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EFFECTS OF AGE AND INSULIN-LIKE GROWTH FACTOR-1 ON RAT NEUROTROPHIN RECEPTOR EXPRESSION AFTER NERVE INJURY

T. DAVID LUO, MD[1], **TIMOTHY B. ALTON, MD**[1], **PETER J. APEL, MD, PhD**[1], **JIAOZHONG CAI, BS**[1], **JONATHAN C. BARNWELL, MD**[1], **WILLIAM E. SONNTAG, PhD**[2], **THOMAS L. SMITH, PhD**[1], and **ZHONGYU LI, MD, PhD**[1]

[1]Department of Orthopaedic Surgery, Wake Forest Baptist Medical Center, Medical Center Boulevard, Winston-Salem, North Carolina 27157, USA

[2]Department of Geriatric Medicine, University of Oklahoma Health Science Center, Oklahoma City, Oklahoma, USA

Abstract

Introduction—Neurotrophin receptors, such as $p75^{NTR}$, direct neuronal response to injury. Insulin-like growth factor-1 receptor (IGF-1R) mediates the increase in $p75^{NTR}$ during aging. The aim of this study was to examine the effect of aging and insulin-like growth factor-1 (IGF-1) treatment on recovery after peripheral nerve injury.

Methods—Young and aged rats underwent tibial nerve transection with either local saline or IGF-1 treatment. Neurotrophin receptor mRNA and protein expression were quantified.

Results—Aged rats expressed elevated baseline IGF-1R (34% higher, $P=0.01$) and $p75^{NTR}$ (68% higher, $P<0.01$) compared with young rats. Post-injury, aged animals expressed significantly higher $p75^{NTR}$ levels (68.5% above baseline at 4 weeks). IGF-1 treatment suppressed $p75^{NTR}$ gene expression at 4 weeks (17.2% above baseline, $P=0.002$) post-injury.

Conclusions—Local IGF-1 treatment reverses age-related declines in recovery after peripheral nerve injuries by suppressing $p75^{NTR}$ upregulation and pro-apoptotic complexes. IGF-1 may be considered a viable adjuvant therapy to current treatment modalities.

Keywords

aging; IGF-1; insulin-like growth factor-1; nerve growth factor; nerve regeneration; neurotrophin receptor; peripheral nerve injury

Peripheral nerve injuries (PNIs) are commonly associated with extremity trauma.[1–4] The patient's age at the time of injury critically influences motor and sensory recovery. Aging is prognostic of worse outcomes after nerve injury.[5,6] Studies in animal models have demonstrated that aging slows axonal regeneration and disrupts peripheral nervous system

Correspondence to: Z. Li; zli@wakehealth.edu.

function.[7–10] Impaired recovery from nerve damage in the elderly has been established clearly, but the underlying molecular mechanisms have not been well studied.[11]

Circulating anabolic factors such as insulin-like growth factor-1 (IGF-1) are known to decline with aging, which may contribute to the observed impairments in nerve recovery.[12,13] IGF-1 is a potent trophic factor known to promote neuronal survival and to stimulate angiogenesis and myogenesis.[14–16] Locally delivered IGF-1 increases axonal number, density, and myelination in aged animals and may have therapeutic benefits in recovery from nerve injury.[11,17]

Neurotrophins (NTs) are a family of proteins that include nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/ 5), and brain-derived neurotrophic factor (BDNF).[18,19] These NTs each interact with specific cell surface neurotrophin receptors (NTRs) of the tyrosine kinase receptor family (TrkA, TrkB, and TrkC) to promote neuronal survival and axonal growth.[18,20,21] In addition, NTs and their precursors may bind to p75 neurotrophin receptor ($p75^{NTR}$), a transmembrane glycoprotein, with varying degrees of affinity.[22–25] During aging, cortical motor neurons undergo a parallel increase in $p75^{NTR}$ and decrease in TrkA protein expression, a process mediated by insulin-like growth factor-1 receptor (IGF-1R), as seen in the pathophysiology of Alzheimer disease.[26,27] To date, there has been no *in vivo* confirmation of this process in spinal cord motor neurons.

The role of $p75^{NTR}$ is wide-ranging and has been implicated in neuronal cell death during development and after injury.[28] As a co-receptor, it forms membrane-bound multimeric complexes with Trk as well as sortilin, a sorting protein. Sortilin and $p75^{NTR}$ mRNA and protein are expressed in motor neuron cell bodies.[29,30] They form cross-links on the cell membrane to initiate neuronal apoptotic pathways.[31–33] This interaction increases 5-fold in the presence of pro–nerve growth factor (proNGF), whereas mature NGF selectively binds to $p75^{NTR}$.[34] The aim of this study was to assess the effect of aging on recovery after peripheral nerve injury. Our previous studies have demonstrated that IGF-1 improves nerve regeneration after injury.[35] We hypothesized that aged peripheral nerves are more vulnerable after injury due to altered NTR expression, and that local IGF-1 would improve regeneration by promoting a pro-survival NTR profile.

METHODS

Animal Model

Male Fischer 344 ×Brown-Norway hybrid rats were obtained from the National Institute for Aging and housed in an animal facility fully approved by the Association for Assessment and Accreditation of Laboratory Animal Care. All experimental procedures were performed with the approval of the institutional animal care and use committee. Rats were allowed 10 days to adapt to the facility and were maintained on a 12-hour light/dark cycle with rat chow and water *ad libitum*. Body weights were used to indicate animal health.

Preliminary Experiments

In 10 strain-matched animals, the tibial nerve was dissected from the lower leg to dorsal and ventral roots. The tibial nerve most reliably corresponds to spinal levels L1–L3. Spinal

segments were evaluated for growth-associated protein (GAP) 43 mRNA expression and found to be elevated as expected, confirming that samples included motor neurons from the correct level. Electrophysiology data showed no nerve conduction by the fourth week post-injury.

Experimental Design and Sample Size

Eighty-four rats, 42 young (4 months) and 42 aged (24 months), were used in the first part of this study. Six young and 6 aged animals were assigned randomly as negative controls, and the remaining 72 animals were assigned randomly to 12 treatment groups of 6 each (Table 1). The 72 treatment group animals underwent left tibial nerve transection and subsequent T-tube repair. Animals were excluded in subsequent experiments if they had died due to surgical complications or if they sustained wound dehiscence or infection. The animals were followed for 1, 2, or 4 weeks, at which time L1–L3 spinal levels were harvested. The specimens were subjected to mRNA extraction of 3 neurotrophins (NGF, NT-3, and BDNF), 4 neurotrophin receptors (p75, TrkB, TrkC, and sortilin), and IGF-1R, followed by 2-step real-time polymerase chain reaction (PCR).

In the second part of the study, 32 2-month-old (young) and 12-month-old (aged) rats were used to evaluate protein expression. Animals that underwent tibial nerve injury or sham surgery (control) were chosen at random to be euthanized at 1, 3, or 10 days after surgery, at which time L1–L3 spinal levels were harvested for Western blot analysis (Table 2).

Surgical Technique

Aseptic surgical technique and isoflurane anesthesia were used to perform the surgery on all animals. Incision was made on the left posterior thigh. Muscles were separated to expose the left sciatic nerve. Under a surgical microscope, the tibial, sural, and common fibular nerve branches were carefully separated. The tibial nerve was transected 1 cm proximal to where it passes deep into the gastrocnemius. Proximal and distal nerve stumps were placed in a custom T-tube with the middle arm attached to a mini-pump. The nerve conduit was constructed from 1016 μm internal diameter, semiporous blood-compatible tubing (Micro-Rena-thane; Braintree Scientific, Braintree, Massachusetts), and the T-arm was constructed from Silastic tubing (Dow Corning, Midland, Michigan). An mini-osmotic pump (ALZET 1002; DURECT Corp., Cupertino, California) delivered recombinant human IGF-1 (Bachem AG, Torrance, California) or saline of 0.10-μg/μl concentration at a rate of 0.25 μl/h and was placed in a subcutaneous dorsal midline pouch. Rats that received saline treatment were used as positive controls. The fascia was closed using absorbable suture, and the skin was closed using staples. Postoperatively, the animals received analgesia in the form of buprenorphine and were returned to their cages for recovery.

Gene Expression

At 1, 2, or 4 weeks, animals were anesthetized with isoflurane, and pump-tube congruity was evaluated. Bilateral laminectomy was performed to expose the spinal cord *in situ*. L1–L3 was identified, excised, stripped of dorsal and ventral roots, hemisected, immersed in liquid nitrogen, and stored at −80°C. Animals were then euthanized. All surgeries and tissue harvests were performed by the same author (T.B.A.). Frozen samples were mechanically

homogenized in liquid nitrogen. Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, California). Reverse transcription was performed using 2 μg total RNA, random hexamers, and Superscript II reverse transcriptase (Invitrogen). Real-time PCR (ABI 7900HT; Applied Biosystems, Foster City, California) was performed in triplicate with 384-well plates and non-template controls for each sample. Assay wells contained 10-μl total reaction volume, 5 μl of 2 ×PCR master mix (Applied Biosystems), 0.5 μl of 20 ×primer probe (Applied Biosystems), and 4.5 μl of cDNA. The amplification thermal profile was set to 50°C for 2 min, 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 1 min. Taq-Man gene expression assays (Applied Biosystems) were used to perform quantitative real-time polymerase chain reaction (qRT-PCR) with glyceraldehyde 3-phosphate dehydrogenase endogenous control. Real-time data were analyzed and normalized to the young control group with Sequence Detection System version 2.1 (Applied Biosystems) software.

Western Blot Analysis

The protein levels of $p75^{NTR}$, TrkB, sortilin, and IGF-1R were analyzed by Western blot. Spinal cord tissue was prepared in buffer as previously described into a lysate solution.[36] Equal amounts (100 μg) of lysate were loaded onto sodium dodecylsulfate–polyacrylamide gels and separated via electrophoresis, then transferred onto a polyvinylidene difluoride membrane (PerkinElmer Life Sciences, Waltham, Massachusetts) overnight. The membrane was blocked with 5% milk–Tris-buffered saline–Tween 20 (Cell Signaling, Danvers, Massachusetts) for 1 hour at room temperature and subsequently incubated overnight at 4°C with a monoclonal rabbit anti–IGF-1 receptor β-antibody (1:1,000; Cell Signaling), a polyclonal rabbit anti-p75 NGF receptor antibody (1:2,000; Abcam, Cambridge, Massachusetts), a monoclonal mouse anti-sortilin antibody (1:1,000; Abcam), or a polyclonal rabbit anti-TrkB antibody (1:500; Abcam). β-actin was used as loading control and was probed with a monoclonal mouse anti–β-actin antibody (1:5,000; Sigma, St. Louis, Missouri). Protein complexes were detected by incubation for 1 hour at room temperature with secondary antibody conjugated to horseradish peroxidase diluted at 1:5,000 in blocking buffer and detected by Enhanced Chemiluminescence Plus (GE Healthcare, Barrington, Illinois).

Statistical Analysis

SigmaStat version 3.11 (Systat Software, Inc., San Jose, California) was used for data analysis. A 1-way analysis of variance (ANOVA) model for each outcome measure was used for young and aged controls. Age and treatment effect analysis using a 2-way ANOVA model was established with age and treatment as the independent variables. For non-parametric data, rank transformation was performed as appropriate. *Post hoc* pairwise comparisons were done using the Student–Newman–Keuls method. Statistical significance was set at $P<0.05$.

RESULTS

Two rats from the first part of the study (from 1-week and 4-week IGF-1 treatment groups) died before study completion. Young and aged rats in the negative control group demonstrated different NTR mRNA expressions at baseline. The relative mRNA levels of

IGF-1R (34% higher, $P=0.01$) and $p75^{NTR}$ (68% higher, $P<0.01$) genes were significantly more elevated in aged uninjured rats compared with the young rats (Fig. 1). Baseline expression of sortilin did not differ significantly between age groups.

The relative mRNA levels of $p75^{NTR}$ and sortilin in the spinal cord from tibial nerve–injured, saline-treated young and aged rats are shown in Fig. 2A and B. In young rats, $p75^{NTR}$ mRNA expression was significantly higher at 2 and 4 weeks relative to baseline ($P=0.03$ and $P=0.04$, respectively), and sortilin expression was significantly higher at 4 weeks relative to baseline ($P<0.01$). In aged rats, $p75^{NTR}$ mRNA expression was significantly upregulated to levels 49.6% and 68.5% higher than baseline at 2 and 4 weeks post-axotomy, respectively ($P<0.01$). Among animals in this positive control group, sortilin levels in aged rats increased at earlier time-points (at 1 and 2 weeks) and to a greater extent relative to baseline than in young rats. Sortilin mRNA remained upregulated at 4 weeks in both young and aged positive control groups. IGF-1R in aged rats from the positive control group was elevated at baseline and did not change significantly over time (data not shown). Conversely, in young saline-treated rats, IGF-1R expression was elevated at 2 and 4 weeks after injury relative to baseline, but this change did not reach statistical significance. NGF, BDNF, NT-3, TrkB, and TrkC all remained near baseline from 1 to 4 weeks (data not shown).

The relative mRNA levels of $p75^{NTR}$ and sortilin from young and aged rats treated with IGF-1 after tibial nerve injury are shown in Figure 2C and D. IGF-1 treatment significantly increased the $p75^{NTR}$ gene expression at 4 weeks in young rats but not in aged rats ($P=0.004$). In aged rats, IGF-1 treatment upregulated $p75^{NTR}$ gene expression by only 9.4% and 17.2% at 2 and 4 weeks, respectively, which indicated significant suppression compared with the saline-treated controls ($P=0.002$ for both time-points). IGF-1 treatment significantly increased sortilin gene expression in both young and aged rats at 2 and 4 weeks relative to baseline levels, but it did not significantly affect any other gene expression (data not shown).

Spinal cord protein expression of IGF-1R, $p75^{NTR}$, sortilin, and TrkB from aged and young animals is shown in Figure 3. IGF-1R protein levels were nearly undetectable in young animals from both uninjured and injured groups and did not vary over time. This corresponded to the earlier reported gene expression data. The protein levels of $p75^{NTR}$ were similarly undetectable in young uninjured and injured animals, corresponding to the gene expression data. Protein levels of sortilin were detectable in both adult and young rats. The intensity of the blot was higher in young rats when compared with adult rats. TrkB protein was similarly expressed in both groups.

DISCUSSION

In this study, we have demonstrated that age and IGF-1 affect NTR expression acutely after peripheral nerve injury. Aging exaggerates baseline and post-injury expression of pro-apoptotic complexes, and IGF-1 treatment ameliorates these changes. Previous studies have shown that, during aging, $p75^{NTR}$ levels increase, whereas Trk levels decrease in a parallel fashion, potentially altering axonal recovery after injury.[26] IGF-1R mediates this switch, leading to neuronal degeneration and Aβ production in Alzheimer disease.[26,27] Because Trk

and $p75^{NTR}$ induce opposite physiologic effects, cell survival and death, respectively, an age-induced switch may place aged motor neurons at a baseline recovery disadvantage.

In this study, mRNA and protein expression of IGF-1R and $p75^{NTR}$ increased with age (Figs. 1 and 3) whereas TrkB levels did not differ significantly between aged and young rats. Our results support earlier findings showing that neuronal $p75^{NTR}$ protein expression increases with age.[37] Although $p75^{NTR}$ increased after nerve injury, IGF-1R did not follow the same trend, which could indicate that post-injury upregulation of $p75^{NTR}$ may be controlled by a separate mechanism.

Aged subjects have a stronger pro-death NTR transcription response to nerve injury than young subjects due to greater upregulation of $p75^{NTR}$ in the presence of upregulated sortilin without changes in Trk expression. Due to the ability of $p75^{NTR}$ to induce apoptosis alone or form pro-apoptotic complexes with sortilin, upregulation of this receptor places aged motor neurons at a baseline disadvantage to recover from the injury.

We successfully showed that age-induced pro-apoptotic response to injury is blunted at the molecular level by local treatment with IGF-1. Trk and sortilin did not seem affected by IGF-1, whereas treatment prevented post-axotomy transcriptional elevation of $p75^{NTR}$ in aged animals at 2 and 4 weeks post-injury (Fig. 2D). Because this molecular environment is rich with the co-receptor sortilin, preventing $p75^{NTR}$ upregulation decreases the potential number of pro-apoptotic crosslinking complexes that can form on the neuronal cell membrane. Aged motor neurons show increased expression of $p75^{NTR}$ after injury, as indicated by our study and others, because of retrograde transport of a yet-to-be-determined positive signal from axons regenerating through damaged or denervated peripheral nervous tissue.[38] Local delivery of IGF-1 to aged animals potentially reverses deleterious effects of age on the neuron's ability to survive injury, likely by changing the microenvironment around the injured nerve and preventing IGF-1R mediation of the $p75^{NTR}$ expression–inducing signal.[38] This is an age-specific effect, as young and aged animals have opposite reactions to the influence of IGF-1. IGF-1 delivery in young rats seemed to upregulate $p75^{NTR}$ mRNA expression more at 4 weeks than saline-treated young rats.

Neurotrophins, such as BDNF, NGF, and NT-3, also undergo retrograde transport after injury.[39,40] Schwann cells are a significant source of these trophic factors as well as a source of IGF-1.[40,41] We did not detect significant elevations in NT expression at the level of the motor neuron cell body after injury. This lends further support to the notion that perineurial Schwann cells play a key role in regulating tissue turnover and repair after injury. Aging also likely alters this physiological process.

This study has several limitations. First, we used homogenized spinal cord samples instead of isolated motor neurons, making it difficult to determine with certainty the origin of the mRNA transcripts and protein products. This was done due to lack of adequate tissue samples around the injured nerves and the difficulty of tissue extraction for full analysis. Second, we used different age groups in the first (4 and 24 months) and second (2 and 12 months) parts of the study. The first part, with 4- and 24-month-old rats (corresponding to 15 and 60 human years, respectively), clearly demonstrated that age affects NT mRNA

expression. We chose 2- and 12-month-old rats (corresponding to 10 and 30 human years, respectively) for protein production analysis as it is well known that nerve regeneration potential declines rapidly after adolescence in humans.[9,42] Despite the age differences, we do not believe this significantly altered the results, as the evaluation of protein expression remains a relative comparison between young and adult animals. Last, we did not perform functional analysis in the subjects, which may have further supported the adverse effect of age on nerve injury recovery. In a previous study, we successfully demonstrated that IGF-1 improved functional recovery by preserving the postsynaptic neuromuscular junction after PNI.[35] Despite the limitations, it is clear from the whole of these data that aging alters baseline and post-axotomy NTR mRNA expression. Aging places motor neurons at a baseline disadvantage. There is a stronger pro-apoptotic transcriptional response to injury in older animals compared with younger animals. Local delivery of IGF-1 ameliorates this response at the molecular level in aged animals only.

The data from these experiments have several implications for future research into nerve injury with aging. $p75^{NTR}$ appears to play a critical role in age-related changes in motor neuron recovery from injury. By altering the local environment around the injured nerve, transcriptional changes within the motor neuron itself can occur. Motor neurons do not upregulate neurotrophin mRNA after injury to a significant extent, highlighting the need for Schwann cells and other perineurial cells to produce the trophic factors essential for motor neuron recovery. Lastly, these findings offer a partial explanation for impaired motor neuron recovery with increased age. Nerve regeneration strategies and therapeutic treatments seeking to improve nerve recovery in older individuals must consider age-related effects on baseline and post-injury NTR expression and the interaction between the injured nerve stump and its local environment.

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Abbreviations

ANOVA	analysis of variance
BDNF	brain-derived neuro-trophic factor
GAP	growth-associated protein
IGF-1	insulin-like growth factor-1
IGF-1R	insulin-like growth factor-1 receptor
NGF	nerve growth factor
NT	neurotrophin
NT-3	neurotrophin-3

NT-4/5	neurotrophin-4/5
NTR	neurotrophin receptor
$p75^{NTR}$	p75 neurotrophin receptor
PCR	polymerase chain reaction
PNI	peripheral nerve injury
proNGF	pro–nerve growth factor
qRT-PCR	quantitative real-time polymerase chain reaction
Trk	tyrosine kinase receptor

References

1. Ciaramitaro P, Mondelli M, Logullo F, Grimaldi S, Battiston B, Sard A, et al. Traumatic peripheral nerve injuries: epidemiological findings, neuropathic pain and quality of life in 158 patients. J Peripher Nerv Syst. 2010; 15:120–127. [PubMed: 20626775]
2. Kouyoumdjian JA. Peripheral nerve injuries: a retrospective survey of 456 cases. Muscle Nerve. 2006; 34:785–788. [PubMed: 16881066]
3. Noble J, Munro CA, Prasad VS, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. J Trauma. 1998; 45:116–122. [PubMed: 9680023]
4. Bekelis K, Missios S, Spinner RJ. Falls and peripheral nerve injuries: an age-dependent relationship. J Neurosurg. 2015; 123:1223–1229. [PubMed: 25978715]
5. Hundepool CA, Ultee J, Nijhuis TH, Houpt P, Hovius SE. Research Group 'ZERO'. Prognostic factors for outcome after median, ulnar, and combined medianulnar nerve injuries: a prospective study. J Plast Reconstr Aesthet Surg. 2015; 68:1–8. [PubMed: 25448370]
6. Ruijs AC, Jaquet JB, Kalmijn S, Giele H, Hovius SE. Median and ulnar nerve injuries: a meta-analysis of predictors of motor and sensory recovery after modern microsurgical nerve repair. Plast Reconstr Surg. 2005; 116:484–494. [PubMed: 16079678]
7. Black MM, Lasek RJ. Slowing of the rate of axonal regeneration during growth and maturation. Exp Neurol. 1979; 63:108–119. [PubMed: 467539]
8. Verdu E, Buti M, Navarro X. The effect of aging on efferent nerve fibers regeneration in mice. Brain Res. 1995; 696:76–82. [PubMed: 8574688]
9. Verdu E, Ceballos D, Vilches JJ, Navarro X. Influence of aging on peripheral nerve function and regeneration. J Peripher Nerv Syst. 2000; 5:191–208. [PubMed: 11151980]
10. Marinelli S, Eleuteri C, Vacca V, Strimpakos G, Mattei E, Severini C, et al. Effects of age-related loss of P/Q-type calcium channels in a mice model of peripheral nerve injury. Neurobiol Aging. 2015; 36:352–364. [PubMed: 25150573]
11. Apel PJ, Alton T, Northam C, Ma J, Callahan M, Sonntag WE, et al. How age impairs the response of the neuromuscular junction to nerve transection and repair: an experimental study in rats. J Orthop Res. 2009; 27:385–393. [PubMed: 18853430]
12. Hochberg MC, Lethbridge-Cejku M, Scott WW Jr, Reichle R, Plato CC, Tobin JD. Serum levels of insulin-like growth factor in subjects with osteoarthritis of the knee. Data from the Baltimore Longitudinal Study of Aging. Arthritis Rheum. 1994; 37:1177–1180. [PubMed: 8053956]
13. Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, et al. Effects of human growth hormone in men over 60 years old. N Engl J Med. 1990; 323:1–6. [PubMed: 2355952]
14. Li L, Oppenheim RW, Lei M, Houenou LJ. Neurotrophic agents prevent motoneuron death following sciatic nerve section in the neonatal mouse. J Neurobiol. 1994; 25:759–766. [PubMed: 8089654]

15. Rabinovsky ED. The multifunctional role of IGF-1 in peripheral nerve regeneration. Neurol Res. 2004; 26:204–210. [PubMed: 15072640]
16. Florini JR, Ewton DZ, Magri KA, Mangiacapra FJ. IGFs and muscle differentiation. Adv Exp Med Biol. 1993; 343:319–326. [PubMed: 8184742]
17. Vijayashankar N, Brody H. A study of aging in the human abducens nucleus. J Comp Neurol. 1977; 173:433–438. [PubMed: 856891]
18. Lykissas MG, Batistatou AK, Charalabopoulos KA, Beris AE. The role of neurotrophins in axonal growth, guidance, and regeneration. Curr Neurovasc Res. 2007; 4:143–151. [PubMed: 17504212]
19. Yin Q, Kemp GJ, Frostick SP. Neurotrophins, neurones and peripheral nerve regeneration. J Hand Surg Br. 1998; 23:433–437. [PubMed: 9726539]
20. Lei L, Parada LF. Transcriptional regulation of Trk family neurotrophin receptors. Cell Mol Life Sci. 2007; 64:522–532. [PubMed: 17192812]
21. Ebadi M, Bashir RM, Heidrick ML, Hamada FM, Refaey HE, Hamed A, et al. Neurotrophins and their receptors in nerve injury and repair. Neurochem Int. 1997; 30:347–374. [PubMed: 9106250]
22. Meeker R, Williams K. Dynamic nature of the p75 neurotrophin receptor in response to injury and disease. J Neuroimmune Pharmacol. 2014; 9:615–628. [PubMed: 25239528]
23. Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci. 2003; 4:299–309. [PubMed: 12671646]
24. Chao MV. The p75 neurotrophin receptor. J Neurobiol. 1994; 25:1373–1385. [PubMed: 7852992]
25. Barbacid M. Neurotrophic factors and their receptors. Curr Opin Cell Biol. 1995; 7:148–155. [PubMed: 7612265]
26. Costantini C, Weindruch R, Della Valle G, Puglielli L. A TrkA-to-p75NTR molecular switch activates amyloid beta-peptide generation during aging. Biochem J. 2005; 391:59–67. [PubMed: 15966860]
27. Costantini C, Scrable H, Puglielli L. An aging pathway controls the TrkA to p75NTR receptor switch and amyloid beta-peptide generation. EMBO J. 2006; 25:1997–2006. [PubMed: 16619032]
28. Kaplan DR, Miller FD. Neurobiology: a move to sort life from death. Nature. 2004; 427:798–799. [PubMed: 14985746]
29. Sarret P, Krzywkowski P, Segal L, Nielsen MS, Petersen CM, Mazella J, et al. Distribution of NTS3 receptor/sortilin mRNA and protein in the rat central nervous system. J Comp Neurol. 2003; 461:483–505. [PubMed: 12746864]
30. Wu W, Li L, Yick LW, Chai H, Xie Y, Yang Y, et al. GDNF and BDNF alter the expression of neuronal NOS, c-Jun, and p75 and prevent motoneuron death following spinal root avulsion in adult rats. J Neurotrauma. 2003; 20:603–612. [PubMed: 12906744]
31. Hempstead BL. Dissecting the diverse actions of pro- and mature neurotrophins. Curr Alzheimer Res. 2006; 3:19–24. [PubMed: 16472198]
32. Nykjaer A, Willnow TE, Petersen CM. p75NTR—live or let die. Curr Opin Neurobiol. 2005; 15:49–57. [PubMed: 15721744]
33. Kraemer BR, Snow JP, Vollbrecht P, Pathak A, Valentine WM, Deutch AY, et al. A role for the p75 neurotrophin receptor in axonal degeneration and apoptosis induced by oxidative stress. J Biol Chem. 2014; 289:21205–21216. [PubMed: 24939843]
34. Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, et al. Sortilin is essential for proNGF-induced neuronal cell death. Nature. 2004; 427:843–848. [PubMed: 14985763]
35. Apel PJ, Ma J, Callahan M, Northam CN, Alton TB, Sonntag WE, et al. Effect of locally delivered IGF-1 on nerve regeneration during aging: an experimental study in rats. Muscle Nerve. 2010; 41:335–341. [PubMed: 19802878]
36. Hervera A, Negrete R, Leanez S, Martin-Campos JM, Pol O. The spinal cord expression of neuronal and inducible nitric oxide synthases and their contribution in the maintenance of neuropathic pain in mice. PLoS One. 2010; 5:e14321. [PubMed: 21179208]
37. Yuan Q, Hu B, So KF, Wu W. Age-related reexpression of p75 in axotomized motoneurons. Neuroreport. 2006; 17:711–715. [PubMed: 16641674]

38. Bussmann KA, Sofroniew MV. Re-expression of p75NTR by adult motor neurons after axotomy is triggered by retrograde transport of a positive signal from axons regrowing through damaged or denervated peripheral nerve tissue. Neuroscience. 1999; 91:273–281. [PubMed: 10336077]

39. Curtis R, Tonra JR, Stark JL, Adryan KM, Park JS, Cliffer KD, et al. Neuronal injury increases retrograde axonal transport of the neurotrophins to spinal sensory neurons and motor neurons via multiple receptor mechanisms. Mol Cell Neurosci. 1998; 12:105–118. [PubMed: 9790733]

40. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. J Cell Biol. 1992; 119:45–54. [PubMed: 1527172]

41. Cheng HL, Randolph A, Yee D, Delafontaine P, Tennekoon G, Feldman EL. Characterization of insulin-like growth factor-I and its receptor and binding proteins in transected nerves and cultured Schwann cells. J Neurochem. 1996; 66:525–536. [PubMed: 8592122]

42. Turturro A, Witt WW, Lewis S, Hass BS, Lipman RD, Hart RW. Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. J Gerontol A Biol Sci Med Sci. 1999; 54:B492–501. [PubMed: 10619312]

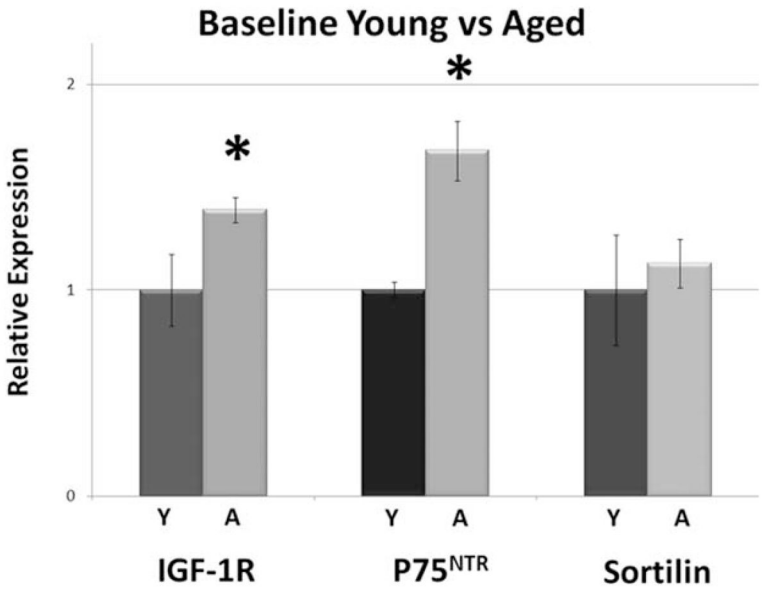

FIGURE 1.
Baseline mRNA expression in young (Y) and aged (A) negative controls. *Significant upregulation in aged animals relative to young animals.

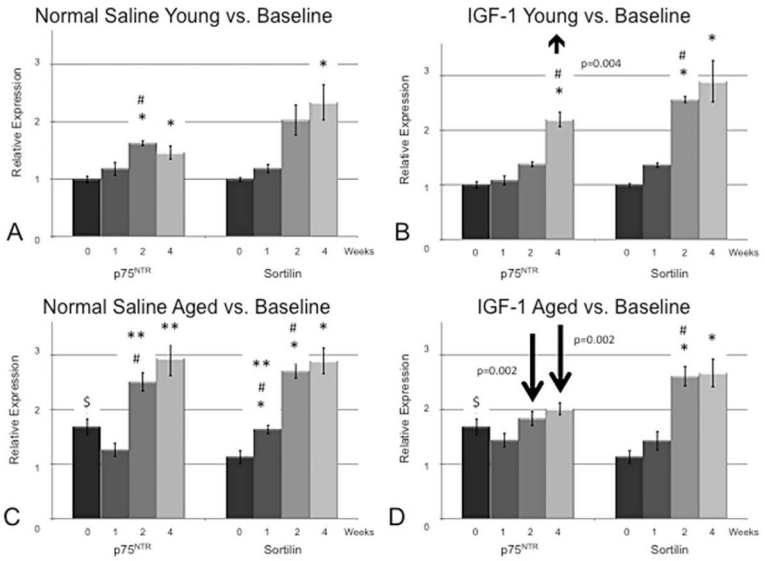

FIGURE 2.

Changes in relative mRNA expression in young and aged rats treated with saline or IGF-1 after nerve injury. Relative expression of 1 =Young baseline. $^{\$}$Upregulation in aged rats at baseline relative to young rats. *Upregulation after nerve injury from age-matched baseline levels. **Significant mRNA upregulation in aged rats relative to young rats in saline-treated controls demonstrating stronger pro-apoptotic response (P <0.01). $^{\#}$Significant upregulation from previous week.

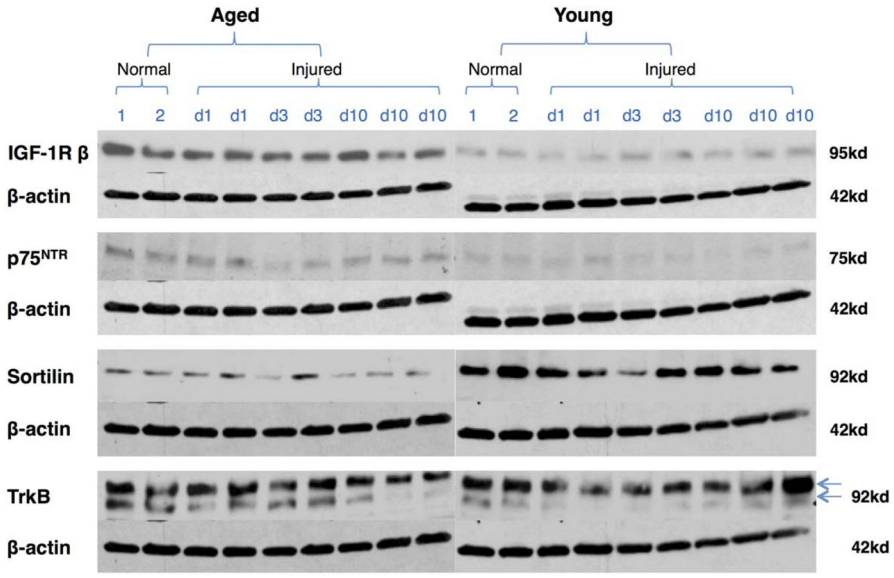

FIGURE 3.

Western blot results of IGF-1R, $p75^{NTR}$, sortilin, and TrkB with β-actin as loading control. Results are shown for uninjured, injured at 1, 3, and 10 days in aged and young rats.

Table 1

Experimental groups in first part of study

	1 week		2 week		4 week	
T-tube nerve repair	**Young**	**Aged**	**Young**	**Aged**	**Young**	**Aged**
Normal saline (n)	6	6	6	6	6	6
Local IGF-1 to repair site (n)	6	6	6	6	6	6

Table 2

Experimental groups in second part of study

		Injured		
	Uninjured	**Day 1**	**Day 3**	**Day 10**
Aged (n)	4	4	4	4
Young (n)	4	4	4	4