the impact of aging on therapeutic responses in PD, especially in preclinical studies, has received sparse attention (for review [73, 74]). It is striking that what is known about the interaction of aging with PD and DA depletion strongly implicates aging as a significant factor in limitations of therapeutics in PD [73]. There are abundant data demonstrating that within the aged, DA depleted striatum, abundant pathology develops. For example, it is well documented in postmortem PD striatum that there are significant alterations of the most prominent neuron population, the MSNs [58, 75, 76], with not only a significant reduction in the length of their dendrites compared to age-matched controls, but also the remaining dendrites often show few to no spines [58].

Research over the past decade has begun to elucidate how and why these pathological changes in the MSNs of the parkinsonian striatum can impact PD therapeutics. Just briefly, the striatal MSNs have an extensive dendritic arbor that is studded with numerous dendritic spines. These spines are critical cytoarchitectural units that receive massive cortical glutamatergic motor information *via* afferent terminals that synapse onto the heads of the spines. The SN DA afferent terminals make synaptic contact onto the necks of the same spines as the cortical afferents, serving to modulate cortical input to the MSNs (for review [77–79]). Multiple studies demonstrate that loss of dendritic spines on striatal MSNs associated with DA depletion is associated with induction of levodopa and graft-induced dyskinesias (for review see [80]). Further, inferior behavioral recovery in aged parkinsonian rats is associated with evidence of inferior synaptic integration between graft and host [81],

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and preserving dendritic spine density on striatal MSNs in the parkinsonian striatum significantly improves therapeutic response to DA neuron replacement strategies [78].

As discussed above, BDNF is a critical factor controlling dendritic spine density [40, 46, 58–60] and sculpting the structure and function of synapses (e.g., [56, 57]). As such, an environment of compromised BDNF signaling would be expected to negatively impact the integrity and/or function of these critical striatal cytoarchitectural elements, impact basal ganglia function, and accordingly impact responsiveness of individuals to therapeutic interventions. What is known about BDNF signaling in the aged, parkinsonian striatum? Existing evidence generally suggests that there is *no* change in BDNF mRNA or protein expression in brain across the lifespan; however, results appear to differ depending on the brain structure and the rat strain studied (see Table 1) [82]. In 1993, Lapchak and colleagues were the first to examine changes in trkB and BDNF mRNA expression in the aging rat brain [83]. Using northern blot and in situ hybridization techniques, Lapchak *et al.* [83] showed that the prevalence and regional distribution of BDNF and trkB mRNA within the hippocampus did not change with age. Similarly, Narisawa-Saito *et al.* [84] showed several years later that BDNF protein expression did not change with age in the hippocampus and frontal cortex of male Fischer 344 (F344) rats, though BDNF mRNA was significantly increased in the aged hippocampus. Croll and colleagues [85] later examined trkB and BDNF expression in various regions of the aging brain of male Sprague Dawley rats and of the multiple structures examined, BDNF protein was significantly decreased only in the midbrain of aged rats compared to young rats, and BDNF mRNA was significantly decreased only in the pons. Additional studies in both rats and human postmortem tissue identified various different, sometimes contradictory, age-related changes in trkB and BDNF expression with age (e.g., [86]). Thus, while there appears to be a the lack of consensus on age-related change in BDNF expression, which appears to vary between brain regions, an age-related decrease in trkB expression has been more consistently reported regardless of the structure examined (Table 1).

Returning to the question of whether there is a change of BDNF signaling in the aged striatum that could impact individuals with PD, it is noteworthy that in our review of the literature only a single study by Croll and colleagues [85] has assessed changes in BDNF and trkB expression within the *aged* striatum, where no significant changes were found. Although current evidence generally supports the idea that BDNF expression in the brain does *not* change with age (e.g., [85], but see Table 1 for additional details), studies have shown impaired ability of the aged brain to produce BDNF in response to stress [87, 88] and reduced expression of the regulators of BDNF transcriptional activation including neuronal PAS domain protein 4 (*Npas4*) and cyclic Adenosine Mono Phosphate (cAMP) responsive element-binding protein (*Creb*) compared to the young brain [86]. Taken together, these data suggest a diminished capacity of the aged brain to transcribe, release, and/or respond to BDNF. Continued systematic investigations are warranted to fully understand the impact of aging in BDNF signaling, and its implication for therapeutics for multiple neurodegenerative conditions.

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Implications of Dysfunctional BDNF for Therapeutics in PD

Within the striatum two main subsets of MSNs exist: those of the direct pathway (dMSNs) and those of the indirect pathway (iMSNs). Though morphologically similar, these two cell populations express distinct receptors and proteins (e.g., dMSNs express D1 DA receptors and iMSNs express D2 DA receptors), and have different projection pathways and physiological functions (e.g., [89]). They also appear to be differentially affected by DA depletion and levodopa treatment in PD (reviewed in [80]). In addition, data from a recent study by Gagnon and colleagues [90] indicate that a smaller population of MSNs exhibiting both D1 and D2 DA receptors (about 1.9% in the dorsal striatum) within the mouse striatum are morphologically distinct, and are impacted by DA depletion in a manner that is distinct from D1 MSNs or D2 MSNs. Interestingly, previous studies have demonstrated that in the nucleus accumbens (NAc) [91], in tissue containing both NAc and striatum used for fluorescence activated cell sorting [92], and in striatum during development [53], the majority of trkB mRNA and protein is in iMSNs. Further, when trkB is selectively deleted in striatal MSNs, only iMSNs are adversely affected, as indicated by a 63–80% loss of these neurons following trkB deletion, while dMSNs remain unaffected [53]. Also, iMSNs have been shown to degenerate first in Huntington's disease (HD), and are more affected by BDNF down regulation in a heterozygous BDNF transgenic mouse model [93, 94]. The work of Baydyuk *et al.* [52, 53] suggests a causative role for differential trkB expression in MSN subtypes in vulnerability to development and disease-associated decline in MSN survival. Recent data from our laboratory support the hypothesis that overall striatal trkB mRNA expression measured using *in situ* hybridization declines with advanced age, and reveal for the first time that this age-related effect is restricted to iMSNs (Figure 1). Of note, this is the population of MSNs that also shows loss of dendritic spines in response to DA depletion ([54]; reviewed in [80]). The importance of this phenomenon for the pathophysiology of PD remains to be elucidated. Additional evidence from our laboratory demonstrates that dendritic spine density on striatal MSNs declines in rats in association with advanced age (Figure 2).

Similar to the sparse data on the impact of age on *striatal* BDNF signaling, there is to the best of our knowledge but a single study examining the impact of advanced age on *striatal* dendritic spine density. This is notable given the abundance of data demonstrating that neurons within the hippocampus, cortex, and NAc show aging-related loss of dendritic spines [95–99]. In the single study examining the impact of advanced age on dendritic spine density in the striatum, Levine and colleagues [100] directly compared changes in striatal MSN dendritic spine density between young and aged cats. They observed a 49% reduction in striatal dendritic spine density of aged (15–18 years old) cats compared to young (1–3 years old) cats. Our recent data (Figure 2) corroborate a loss of dendritic spines in the aged striatum of rats. Further, while spine loss related to DA depletion can be prevented pharmacologically with CaV1.3 calcium channel antagonists [54, 78], our data demonstrate for the first time that *aging-related spine loss is NOT* compensated for through this mechanism (Figure 2). Due to our observation that trkB mRNA was significantly decreased only in iMSNs of aged rats (Figure 1) and further evidence indicating that MSN spine density is decreased in BDNF knock-out animals [101–103], it is reasonable to suggest that

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age-related loss of spine density would be more significantly reduced in iMSNs than dMSNs. Considering that iMSNs and dMSNs have been shown to respond differently to DA depletion and levodopa treatment in animal models of PD (e.g., [104, 105] but also see [80]), there is reason to infer that differential age-related changes in trkB expression may contribute to this phenomenon. A thorough characterization of this phenomenon is needed in the important preclinical rat model to understand 1) which MSN population is affected in the aged striatum, 2) whether a decrease in BDNF signaling, specifically the age-related decrease in trkB, underlies this age-related striatal spine loss, and 3) what the consequences of striatal synaptopathology secondary to dendritic spine loss are in clinical medicine.

Summary

In PD, for which the primary risk factor is aging, there is progressive loss of dendritic spines on MSNs. Dopamine depletion has a well-established link to striatal spine retraction (e.g., [54]; reviewed in [78]). However, data from our lab and Levine *et al.* [100] demonstrate that there also is loss of striatal spine density in association with advanced age, and our data reveal that the yet unknown mechanism is independent of CaV1.3 calcium channel activity. There is strong rationale to suggest that impaired BDNF signaling exists in the aged striatum, and that this may be further exacerbated in individuals with the rs6265 *BDNF* SNV. The critical nature of BDNF signaling in the nigrostriatal system, particularly its role in maintaining dendritic spine density and synapse function, would predict that impaired BDNF signaling in association with aging and/or the rs6265 SNV would significantly and negatively impact plasticity and function of the basal ganglia in health and disease. As discussed above, the loss of dendritic spines is associated with waning efficacy of levodopa and dyskinesia side-effect development, as well as abnormal “re-wiring” in experimental regenerative approaches of grafting of embryonic DA neurons into the parkinsonian striatum [58, 77–79, 81, 106]. Despite the practical issues associated with aging animals in preclinical research, there remains a great need for exploration of the impact of aging on BDNF signaling in the nigrostriatal system and its impact on mainstream pharmacotherapies as well as experimental regenerative therapies in PD that continue to be of significant interest in the field. Continued investigations will be critical to the development of more efficacious therapeutics for the treatment of aging-related neurodegenerative diseases affecting the striatum, including PD.

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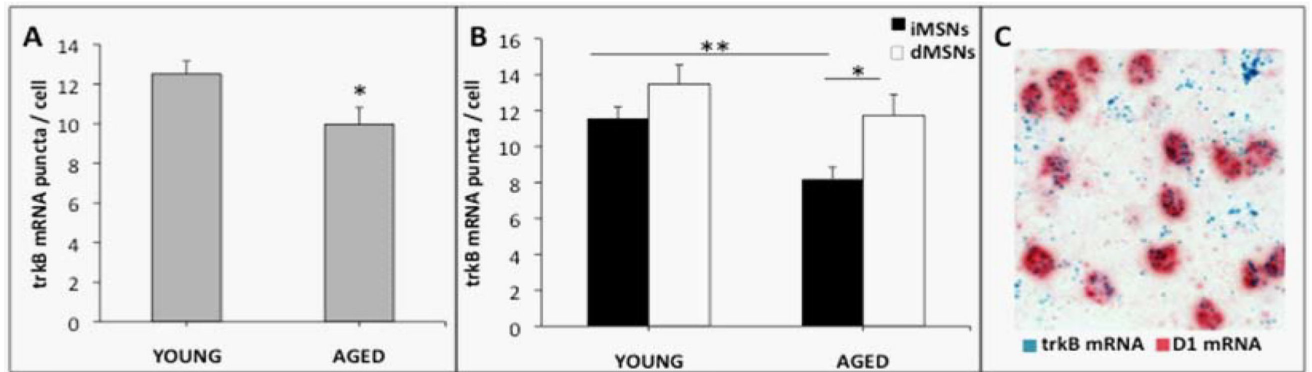

Figure 1.

Dual-label RNAscope® *in situ* hybridization shows a selective loss of trkB mRNA in D2 receptor containing iMSNs in the aged striatum. A) Total trkB mRNA expression is significantly reduced in MSNs of the dorsolateral striatum in aged compared to young Fischer 344 rats. B) TrkB mRNA decreased significantly with age in indirect pathway MSNs containing D2 receptor mRNA, but not direct pathway MSNs containing D1 receptor mRNA. C) Representative 60x brightfield microscopy image of dual-labeled striatal D1 DA receptor and trkB mRNA. Experimental Details: Young (4 m.o.) and aged (20 m.o.) F344 rats were perfused with heparinized saline followed by 1.5% paraformaldehyde. Brains were post-fxed in 4% paraformaldehyde and cryoprotected before sectioning on a microtome. 20-μm sections were dual-labeled for trkB and either D1 or D2 dopamine receptor RNA with the RNAscope® 2.5 Duplex manual assay for *in situ* hybridization. Images of the dorsolateral striatum were taken at 60x magnification and the number of blue trkB mRNA puncta per cell was manually quantified using ImageJ software. Four images per animal were used for analysis of each dopamine receptor probe.

*$p < 0.05$, **$p < 0.01$; Young N = 5, Aged N = 5.

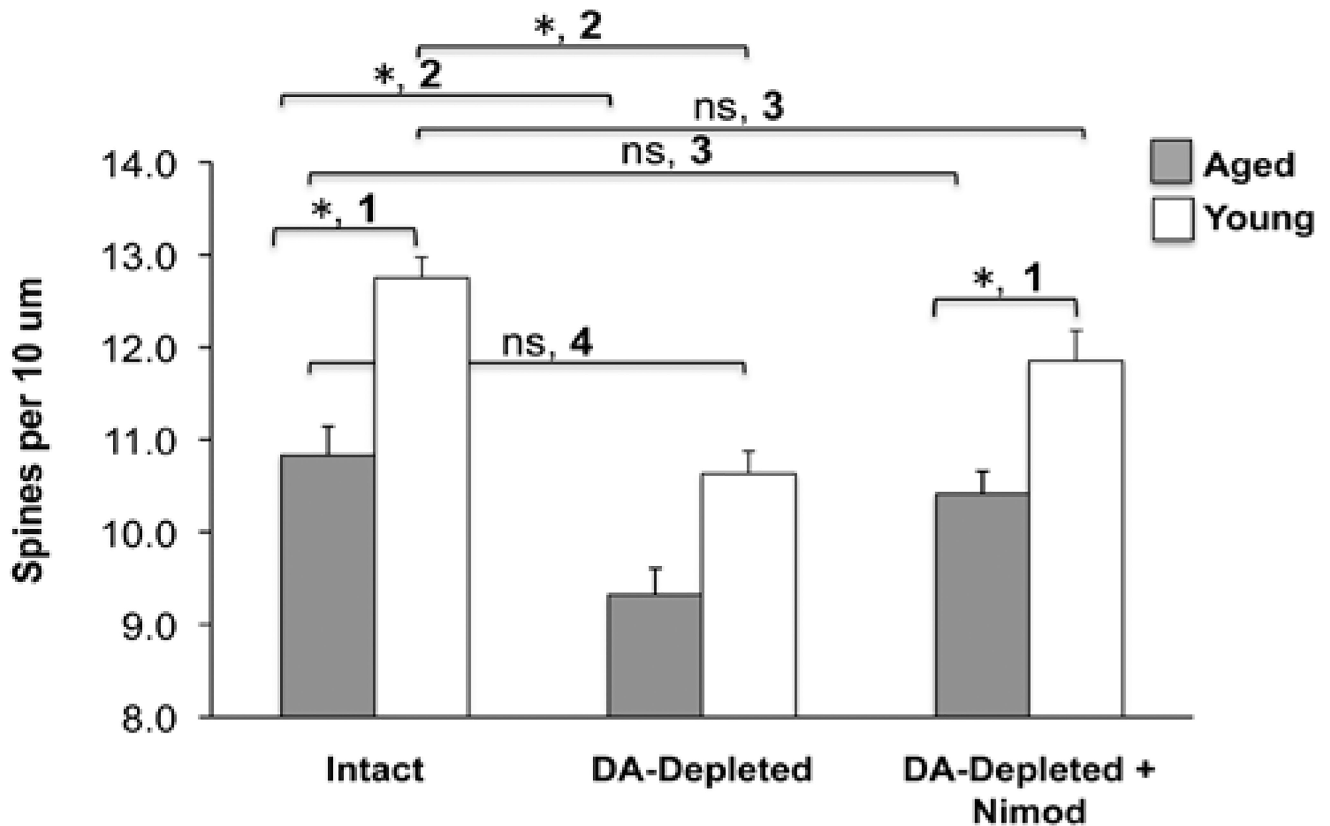

Figure 2.

Dendritic spine dynamics in young and aged rats. '1': Young rats (3mo) have significantly more spines compared to aged rats (20mo). '2, 3': DA-depletion related spine loss ('2') is reversible with CaV1.3 calcium channel antagonism ('3'). '4': The aged "normal" striatum has the same density of dendritic spines as the young parkinsonian striatum. Experimental Details: Young and aged male Sprague Dawley rats were lesioned with 6OHDA. One cohort from each age group received vehicle pellets and a second received slow-release subcutaneous pellets containing the CaV1.3 calcium channel inhibitor nimodipine. Pellets were implanted 10 days after lesion, a time when striatal MSNs show significant dendritic spine loss. Rats were sacrificed 3wks after pellet implantation for spine density analysis with Golgi impregnation techniques. MSNs were first visualized at low-power magnification and selected for reconstruction based on the quality of Golgi–Cox impregnation. Neurons selected for analysis were required to have at least four primary dendrites that radiated from the soma in an arc encompassing 360° and to not have excessive dendritic overlap with neighboring cells. Spine density was quantified on three to six dendrites in N=3 young intact, N=3 DA-depleted and N=4 DA-depleted+Nimod rats, and or 6, 5, or 8 N=6 aged intact, N=5 DA-depleted and N=8 DA-depleted+Nimod rats.

* = significant difference $p<0.05$; ns = not significant; one-way ANOVA with post hoc Tukey's multiple comparisons test. Nimod = Nimodipine

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Table 1

Summary of studies examining age-related changes in BDNF and trkB expression.

Publication	Subjects	Region Studied	TrkB studied?	BDNF studied?	Change with Age
Lapchak *et al.* (1993)	Male SD & F344 rats	Hippocampus	mRNA	mRNA	No change
Narisawa –Saito *et al.* (1996)	Male F344 rats	Hippocampus Frontal Cortex		Protein/ mRNA	↑BDNF mRNA in hippocampus
					No change in BDNF protein in either structure
Croll *et al.* (1998)	Male SD rats	various regions throughout brain, including striatum	mRNA	Protein/ mRNA	↓BDNF protein in midbrain only
					↓BDNF mRNA in pons only
					↓trkB Mrna in various regions
Romankzyk *et al.* (2002)	Postmortem human tissue	Prefrontal Cortex	mRNA		↓ full-length trkB mRNA with aging in all cortical layers
Webster *et al.* (2002)	Postmortem human tissue	Prefrontal Cortex		mRNA	No change
Silhol *et al.* (2005)	Male SD rats	Hippocampus Hypothalamus	Protein/ mRNA	Protein/ mRNA	↓ full-length trkB protein, no change in trkB Mrna
					No change in BDNF protein or mRNA in either structure
Webster *et al.* (2006)	Postmortem human tissue	Hippocampus Temporal Cortex	mRNA	mRNA	trkB RNA in both structures
					↓ BDNF mRNA in cortex
					No change in BDNF mRNA in hippocampus
Rage *et al.* (2007)	Male SD rats	Pitutary	Protein/ mRNA	Protein/ mRNA	trkB protein
					↑ BDNF mRNA
					No change in BDNF protein or trkB mRNA
Chapman *et al.* (2012)	F344/Brown Norway hybrid rats	Hippocampus		mRNA	↓ BDNF mRNA in CA1 and CA3
					No change in dentate gyrus
Calabrese *et al.* (2013)	Male Wistar Han rats	Hippocampus Prefrontal Cortex	Protein	Protein/ mRNA	↓ BDNF mRNA in both structures
					↓ BDNF protein in both structures
					↓ trkB protein in both structures
Perovic *et al.* (2013)	Wistar rats	Hippocampus Cortex	Protein	Protein/ mRNA	BDNF mRNA in cortex
					↓ BDNF mRNA in hippocampus
					↓ full-length trkB protein in both structures
					No change in BDNF protein

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Publication	Subjects	Region Studied	TrkB studied?	BDNF studied?	Change with Age
Tong *et al.* (2015)	Adult cats	Lateral Geniculate Nucleus	Protein	Protein	BDNF and trkB proteins

SD = Sprague Dawley; F344 = Fischer 344; BDNF = brain-derived neurotrophic factor; trkB = tyrosine receptor kinase B