# ANALYSIS OF LOCALIZATION AND TRAFFICKING OF TRKB AFTER BDNF TREATMENT IN RAT HIPPOCAMPAL NEURONS

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#### ABSTRACT OF THE DISSERTATION

# Analysis of localization and trafficking of TrkB after BDNF treatment in rat hippocampal

neurons

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Neurotrophins regulate neuronal cell survival and death in hippocampal neurons via two distinct receptors, TrkB, a member of the Trk family of receptor tyrosine kinases and the p75, neurotrophin receptor (p75NTR). When BDNF binds to TrkB, the association with p75NTR is enhanced. Yet, the purpose of the association of these two receptors is unknown. This thesis investigated whether the association with p75NTR directs the trafficking of TrkB to specific endosomal compartments. To determine whether the treatment with BDNF in the presence or absence of p75NTR alters the trafficking of TrkB though the endosomal pathway, we examined and analyzed the localization of TrkB and pAkt, a downstream effector of the PI3K pathway, in wild type and p75<sup>-/-</sup> rat hippocampal neurons using immunocytochemistry and confocal microscopy. Our data suggested that the addition of BDNF increased trafficking of pTrkB to the early and recycling endosomes. However, in the absence of p75NTR, more TrkB appeared to traffic to the lysosome, suggesting that p75NTR may participate in directing trafficking of TrkB to the endosomal pathway and preventing its degradation. Additionally, activation of pAkt localized in the late and recycling endosomes in the wild type neurons. These results suggested that p75NTR may direct the internalized TrkB through the endosomal pathway.

#### Preface

This dissertation is based on the investigation of the mechanisms of neurotrophin signaling and the consequences for neuronal function. All of the work presented henceforth was conducted in the Life Science Center at the Rutgers University, Newark campus. None of the text of the dissertation is taken directly from previously published or collaborative articles.

The dissections of the embryonic day 18 rats and incubations of the rat hippocampal neurons were conducted by Dr. Marta Volosin. The immunochemistry used to fix cells and applied them with primary and secondary antibodies were conducted by Dr. Marta Volosin and Dr. Juan Zanin. I conducted the statistical and morpholgical analyses and confocal microscopy of the quantification analysis using ImageJ with assistance from Dr. Marta Volosin and Dr. Wilma Friedman.

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#### Introduction

Brain-derived neurotrophic factor (BDNF) is a member of a family of neurotrophic factors including nerve growth factor (NGF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4) (Hallböök 1999; Hallböök et al. 1991; Bronfman et. al. 2007). BDNF can bind specifically to a member of the Trk family of receptor tyrosine kinases, TrkB, and to p75NTR on the plasma membrane (Ohta et al., 2011; He et al., 2013). When TrkB is activated by BDNF, the receptor is internalized and traffics to endosomal compartments. There are two endosomal pathways the receptor complex may traffic to, one is the endosomal recycling system where receptors that travel to the recycling endosome are either transported back to the same domain, remain in the recycling endosome, or are transported to a different domain on the plasma membrane (Di Guglielmo et al., 1994; Bronfman et al., 2007). Alternatively, rather than trafficking to the recycling endosome, internalized receptors can travel to the early endosome and from there, may get transported to the late endosome and then to the lysosome for degradation (Di Fiore and de Camilli, 2001; Sorkin and Von Zastrow, 2002; Miaczynaska et al., 2004; Bronfman et al., 2007). These vesicles carrying the receptors enter the endosomes through facilitation and regulation by Rab GTPases, located on the membrane of endosomes (Zerial and McBride, 2001; Schimmoller et al., 1998; de Renzis, Sönnichsen, & Zerial et al., 2002; Jordens et al., 2005; Bronfman et al., 2007).

When BDNF binds to the high affinity TrkB neurotrophin receptor, the receptor undergoes homodimerization and phosphorylation on tyrosine residues, which act as a scaffold for the binding of intracellular signaling proteins (Minichiello et al., 1998; Huang and Reichardt, 2003; He et al., 2013). The complex recruits many downstream proteins to activate a series of pathways including mitogen activated protein (MAP) kinase, phosphatidylinositol 3-kinase (PI3K)/Akt, and phospholipase C- $\gamma$  signaling pathways (English et al., 1999; Kaplan and Miller, 2000; Datta, Brunet, and Greenberg, 1999; Jaworski et al., 2005; Kumar et al., 2005; Zheng et al., 2008; Lazo et al., 2013; Al-Qudah et al., 2014; Sciarretta et al., 2010). In dendrites, binding of the BDNF ligand also leads to the internalization of TrkB, which traffics to the recycling endosome after phosphorylation to increase BDNF signaling (Lazo et al., 2013). In contrast, when BDNF binds to p75NTR in the absence of TrkB, its intracellular death domain can activate apoptosis through activation of c-Jun, N-terminal kinase (JNK), mitochondrial release of cytochrome c and activation of the caspase-9 signaling cascade (Roux and Barker, 2002; Kafitz et al., 1999; He et al., 2013; Troy, Friedman, and Friedman, 2002; Liu et al., 1996). p75NTR can associate with many different co-receptors. Even though BDNF binding to p75NTR can promote apoptosis, proneurotrophins such as proBDNF or proNGF are more effective ligands for p75NTR-mediated apoptosis, due to the association of p75NTR with the co-receptor sortilin, a type-1 receptor that acts as a co-receptor with p75NTR to enhance affinity of the binding complex for proneurotrophins. Activation of this receptor complex by proNGF or proBDNF can induce cell death in basal forebrain and hippocampal neurons (Song et al., 2010; Njkjaer et al., 2004; Volosin et al., 2006). In contrast, when p75 associates with Trk receptors, including TrkB (Bibel, Hoppe, and Barde, 1999; Huang and Reichardt, 2003; Bronfman et al., 2007) there is greater selectivity and affinity for mature neurotrophins, which activate Trk signaling and downstream pathways (Roux and Barker, 2002; He et al., 2013). The assistance of p75NTR supporting TrkB allows p75NTR to have a dual role in the regulation of

neuronal cell survival, facilitating survival when it associates with TrkB and promoting apoptosis when it associates with sortilin.

It is intriguing how cell survival can be regulated by the interaction between TrkB and p75NTR. Our preliminary studies indicated that the maximal association of p75NTR with TrkB occurs 15-30 minutes after BDNF treatment, suggesting that the association of the two receptors is not required for initial binding and activation of TrkB, which occurs within 5 minutes (Ip et al., 1993; Squinto et al., 1991; Dechant et al., 1993). Therefore, we investigated whether the association of p75NTR with TrkB regulates TrkB trafficking to different endosomal compartments to activate specific signaling pathways affecting cell survival. In this study, to examine what controls the fate of TrkB and overall regulation of neuronal cell survival and death, analyses were conducted to examine the trafficking of TrkB and downstream effector of the PI3K/Akt pathway, pAkt. Phosphorylated by phosphoinositide-dependent protein kinase 1 (PDKI) and mTOR, a serine/threonine protein kinase (Datta, Brunet, and Greenberg, 1999; Yan and Yankner, 2000; Patapoutian and Reichardt, 2001), pAkt phosphorylates other effector proteins involved in promoting neuronal cell survival including the inhibition of Bcl-2/Bcl-zassociated death promoter (BAD) and the activation of forkhead transcription factor FKHRL1 and glycogen synthase kinase  $3-\beta$  (GSK  $3-\beta$ ) (Brunet et al., 1997; Yan and Yankner, 2000; Datta, Brunet, and Greenberg, 1999; Patapoutian and Reichardt, 2001). To assess the trafficking of activated TrkB (pTrkB) and its downstream effectors, we analyzed the colocalization of pAkt or pTrkB with different Rab GTPases as markers for specific endosomal compartments, lysotracker, a specific marker for mature lysosomes,

and early endosome antigen 1 (EEA1) after treatment with BDNF in wild type and  $p75^{-/-}$  cultured rat hippocampal neurons.

#### Materials and methods

#### Reagents

BDNF was a gift from C.F. Ibáňez (Karolinska Institute, Stockhelm, Sweden). The chicken anti-TrkB<sub>in</sub> was purchased from Promega (Madison,WI) (1:500 dilution). Eagle's MEM, Ham's F12, and penicillin-streptomycin were purchased from Invitrogen (Gaithersburg, MD). Polylysine, glucose, insulin, putrescine, progesterone, transferrin, and selenium were obtained from Sigma (St.Louis, MO). Mouse anti-EEA1 antibody was obtained from BD Transduction (USA) (monoclonal) (1:200 dilution). The rabbit anti-Rab5, anti-Rab7, anti-Rab11, and anti-phospho-TrkB (monoclonal) (1:200 dilution); mouse IgG2b anti-pAkt (Ser 473) (1:5 dilution) and anti-ERK (1:50 dilution) (monoclonal) were obtained from Cell Signaling (Beverly,MA). Lysotracker was obtained from Life Technologies (Grand Island, NY). Secondary antibodies used for immunostaining were Alexa 488 and Alexa 555 anti-mouse and anti-rabbit 555 antibodies were obtained from Invitrogen (Gaithersburg, MD) (1:300 dilution) (Volosin et al.,2006).

#### Hippocampal neuron culture

Pregnant rats were killed by exposure to CO<sub>2</sub> and soaked in 80% ethanol for 10 minutes. Embyronic day 18 (E18) rat fetuses were removed under sterile conditions and kept in PBS on ice. Hippocampi were dissected and dissociated in serum-free medium composed of a 1:1 mixture of Eagle's MEM and Ham's F-12 supplemented with glucose (6 mg/ml), putresine (60µm), progesterone (20 nm), transferrin (100 µg/ml), selenium (30 nm), penicillin (0.5 U/ml), and streptomycin (0.5 µg/ml). The cells were then plated on tissue culture dishes that were precoated overnight with poly-D-lysine (0.1 mg/ml). The cells were maintained in serum-free medium for 5 days at 37°C (Song et al.,2010; Farinelli et al., 1998; Friedman et al., 1993).

#### Immunocytochemistry

Cultured cells were fixed with 4% paraformaldehyde, blocked with PBS/0.3% Triton X-100/10% goat serum with 1% BSA in PBS for 30 minutes at room temperature. Primary antibodies were applied to the cells overnight 4°C. The cells were then washed three times with PBS for 15 minutes. The secondary antibodies anti-rabbit Alexa 488 (green) and anti-mouse (Alexa 555) (red) (1:300 dilution) were applied to cells in the dark for 1 hour, washed three times with PBS for 15 minutes, and mounted. Cells were coverslipped with anti-fading medium (ProLong Gold; Invitrogen) and analyzed by confocal microscopy (Carl Zeiss). No immunostaining was seen in controls with omission of the primary antibodies (Volosin et al., 2006; Volosin et al., 2008).

#### Morphological analysis

In our quantification analysis, we used Wright Cell Imaging Facility Colocalization Plugins in ImageJ, an image analysis software developed by Toronto Western Research Institute. This software is used to determine the amount of signal from an image channel that occurs in the same location as another channel. If the channels represent specific objects such as fluorescence tagged proteins, colocalization analysis will quantify whether the different objects are found in the same location.

(http://www.uhnres.utoronto.ca/facilities/wcif).

#### Statistical analysis

The analysis was conducted on a scatterplot that measured and compared the intensity of pixels in different images using Manders' original coefficient using thresholds, which is represented as tM1. Manders' original coefficient measured the percentage of pixel intensities of images overlapping each other where the threshold eliminates the background intensity (Manders, Verbeek, and Aten, 1993; Dunn, Kamocka, and McDonald, 2011). The scale of Manders' coefficient ranges from 0 to 1 where 0 is no colocalization and 1 is perfect colocalization. tM1 is the percentage of green pixels in one image that overlap the percentage of red pixels in another image.

#### Results

#### pTrkB traffics to the early endosome

p75NTR can interact with many co-receptors to form a complex, resulting in different cellular outcomes. BDNF treatment induced the association of TrkB with p75NTR, however the purpose of this association is unclear. Since activation of TrkB occurs rapidly, prior to association with p75NTR, the formation of the complex does not appear to be required for ligand binding. In this study, we analyzed whether the interaction of TrkB with p75NTR was required for appropriate trafficking of the receptor. The wild type, E18 hippocampal neurons were cultured and treated with BDNF for the indicated

time points. The treated hippocampal neurons were then fixed and stained using anti-p-TrkB and anti-EEA1 antibodies.

To determine if BDNF signaling guides TrkB to the early endosome, the colocalization of pTrkB with EEA1 was analyzed by ImageJ using Manders' coefficient correlation analysis, tM2. The data indicated that compared to the control (the Manders' original coefficient using calculated thresholds (tM2) between pTrkB and EEA1 is 25.1%) there was a 34.6% colocalization between pTrkB and EEA1 at the 15 minute time point, and a substantial decrease in association at the 30 minute time point, suggesting that pTrkB localization to the early endosome may be transiently increased by BDNF (Fig.1A-C).

#### pTrkB traffics to the recycling endosome in soma and processes

In order to determine if pTrkB traffics to the recycling endosome during BDNF treatment, the colocalization of pTrkB with Rab11 was analyzed in cultured wild type and p75<sup>-/-</sup> hippocampal neurons treated without or with BDNF for the indicated time points. Neurons were fixed and stained using anti-pTrkB and anti-Rab11 antibodies. In the somas of the wild type neurons, compared to the control (the Manders' original coefficient using calculated thresholds (tM1) between pTrkB and Rab11 is 35.2%), there was 57.2% of colocalization between pTrkB and Rab11 at the 5 minute time point, 43.9% at the 15 minute time point, and the 47.1% at the 30 minute time point (Fig.2A-C). Then the trend decreased to control levels at 30 and 60 minute time points. In contrast, in the processes, compared to the control, where there was 38.7% colocalization, there was a colocalization of 40.7% at the 5 minute time point, 53% at the 15 minute time point, and 47.6% at the 1 hour time point (Fig.2A, D-E). Conversely, in the p75<sup>-/-</sup> hippocampal

neurons, in the somas, compared to the control (the Manders' original coefficient using calculated thresholds (tM1) between pTrkB and Rab11 is 31.2%), there is 40% colocalization at the 5 minute time point, 42.2% at the 60 minute time point, and 38.3% at the 2 hour time point (Fig. 3A-C), suggesting that in the absence of p75NTR, there is little trafficking of TrkB to the recycling endosome. More variability was observed in the processes, comparing the control (the Manders' original coefficient using calculated thresholds (tM1) between pTrkB and Rab11 is 41.7%) there was a colocalization of 53% at the 5 minute time point, 64.5% at the 30 minute time point, and 62.2% at the two hour time point (Fig.3A, D-E). BDNF appeared to induce more localization of pTrkB to the recycling endosomes in the processes compared to the soma in wild type and p75<sup>-/-</sup> hippocampal neurons. However, in the wild type neurons, there was more colocalization of pTrkB and Rab11 after BDNF treatments than in the p75<sup>-/-</sup> hippocampal neurons.

## TrkB traffics to the lysosome in p75<sup>-/-</sup> hippocampal neurons

To determine whether TrkB traffics to the lysosomes during BDNF treatment, we examined colocalization of TrkB with lysotracker in cultured wild type and p75<sup>-/-</sup> hippocampal neurons treated without or with BDNF for the indicated time points. Neurons were fixed and stained using anti-pTrkB antibodies and lysotracker. In the wild type neurons, compared to the control (the Manders' original coefficient using calculated thresholds (tM1) between TrkB and lysotracker is 30.3%), there was a slight increase in colocalization between TrkB and lysotracker at the 5 minute time point of BDNF treatment (32.9%) (Fig.4A-C), and colocalization decreased with increasing duration of BDNF treatment, suggesting that there was less localization of TrkB trafficking toward

the lysosome. Conversely, in the p75<sup>-/-</sup> type neurons, there was an increase in colocalization between TrkB and lysotracker during the BDNF treatment compared to the control (Manders' original coefficient between TrkB and lysotracker is 15.2%) (Fig.5A-C). These data suggest that p75NTR may play a role in guiding TrkB toward the endosomal pathway rather than the lysosomal pathway.

#### pAkt is not activated in the early endosome

As mentioned above, when BDNF binds TrkB, it elicits activation of the PI3K/Akt signaling pathway, which promotes neuronal survival (Ohta et al., 2011). In order to investigate whether pAkt localized to the early endosome upon BDNF treatment, colocalization of pAkt with Rab5 was analyzed by ImageJ in wild type, E18 hippocampal neurons. The neurons were cultured and treated without or with BDNF at the indicated time points, then were fixed and stained using anti-pAkt and anti-Rab5 antibodies. Compared to the control (the Manders' original coefficient using calculated thresholds (tM1) between pAkt and Rab5 is 28%), there was colocalization of 30.4% at the 5 minute time point between pAkt and Rab5 and then the trend decreased the 30 minute time point at 24.5% colocalization (Fig.6A-C), suggesting that PI3K pathway is not activated in the early endosome.

#### pAkt is activated in the recycling endosome

It has been demonstrated that when neurotrophins bind to receptors and undergo endocytosis, the activated receptor and neurotrophin complexes and the components of the signaling pathways remain activated within endosomal compartments rather than terminate signaling (Grimes et al., 1996; Grimes, Beattie, and Mobley., 1997; Di Fiore and de Camilli., 2001; Bucci, Alifano, and Cogil., 2014). To determine if pAkt can be activated in the recycling endosome, the colocalization of pAkt with Rab11 was analyzed in wild type, E18 hippocampal neurons. Neurons were cultured and treated with BDNF for the indicated time points. The controls and the BDNF-treated neurons were fixed and stained using anti-pAkt and anti-Rab11 antibodies. Two experiments were conducted, yet, one of the experiments failed to elicit activation of pAkt due to issues with the BDNF treatment. However, in the other experiment compared to the control (the Manders' original coefficient using calculated thresholds (tM1) between pAkt and Rab11 is 17.9%), colocalization increased during the BDNF treatment (Fig.7A-C). These data suggest that the PI3K pathway may be activated in the recycling endosome.

#### pAkt is activated in the late endosome

To establish if pAkt is activated in the late endosome, the colocalization of pAkt with Rab7 in BDNF treated cultured hippocampal neurons was analyzed by ImageJ in wild type, E18 hippocampal neurons. Neurons were cultured and treated with BDNF as indicated. The control and the BDNF-treated neurons were fixed and stained using anti-pAkt and anti-Rab7 antibodies. The Manders' original coefficient using calculated thresholds (tM1) between pAkt and Rab7 was 24.3%. After the addition of BDNF treatment, there was a colocalization of 33.2% at the 60 minute time point between pAkt and Rab7 (Fig.8A-C). The data suggest that there may be a longer time duration for the PI3K pathway to activate in the late endosomes.

#### Discussion

BDNF binds to TrkB and activates a variety of signaling pathways that regulate neuronal survival and function. Where the activated intracellular signaling proteins are localized is not known. Previous studies from our lab showed that treatment of hippocampal neurons with BDNF elicited an association of TrkB with p75NTR after 15-30 minutes of treatment, a time after which the TrkB receptor is already activated. We hypothesized that the association of p75NTR with TrkB may influence the trafficking of TrkB after internalization of the receptor. Therefore, cultured hippocampal neurons from wild type or p75<sup>-/-</sup> rats were treated with BDNF for different periods of time and analyzed by immunostaining for pTrkB, and pAkt with markers of specific endosomal or lysosomal compartments.

The degree of colocalization of TrkB and pAkt with specific markers was evaluated using Manders' correlation coefficient, with calculated thresholds, rather than use Pearson's correlation coefficient . The advantages of using Manders' coefficient were the insensitivity of intensity for overlapping pixels, great measure for colocalization, and quantity of interest, despite its sensitivity background threshold (Dunn, Kamocka, and McDonald, 2011). Manders' coefficient using calculated thresholds was used to calculate and analyze the interaction of pTrkB and pAkt with the Rab GTPases, EEA1, and lysotracker. Manders' correlation coefficient was the preferred correlation analysis to be presented in this paper. In the wild type, the trend of the colocalization between pTrkB and EEA1 illustrated more localization of pTrkB in compartments that contained EEA1 with the addition of BDNF, suggesting that pTrkB localized to early endosomes. Similar results demonstrated that pTrkB traffics to the recycling endosome in wild type and p75<sup>-/-</sup>

hippocampal neurons, most likely to enhance BDNF signaling. Additionally, with BDNF treatment, there seems to be an increase in colocalization between pTrkB and Rab11 in the processes compared to the soma in both wild type and p75<sup>-/-</sup> type neurons. The importance of Rab11-recycling endosomes trafficking TrkB has been shown to induce dendritic branching (Lazo et al., 2013).

To assess whether the association of p75NTR with TrkB was altered, we compared wild type and p75<sup>-/-</sup> neurons for trafficking to the degradative pathway to the lysosome. Interestingly, there was little colocalization between TrkB and lysotracker in the wild type neurons after the addition of BDNF compared to the p75<sup>-/-</sup> type neurons, where there was an increase in colocalization between TrkB and lysotracker as the duration of the treatment increased, suggesting that p75NTR may have a role in diverting TrkB form the lysosome to the endosomal pathway and the directing towards the activation of survival pathways.

The binding of BDNF to TrkB and p75NTR is known to activate the PI3K/Akt pathway. Therefore, we examined the localization of pAkt, the downstream effector, in different endosomal compartments. So far, the findings showed that in the early endosome, there was no colocalization between pAkt and Rab5 after BDNF treatment in the wild type neurons. However, colocalization between pAkt and Rab11 increased after 15 minutes of BDNF treatment. Interestingly, the detection of pAkt signaling in recycling endosome is consistent with previous data of the trafficking of pTrkB to the recycling endosome. Finally, colocalization between pAkt and Rab7 was observed after 60 minutes of BDNF treatment. It has been demonstrated that the association of Rab7 and TrkB are involved in retrograde transport of endosomes by cytoplasmic dynein, promoting neuronal survival. (Mitchell et al., 2012; Heersen, Pazyra, and Segal, 2004; Beattie et al., 1996; Claude et al., 1982; Watson et al., 1999; Korsching and Thoenen, 1983).

Although, these data illustrated the colocalization of pTrkB and pAkt with the Rab GTPases and lysotracker, these independent experiments had small sample sizes. Therefore, we cannot draw a definite conclusion yet based on the colocalization analysis. However, additional wild type and p75<sup>-/-</sup> experiments of the colocalization of pTrkB and pAkt with the Rab GTPases and lysotracker will be conducted and the outcome of the data will be compared to observe any differences or similarities of the endosomal transport of TrkB and pAkt. Also, we will examine another downstream effector of the MAP kinase pathway, pErk, that is activated by Trk receptors and Ras GTPases (Borasio et al., 1993; Nobes and Tolkovsky., 1995; Bonni et al., 1999; Kaplan and Miller., 2000; Bucci, Alifano, and Cogil., 2014). Phosphorylated by MEK, pErk transduces intracellular signals from the cell surface thorough phosphorylation of substrates and are involved cell survival and differentiation (English et al., 1999; Wortzel and Seger, 2011; Roskoski Jr. 2012). By then, we can determine the factors that regulate the signaling pathways for survival and death and whether the association of p75NTR or the influence under BDNF signaling controls the internalization of TrkB trafficking to the endosomal compartments.

#### References

- Al-Qudah, M. et al. "Brain-derived neurotrophic factor enhances cholinergic contraction of longitudinal muscle of rabbit intestine via activation of phospholipase C." <u>American Journal of Physiology-Gastrointestinal and Liver Physiology</u> 306 Feb 2014: G328-G337. Dana Lib., Newark, NJ. Web. 15 May. 2014.
   <a href="http://ajpgi.physiology.org/content/306/4/G328">http://ajpgi.physiology.org/content/306/4/G328</a>>.
- Beattie, E.C. et al. "A signaling endosome hypothesis to explain NGF actions: potential implications for neurodegeneration." <u>Cold Spring Harb. Symp. Quant. Biol.</u> 61 1996: 389-406. NCBI. Dana Lib., Newark, NJ. Web. 15 Nov. 2014. < <u>http://www.ncbi.nlm.nih.gov/pubmed/9246468?dopt=Abstract&holding=npg></u>.
- Bibel, Miriam, Edmund Hoppe, Yves-Alain Barde. "Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR." <u>The EMBO Journal</u> 18.3 Feb 1999: 616-622. EMBO Press. Dana Lib., Newark, NJ. Web. 27 Oct. 2014. < <u>http://emboj.embopress.org/content/18/3/616>.</u>
- Bonni, Azad et al. "Cell Survival Promoted by the Ras-MAPK Signaling Pathway by Transcription-Dependent and -Independent Mechanisms ." <u>Science</u> 286.5443 Nov 1999: 1358-1362. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://www.sciencemag.org/content/286/5443/1358.long</u>>.
- Borasio, Gian D. et al. "Involvement of ras p21 in neurotrophin-induced response of sensory but not sympathetic neurons." <u>The Journal of Cell Biology</u> 121.3 May 1993: 665-672. PMC. Dana Lib., Newark, NJ. Web. 28 Nov. 2014.
  <a href="http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2119571/">http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2119571/</a>>.
- Bronfman, Francisca et al. "Endosomal transport of neurotrophins: Roles in signaling and neurodegenerative diseases." <u>Developmental Neurobiology</u> 67.9 August 2007: 1183-1203. Wiley Online Library. Dana Lib., Newark, NJ. Web. 7 May. 2014. <<u>http://onlinelibrary.wiley.com/doi/10.1002/dneu.20513/abstract></u>.
- Brunet, Anne et al. "Akt Promotes Cell Survival by Phosphorylating and Inhibiting a Forkhead Transcription Factor ." <u>Cell</u> 96.6 Mar 1999: 857-868. Science Direct. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://www.sciencedirect.com/science/article/pii/S0092867400805954</u>>.
- Bucci, Cecilia, Pietro Alifano, and Laura Cogli." The Role of Rab Proteins in Neuronal Cells and in the Trafficking of Neurotrophin Receptors." <u>Membranes</u> 4.4 Oct. 2014: 642-677. Dana Lib., Newark, NJ. Web. 26 Nov. 2014. <<u>http://www.mdpi.com/2077-0375/4/4/642/htm</u>>.
- Datta, Sandeep R., Anne Brunet, and Michael E. Greenberg. "Cellular survival: a play in three Akts." <u>Genes & Development</u> 13 1999: 2905-2927. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://genesdev.cshlp.org/content/13/22/2905.long</u>>.

- Dechant, G. et al. "Expression and binding characteristics of the BDNF receptor chick trkB. Development." Development 119.2 Oct. 1993: 545-558. Dana Lib., Newark, NJ. Web. 25 Nov. 2014. < http://dev.biologists.org/content/119/2/545.long>.
- Di Fiore, PP and P. De Camilli. "Endocytosis and signaling. An inseparable relationship." Cell 106.1 July 2001: 1-4. Science Direct. Dana Lib., Newark, NJ. Web. 7 May. 2014. < http://www.sciencedirect.com/science/article/pii/S0092867401004287>.

- Di Guglielmo, G.M. et al. "Compartmentalization of SHC, GRB2 and mSOS, and hyperphosphorylation of Raf-1 by EGF but not insulin in liver parenchyma." The EMBO Journal 13.18 Sept 1994: 4269-4277. PMC. Dana Lib., Newark, NJ. Web. 27 Oct. 2014. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC395354/>.
- Dunn, Kenneth W., Malgorzata M. Kamocka, and John H. McDonald. "A practical guide to evaluating colocalization in biological microscopy." American Journal of Physiology Cell Physiology 300 Apr 2011: C723-C742. Dana Lib., Newark, NJ. Web. 7 May. 2014. http://ajpcell.physiology.org/content/300/4/C723.long.
- English, Jessie et al. "Neuroprotective New Insights into the Control of MAP Kinase Pathways." Experimental Cell Research 253.1 Nov 1999: 255–270. Science Direct. Dana Lib. Newark, NJ. Web. 27 Oct. 2014. < http://www.sciencedirect.com/science/article/pii/S0014482799946876>.
- Farinelli, Stephen E., Lloyd A. Greene, and Wilma J. Friedman."Neuroprotective Actions of Dipyridamole on Cultured CNS Neurons." Journal of Neuroscience 18.14 Jul 1998: 5112-5123. Dana Lib. Newark, NJ. Web. 8 May. 2014. < http://www.jneurosci.org/content/18/14/5112.full>.
- Florez-McClure, M.L. et al."The p75 neurotrophin receptor can induce autophagy and death of cerebellar Purkinje neurons." Journal of Neuroscience 24.19 May 2004: 4498-4509. Dana Lib., Newark, NJ. Web. 8 May. 2014. < http://www.jneurosci.org/content/24/19/4498.long>.
- Friedman, W.J. et al. "Differential Actions of Neurotrophins in the Locus Coeruleus and Basal Forebrain." Experimental Neurobiology 119.1 Jan 1993: 72-78. Science Direct. Dana Lib., Newark, NJ. Web. 8 May. 2014. < http://www.sciencedirect.com/science/article/pii/S0014488683710071>.
- Grimes, Mark L. Eric Beattie, and William C. Mobley. "A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA." Proceedings of the National Academy of Sciences of the United States of America 94.18 Sept. 1997: 9909–9914. Dana Lib., Newark, NJ. Web. 26 Nov. 2014. < http://www.pnas.org.proxy.libraries.rutgers.edu/content/94/18/9909.full>.
- Grimes, Mark L. et al."Endocytosis of Activated TrkA: Evidence that Nerve Growth Factor Induces Formation of Signaling Endosomes." The Journal of Neuroscience 16.24 Dec. 1996: 7950-7964. Dana Lib., Newark, NJ. Web. 26 Nov. 2014. < http://www.jneurosci.org.proxy.libraries.rutgers.edu/content/16/24/7950.full>.

- He, Yan-Yan et al. "Role of BDNF in Central Motor Structures and Motor Diseases." <u>Molecular Neurobiology</u> 48.3 Dec 2013: 783-793. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://link.springer.com/article/10.1007%2Fs12035-013-8466-y</u>>.
- Hallböök, Finn. "Evolution of the vertebrate neurotrophin and Trk receptor gene families." <u>Current Opinion in Neurobiology</u> 9.5 Oct 1999: 616-621. Science Direct. Dana Lib., Newark, NJ. Web. 27 Oct. 2014. ≤ http://www.sciencedirect.com/science/article/pii/S0959438899000112>.
- Hallböök, Finn et al. "Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in xenopus ovary." <u>Neuron</u> 6.5 May 1991: 845-858. Science Direct. Dana Lib., Newark, NJ. Web. 27 Oct. 2014.
  <a href="http://www.sciencedirect.com/science/article/pii/0896627391901808">http://www.sciencedirect.com/science/article/pii/0896627391901808</a>>.
- Heerssen, Heather M., Maria F. Pazyra, and Rosalind A. Segal. "Dynein motors transport activated Trks to promote survival of target-dependent neurons". <u>Nature Neuroscience</u> 7 May 2004: 596 - 604. Dana Lib., Newark, NJ. Web. 15 Nov. 2014. < <u>http://www.nature.com/neuro/journal/v7/n6/full/nn1242.html#B7>.</u>
- Huang, E.J. and L.F. Reichardt. "TRK Receptors: Roles in neuronal signal transduction." <u>Annual Reviews Biochemistry</u> 72 Mar 2003: 609-642. Dana Lib., Newark, NJ. Web. 7 May. 2014. < <u>http://www.annualreviews.org/doi/full/10.1146/annurev.biochem.72.121801.1</u> <u>61629?url\_ver=Z39.88-</u> 2003&rfr\_id=ori:rid:crossref.org&rfr\_dat=cr\_pub%3dpubmed>.
- Ip, Nancy Y. et al. "Cultured Hippocampal Neurons Show Responses to BDNF, NT-3, and NT-4, but Not NGF." <u>The Journal of Neuroscience</u> 13.8 Aug. 1993: 3394-3305. Dana Lib., Newark, NJ. Web. 25 Nov. 2014. < <u>http://www.jneurosci.org/content/13/8/3394.long>.</u>
- Jaworski, J. et al. "Control of dendritic arborization by the phosphoinositide-3'-kinase-Akt-mammalian target of rapamycin." <u>The Journal of Neuroscience</u> 25.49 Dec 2005: 11300-11312. Dana Lib., Newark, NJ. Web. 7 May. 2014. <<u>http://www.jneurosci.org/content/25/49/11300.long>.</u>
- Jordens et al. "Rab proteins, connecting transport and vesicle fusion." <u>Traffic 6.12 Nov</u> 2005: 1070-1077. Wiley Online Library. Dana Lib., Newark, NJ. Web. 7 May. 2014. <<u>http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0854.2005.00336.x/abstract;jsessionid=F88AC745962BFC38CA78844EDBD3C13F.f</u> 03t02>.
- Kaplan, David R and Freda D. Miller. "Neurotrophin signal transduction in the nervous system." <u>Current Opinion in Neurobiology</u> 10.3 Jun 2000: 381–391. Science Direct. Dana Lib., Newark, NJ. Web. 27 Oct. 2014. < <u>http://www.sciencedirect.com/science/article/pii/S0959438800000921></u>.
- Korsching, Sigrun and Hans Thoenen." Quantitative demonstration of the retrograde axonal transport of endogenous nerve growth factor." <u>Neuroscience Letters</u> 39.1 Aug.

1983: 1-4. Science Direct. Dana Lib., Newark, NJ. Web. 15 Nov. 2014. <<u>http://www.sciencedirect.com/science/article/pii/0304394083901556></u>.

- Kumar, V. et al. "Regulation of dendritic morphogenesis by Ras-PI3k-Akt-mTor and Ras-MAPK signaling pathways." <u>The Journal of Neuroscience</u> 25.49 Dec 2005: 11288-11299. Dana Lib., Newark, NJ. Web. 7 May. 2014. <<u>http://www.jneurosci.org/content/25/49/11288.long>.</u>
- Lazo, Oscar M. et al. "BDNF Regulates Rab11-Mediated Recycling Endosome Dynamics to Induce Dendritic Branching." <u>The Journal of Neuroscience</u> 33.14 Apr 2013: 6112-6122. Dana Lib., Newark, NJ. Web. 7 May. 2014. <<u>http://www.jneurosci.org/content/33/14/6112.long>.</u>
- Liu, Xuesong et al. "Induction of Apoptotic Program in Cell-Free Extracts: Requirement for dATP and Cytochrome c ." <u>Cell</u> 86.1 Jul 1996: 147-157. Science Direct. Dana Lib., Newark, NJ. Web. 15 May. 2014. http://www.sciencedirect.com/science/article/pii/S0092867400800859#>.
- Manders, E.M.M, F.J. Verbeek, and J.A. Aten. "Measurement of colocalization of objects in dual-color confocal images." <u>Journal of Microscopy</u> 169.3 Mar 1993: 375-382. Dana Lib., Newark, NJ. Web. 7 May. 2014. <a href="http://fiji.sc/wiki/images/2/24/Manders.pdf">http://fiji.sc/wiki/images/2/24/Manders.pdf</a>>.
- Miaczynska, M., L. Pelkmans, and M. Zerial. "Not just a sink: Endosomes in control of signal transduction." <u>Current Opinion in Cell Biology</u> 16.4 Aug 2004: 400-406.
  Science Direct. Dana Lib., Newark, NJ. Web. 7 May. 2014.
  <a href="http://www.sciencedirect.com/science/article/pii/S0955067404000742">http://www.sciencedirect.com/science/article/pii/S0955067404000742</a>>.
- Minichiello, Liliana et al. "Point Mutation in trkB Causes Loss of NT4-Dependent Neurons without Major Effects on Diverse BDNF Responses." <u>Neuron</u> 21.2 Aug 1998: 335–345. Science Direct. Dana Lib., Newark, NJ. Web. 27 Oct. 2014. <<u>http://www.sciencedirect.com/science/article/pii/S0896627300805437></u>.
- Mitchell, David J. et al. "Trk Activation of the ERK1/2 Kinase Pathway Stimulates Intermediate Chain Phosphorylation and Recruits Cytoplasmic Dynein to Signaling Endosomes for Retrograde Axonal Transport." <u>The Journal of Neuroscience</u> 32.44 Oct. 2012: 15495-15510. Dana Lib., Newark, NJ. Web. 15 Nov. 2014. < http://www.jneurosci.org/content/32/44/15495.long>.
- Njkjaer, Anders. et al. "Sortilin is essential for pro-NGF-induced neuronal cell death." <u>Nature</u> 427 Feb 2004: 843-848. Dana Lib., Newark, NJ. Web. 16 May. 2014. < <u>http://www.nature.com/nature/journal/v427/n6977/full/nature02319.html</u>>.
- Nobes, Catherine D. and Aviva M. Tolkovsky. "Neutralizing Anti-p21ras Fabs Suppress Rat Sympathetic Neuron Survival Induced by NGF, LIF, CNTF and cAMP." <u>European Journal of Neuroscience</u> 7.2 Feb. 1995: 344–350. Wiley Online Library. Dana Lib., Newark, NJ. Web. 28 Nov. 2014. < http://onlinelibrary.wiley.com/doi/10.1111/j.1460-9568.1995.tb01069.x/full>.

- Ohta, H. et al. "Neurotrophic effects of a cyanide dye via the PI3K-Akt pathway: attenuation of motor discoordination and neurodegeneration in an ataxic animal model." <u>PLoS</u> 6.2 Feb 2011: 1-12. Ebscohost. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://eds.b.ebscohost.com/ehost/detail?sid=3db1f9c7-afaf-4138-9923-</u> <u>d09bb7ce259a%40sessionmgr112&vid=1&hid=105&bdata=JnNpdGU9ZWhvc3QtbG</u> 12ZQ%3d%3d#db=aph&AN=73937616>.
- Patapoutian, Ardem and Louis F. Reichardt. "Trk receptors: mediators of neurotrophin action ." <u>Current Opinion in Neurobiology</u> 11.3 Jun 2001: 272-280. Science Direct. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://www.sciencedirect.com/science/article/pii/S0959438800002087>.</u>
- Philippa, Claude et al. "Binding, internalization, and retrograde transport of 125I-nerve growth factor in cultured rat sympathetic neurons." <u>The Journal of Neuroscience</u> 2.4 Apr. 1982: 431-442. Dana Lib., Newark, NJ. Web. 15 Nov. 2014. < <u>http://www.jneurosci.org/content/2/4/431.long></u>.
- Roskoski Jr., Robert. "ERK1/2 MAP kinases: Structure, function, and regulation." <u>Pharmacological Research</u> 66.2 Aug 2012: 105-143. Science Direct. Dana Lib., Newark, NJ. Web. 24 Oct. 2014. <<u>http://www.sciencedirect.com/science/article/pii/S1043661812000977</u>>.
- Roux, P.P. and P.A. Barker. "Neurotrophin signaling through the p75 neurotrophin receptor." <u>Progress in Neurobiology</u> 67.3 Jun 2002: 203-233. Science Direct. Dana Lib., Newark, NJ. Web. 8 May. 2014.
  <a href="http://www.sciencedirect.com/science/article/pii/S0301008202000163">http://www.sciencedirect.com/science/article/pii/S0301008202000163</a>>.
- Schimmöller, Frauke, Iris Simon, and Suzanne R. Pfeffer. "Rab GTPases, Directors of Vesicle Docking." <u>The Journal of Biological Chemistry</u> 273 Aug 1998: 22161-22164. Science Direct. Dana Lib., Newark, NJ. Web. 27 Oct. 2014. <u>< http://www.jbc.org/content/273/35/22161.long>.</u>
- Sciarretta, Carla et al. "PLCγ-activated signalling is essential for TrkB mediated sensory neuron structural plasticity." <u>BMC Developmental Biology</u> 10.103 Oct 2010: 1-15. Dana Lib., Newark, NJ. Web. 15 May. 2014. < <u>http://www.biomedcentral.com/1471-213X/10/103</u>>.
- Shindler, Kenneth S. et al. "Trophic support promotes survival of bcl-x-deficient telencephalic cells in vitro ." <u>Cell Death and Differentiation</u> 5.10 Oct 1998: 901-910. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://www.nature.com/cdd/journal/v5/n10/abs/4400421a.html></u>.
- Song, Wenyu et al. "ProNGF Induces PTEN via p75NTR to Suppress Trk-Mediated Survival Signaling in Brain Neurons." <u>Journal of Neuroscience</u> 30.46 Nov 2010: 15608-15615. Dana Lib., Newark, NJ. Web. 8 May. 2014. <<u>http://www.jneurosci.org/content/30/46/15608.long#sec-2</u>>.
- Sorkin A. and M. Von Zastrow. "Signal transduction and endocytosis: Close encounters of many kinds." <u>Nature Reviews Molecular Biology</u> 3 Aug 2002: 600-614. Dana Lib.,

Newark, NJ. Web. 7 May. 2014. < <u>http://www.nature.com/nrm/journal/v3/n8/abs/nrm883.html>.</u>

- Squinto, S.P. et al." trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor." <u>Cell</u> 65.5 May 1991: 885–893. Science Direct. Dana Lib., Newark, NJ. Web. 25 Nov. 2014. <<u>http://www.sciencedirect.com/science/article/pii/009286749190395F></u>.
- Stefanis, Leonidas et al."Caspase-Dependent and –Independent Death of Camptothecin-Treated Embryonic Cortical Neurons." <u>Journal of Neuroscience</u> 19.15 Aug 1999: 6235-6247. Dana Lib., Newark, NJ. Web. 8 May. 2014. < <u>http://www.jneurosci.org/content/19/15/6235.full</u>>.
- De Renzis, Stefano, Birte Sönnichsen & Marino Zerial. "Divalent Rab effectors regulate the sub-compartmental organization and sorting of early endosomes" <u>Nature Cell</u> <u>Biology</u> 4 Jan 2002: 124 - 133. Dana Lib., Newark, NJ. Web. 27 Oct. 2014 <u>http://www.nature.com/ncb/journal/v4/n2/full/ncb744.html</u>>.
- Troy, Carol M. J.E. Friedman, and W.J. Friedman. "Mechanisms of p75-mediated Death of Hippocampal Neurons: Role of Caspases." <u>The Journal of Biological Chemistry</u> 277 Sept 2002: 34295-34302. Dana Lib., Newark, NJ. Web. 15 May. 2014. <<u>http://www.jbc.org/content/277/37/34295.full</u>>.
- Volosin, Marta et al."Induction of Proneurotrophins and Activation of p75NTR-Mediated Apoptosis via Neurotrophin Receptor-Interacting Factor in Hippocampal Neurons after Seizures." <u>Journal of Neuroscience</u> 28.39 Sept 2008: 9870-9879. Dana Lib., Newark, NJ. Web. 8 May. 2014. <<u>http://www.jneurosci.org/content/28/39/9870.long#sec-2</u>>.
- Volosin, Marta et al."Interaction of Survival and Death Signaling in Basal Forebrain Neurons: Roles of Neurotrophins and Proneurotrophins." <u>Journal of Neuroscience</u> 26.29 Jul 2006: 7756-7766. Dana Lib., Newark, NJ. Web. 8 May. 2014.
   <a href="http://www.jneurosci.org/content/26/29/7756.long#sec-2">http://www.jneurosci.org/content/26/29/7756.long#sec-2</a>>.
- Watson, Fiona L. et al. "Rapid Nuclear Responses to Target-Derived Neurotrophins Require Retrograde Transport of Ligand–Receptor Complex." <u>The Journal of</u> <u>Neuroscience</u> 19.18 Sept.1999: 7889-7900. Dana Lib., Newark, NJ. Web. 15 Nov. 2014 <<u>http://www.jneurosci.org/content/19/18/7889.long</u>>.
- Wortzel, Inbal and Rony Seger. "The ERK cascade: distinct functions within various subcellular organelles." <u>Genes Cancer</u> 2.3 Mar 2011:195-209. PMC. Dana Lib., Newark, NJ. Web. 24 Oct. 2014. <<u>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3128630/</u>>.
- Yuan, Junying and Bruce A. Yankner. "Apoptosis in the nervous system ." <u>Nature</u> 407 Oct 2000: 802-809. Dana Lib., Newark, NJ. Web. 8 May. 2014. <<u>http://www.nature.com/nature/journal/v407/n6805/full/407802a0.html</u>>.

- Zerial, Marino & Heidi McBride. "Rab proteins as membrane organizers." <u>Nature</u> <u>Reviews Molecular Cell Biology</u> 2 Feb 2001: 107-117. Dana Lib., Newark, NJ. Web. 27 Oct. 2014. < <u>http://www.nature.com/nrm/journal/v2/n2/full/nrm0201\_107a.html</u>>.
- Zheng, J. et al. "Clathrin-dependent endocytosis is required for TrkB-dependent Aktmediated neuronal protection and dendritic growth." <u>The Journal of Biological</u> <u>Chemistry</u> 283 May 2008: 13280-13288. Dana Lib., Newark, NJ. Web. 7 May 2014. <u>http://www.jbc.org/content/283/19/13280.long.</u>

## Appendix

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