Role of p75 Neurotrophin Receptor in the development of neuronal and oligodendroglial progenitors of the rat postnatal Subventricular Zone

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A dissertation submitted to the

Graduate School-Newark

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Biological Sciences

Written under the direction of

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And approved by

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Newark, New Jersey

May, 2022
ABSTRACT OF THE DISSERTATION

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The p75 neurotrophin receptor is widely expressed in the brain during embryonic development (Frade & Barde, 1998). In neonatal and adult ages its expression in the central nervous system gets restricted to specific cell populations including cholinergic neurons of the basal forebrain, olfactory ensheathing glia, progenitors of the hippocampus and progenitors of the cerebellum where it plays a multitude of roles. While some studies have also reported that the subventricular zone (SVZ) expresses p75NTR in postnatal and adult stages (Galvão, RP, Garcea-Verdugo, JM, Alvarez-Buylla, 2008; Giuliani et al., 2004; van Strien et al., 2014; Young, Merson, et al., 2007), its exact role in this germinal niche remains unknown. Further, the expression of p75NTR has been reported at various ages in the rat and human SVZ but it is not observed in mice (Galvão, RP, Garcea-Verdugo, JM, Alvarez-Buylla, 2008). Although species differences remain unexplained, no comprehensive studies of p75NTR, regarding its role in the rat or human SVZ, have been conducted thus far.

The SVZ serves as a neurogenic niche for the olfactory bulb in adult animals (Alvarez-Buylla et al., 2002; Bath & Lee, 2010; Faigle & Song, 2013). For a brief period postnatally, it also generates glial cells, predominantly oligodendrocytes for the corpus callosum, cortex and striatum (Kessaris et al., 2006.; Kuhn et al., 2019; Luskin & McDermott, 1994; Menn et al., 2006; Naruse et al., 2017). Initial characterization showed
expression of p75NTR in the dorsolateral SVZ throughout postnatal development in rats with maximal expression observed during the period of gliogenesis. Therefore, I hypothesized that p75NTR may be involved in the regulation of SVZ progenitor development during postnatal development.

This study shows that p75NTR regulates both neuronal and oligodendroglial progenitors in the postnatal SVZ. Progenitors expressing p75NTR proliferate longer than cells in the dSVZ that lack p75NTR. I also determine that in the absence of p75NTR, postnatal oligodendrocyte progenitor development was accelerated in comparison to that observed under normal conditions. Consequently, I observed premature maturation of oligodendrocytes at postnatal ages which could have implications for myelination.

Furthermore, I describe a role for p75NTR in neurogenesis where p75NTR defines a specific neuronal subpopulation expressing the transcription factor Pax6. I show that, postnatally, p75NTR is expressed by proliferating cells present in the rostral migratory stream extending from the lateral ventricle to the olfactory bulb. In younger animals, absence of p75NTR did not alter the cytoarchitecture of the olfactory bulb and did not affect odor discrimination. However, lack of p75NTR functionally affected odor discrimination in aged rats and led to changes in the olfactory bulb circuitry.

Overall, this study defines a novel role for the multifunctional receptor p75NTR in the SVZ in regulation of oligodendrocyte progenitors in vivo during postnatal development. Further, I provide evidence that the role of p75NTR is not limited to a single subset of oligodendrocyte lineage committed progenitors but could have additional roles in olfactory bulb neurogenesis as a result of ageing.
Acknowledgements

I would like to thank my advisor, Dr. Wilma Friedman, for all her guidance, support, and insights throughout my graduate career. Thank you for letting me stubbornly stick with projects and for encouraging and motivating me whenever I needed it. I would also like to extend my thanks to members of my thesis committee who have helped shape my project, Dr. Tran, Dr. Kim, Dr. Levison, Dr. Wood and for a brief period Dr. Etchegaray. Thank you for guiding me in the right direction and for motivating me throughout. I would also like to thank Dr. Shiflett for all his help with the behavioral experiments in my project.

I owe enormous thanks to Dr. Juan Zanin for being a mentor, teacher, friend, and confidant. He trained me and continues to be invested in my progress, both professional and personal. I am equally thankful to Dr. Laura Montroull who helped me refine my skills professionally and is one of my closest friends. Laura, you made surviving and thriving in difficult situations infinitely easier. I admire you both immensely and will always take all your advice with me whenever I go. It would be remiss on my part to not mention Srestha Dasgupta, a fellow graduate student in the lab and very close friend. I thank you for all the brainstorming sessions, coffee breaks, advice, and mad jokes. I could not have asked for a better cohort of people to exist with during my graduate studies.

Lastly, I want to thank all my friends and family, my mum and dad, my sister, and my grandparents for all their encouragement, motivation, love, and support. I also want to thank Jiaxin, Swayam, Subhash and Manpreet for being my family so far from home.
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I. Introduction

1. Development of the Subventricular zone

The subventricular zone (SVZ) is a germinal niche surrounding the lateral ventricles in the forebrain present in postnatal and adult mammals. The SVZ matures from the ventricular zone (VZ) which is present embryonically and is a site of neurogenesis and gliogenesis (Levison & Goldman, 1993; Paterson et al., 1973). The VZ is composed of cells with a bipolar, elongated morphology known as radial glial cells (RG). RG cells contact the ventricle at their basal end and the developing neuroepithelium at the apical end.

RG cells are the principal neural stem cells (NSC) that generate neurons and astrocytes for developing cortex through direct transformation throughout embryonic development (Kriegstein & Alvarez-Buylla, 2009). In postnatal and adult stages, RG cells gradually acquire an astrocytic morphology and form type B1 astrocytic cells that continue to function as NSCs. Type B1 cells consist of a subset of quiescent NSCs that express glial fibrillary acidic protein (GFAP) and a subset of active NSCs that express nestin, a class VI intermediate filament protein, brain lipid-binding protein (BLBP or Fabp7) and epidermal growth factor receptor (EGFR) (Codega et al., 2014; Giachino et al., 2014). Type B1 cells are characterized as a slowly dividing stem cell population. Aside from producing a small proportion of nonneurogenic astrocytes, activated type B1 cells generate rapidly dividing, transit amplifying or type C intermediate progenitor cells. Type C cells have a shorter cell-cycle as determined by pulse chase studies utilizing thymidine analogs such as $[^3]$H-thymidine or 5-bromo-2’-deoxyuridine (BrdU) to label the dividing cells in the S-phase of the cell cycle in vivo (Doetsch et al., 1997, 2002). Type C cells can be of both neuronal and oligodendroglial lineage. NSCs of the neuronal lineage are characterized by markers such
as achaete-scute homolog 1 (Acs11 or Mash1) (Parras et al., 2004), transcription factors Dlx1 and Dlx2 (Petryniak et al., 2007), paired box protein 6 (Pax6) (Brill et al., 2008; Kohwi et al., 2005) and T-box brain protein 2 (Tbr2) (Englund et al., 2005; Hevner, 2019). While Mash1 also defines cells of the oligodendroglial lineage (Parras et al., 2004), oligodendrocyte transcription factor 2 (Olig2) serves as a more specific marker (Menn et al., 2006). RG cells also produce multiciliate ependymal or type E cells which line the lateral ventricles and contact the circulating cerebrospinal fluid separating the developing SVZ from the CSF. Additionally, NSCs of the SVZ are responsible for olfactory bulb (OB) neurogenesis and generate type A migrating neuroblasts that migrate rostrally to the OB and give rise to several subtypes of inhibitory interneurons (Fiorelli et al. 2015).

Reporter mouse lines and retroviral lineage tracing studies have outlined the migration of NSCs from the SVZ and reveal differences in NSC migratory patterns at different stages of development (Azim et al., 2012; Levison et al., 1993). While majority of the anterior SVZ NSCs migrate rostrally to the OB, caudal NSCs can undergo both tangential as well as radial migration colonizing the overlaying white and gray matter. SVZ NSCs cells labeled at postnatal day 2 (P2) colonize both gray and white matter while NSCs retrovirally labeled at P14 show restricted migration to the central and medial white matter (Levison et al., 1993) consistent with migration of oligodendroglial progenitors from the SVZ during the postnatal gliogenesis period.

Progenitors from the SVZ can express more than one stem cell marker at the same time (Zhang & Jiao, 2015) or change the expression of specific markers as they become different cell types (Englund et al., 2005). The complexity of the postnatal or adult SVZ is enhanced by lineage specific microdomains (Fiorelli et al., 2015) and localization of cells in the SVZ.
along the rostro-caudal axis (Azim et al., 2012) as well as the dorsal, medial and lateral walls (Fiorelli et al., 2015). In the rostral SVZ the dorsal, medial and lateral walls generate neurons. However, in the caudal SVZ only the lateral walls are neurogenic (Azim et al., 2012). Studies utilizing proliferation markers such as Ki67, or thymidine analogs elucidate the differences of NSCs based on their cycling properties throughout the SVZ. In adult mice, a greater number of Ki67 expressing cells were found in the rostral SVZ relative to the caudal SVZ and very few Ki67 expressing cells were detected medially (Azim et al., 2012). In another independent study it was reported that anterior SVZ progenitor cells have a shorter cell cycle of approximately 14 hours, while posterior or caudal regions of the SVZ, believed to be predominantly gliogenic, have a longer cell cycle rate of 18.6 hours (Smith & Luskin, 1998) which further elucidates the heterogeneity of NSCs this brain region.

2. SVZ and gliogenesis

Typically, in rodents, oligodendrocyte progenitor cells (OPCs) from the SVZ undergo expansion approximately a week after birth and begin to migrate to the overlaying white matter. Lineage tracing studies show that cells from the postnatal SVZ can generate both oligodendrocyte (OL) and astrocytes. NSCs migrate from the SVZ to the cortex where they differentiate into OLs and astrocytes in tightly-knit clusters. A majority of the NSCs that migrate to the corpus callosum yield OLs and generate very few astrocytes (Levison & Goldman, 1993). After migration, OPCs sit along fiber tracts that will later become white matter tracts and transform into pre-oligodendrocytes (pre-OLs) that have multiple processes. Pre-OLs then differentiate into immature OLs which go onto become fully mature myelinating OLs by turning on the expression of myelin genes (Kuhn et al., 2019).
As OPCs progress through the OL lineage they express specific markers that reflect their developmental stage. Transcription factors such as oligodendrocyte transcription factor 1 (Olig1) and oligodendrocyte transcription factor 2 (Olig2) are expressed throughout OL development. OPCs can be further characterized by cell surface markers such as A2B5, platelet derived growth factor receptor- alpha (PDGFRα) and proteolipid protein (PLP). Pre-OLs express O4, 2’, 3’-cyclic-nucleotide 3’-phosphodiesterase (CNPase) and O1. Mature myelinating OLs express myelin associated proteins such as myelin basic protein (MBP), myelin associated glycoprotein (MAG), and galactocerebroside (GalC) (Baumann & Pham-Dinh, 2001). An additional, novel, myelin associated protein called breast carcinoma amplified sequence 1 (BCAS1) has recently been described as a marker for myelinating OLs (Ishimoto et al., 2017).

Several factors regulate both the total numbers as well as the differentiation process of OPCs along the OL lineage. Intrinsic epigenetic and transcriptional factors define lineage specification (Emery & Lu, 2015). Olig2 has been reported to actively repress the genes required for promoting NSCs to differentiate towards the neuronal lineage (Hack et al., 2004; Marshall et al., 2005). A number of cytoskeletal proteins (Nawaz et al., 2015) and growth factors (el Waly et al., 2014) including platelet derived growth factor (PDGF) (Fruttiger et al., 1999), epidermal growth factor (EGF) (Gonzalez-Perez et al., 2009; Yang et al., 2017), fibroblast growth factor (FGF) (Fortin et al., 2005; Bögler et al. 1990; McKinnon et al. 1990), insulin-like growth factor 1 (Mozell & McMorris, 1991), neuregulins (Canoll et al., 1996) and neurotrophins (Barres, Raff, et al., 1994; Du et al., 2003) promote survival, migration and differentiation of OPCs under normal as well as pathological conditions.
OPCs can divide only a limited number of times before they exit the cell cycle and start to progress through the OL lineage (Temple & Raff, 1986). OPC differentiation is strongly associated with withdrawal of the cells from the cell cycle. Studies conducted on OPCs isolated from postnatal rat optic nerve showed that a cell intrinsic timer directs the time for which an OPC proliferates and subsequently stops before initiating differentiation (Ibarrola et al., 1996). PDGF is required for the timing component of this intrinsic developmental clock Noble et al., 1988; Raff et al., 1988) while thyroid hormone regulates the differentiation process (Barres, Lazar, et al., 1994). It has been observed that in the absence of PDGF OPCs stop dividing and prematurely differentiate into OLs (Noble et al., 1988; Temple & Raff, 1986). In the absence of thyroid hormones, but the presence of PDGF in vitro, OPCs continue to proliferate and do not initiate differentiation (Barres, Lazar, et al., 1994; Gao et al., 1998). There is evidence that the cyclin-dependent kinase inhibitor P27/Kip1 (Durand et al., 1997) and the b1 thyroid hormone receptor (Gao et al., 1998) are both involved in regulating the timing of OPC differentiation.

Each fully differentiated OL can myelinate multiple axons. Contact dependent axonal signals including interaction with specific cell adhesion molecules facilitate OL survival and act as positive signals for myelination (Barres & Raff, 1999; Bozzali & Wrabetz, 2004; Coman et al., 2005).

3. **SVZ and neurogenesis**

In the first three weeks of postnatal development, as the RG cells are differentiating into ependymal cells, type B1 NSCs and type A neuroblasts, the open olfactory ventricle present embryonically closes to form the rostral migratory stream (RMS) (Peretto et al., 2005) and
all the mitotically active cells that are distributed along the lateral ventricle-SVZ-OB axis get confined to the RMS (Lemasson et al., 2005).

Type A cells, or neuroblasts, can be distinguished from other SVZ stem cell populations based on their expression of markers such as doublecortin (DCX) or polysialylated neural adhesion molecule (PSA-NCAM). Neuroblasts from the anterior SVZ migrate in chains through the RMS (Doetsch & Alvarez-Buylla, 1996) to the OB where they disperse radially and differentiate into different populations of interneurons. There are two main populations of OB neurons generated in postnatal or adult stages. These include the dopaminergic or GABAergic granule cells located in the OB granule cell layer and periglomerular interneurons located peripherally in the glomerular layer. Adult-born PGCs are categorized based on their expression of tyrosine hydroxylase (TH), glutamic acid decarboxylase (GAD) or the calcium binding proteins calbindin (CB) and calretinin (CR) (Lledo et al., 2008).

These distinct OB interneurons originate from specific microdomains of the postnatal SVZ. Gene fate-mapping, retroviral injection and electroporation studies have shown that ventral SVZ NSCs preferentially generate the CB expressing PGCs (Fernández et al., 2011; Merkle et al., 2007; Young, Fogarty, et al., 2007), the medial SVZ produces periglomerular interneurons immunoreactive for CR (Fernández et al., 2011; Merkle et al., 2007) and the dorsal SVZ predominantly generates TH expressing PGC interneurons (Brill et al., 2008; de Chevigny et al., 2012; Fernández et al., 2011; Merkle et al., 2007; Young, Fogarty, et al., 2007).

With aging, differences in the timing of production of various populations of PGCs has been observed. At birth, or P0, equivalent numbers of CB, CR and TH expressing cells are
produced. However, very few PV expressing cells are produced. As the animal ages CR+ cells become more predominant while the TH+ cells decline (Batista-Brito et al., 2008). There is a decrease in the total number of proliferating NSCs in rodents by 6-12 months of age (Enwere et al., 2004; Molofsky et al., 2006; Shook et al., 2012), however, the ratio of the newly generated PGC subtypes does not change between 3 months and 12 months, and the loss of NSC population is not lineage dependent (Shook et al., 2012).

4. p75 neurotrophin receptor biology: ligands and co-receptors

The p75 neurotrophin receptor (p75NTR) is a transmembrane receptor member of the tumor necrosis factor family of death receptors that is widely expressed during development of the nervous system (Frade & Barde, 1998). The expression of p75NTR is downregulated in the adult brain (Bibel et al., 2004) and is observed only in certain neuronal populations throughout life, such as the cholinergic cells of the basal forebrain (Volosin et al. 2006). This receptor is re-expressed in pathological conditions such as traumatic brain injury, seizures, ischemia, oxidative stress, axonal injury, and Alzheimer’s disease in adults (Coulson, 2006; Dechant & Barde, 2002).

p75NTR serves as the common receptor for neurotrophins (NT), a family of growth factors, and their immature precursor forms, the pro-neurotrophins (proNT). NTs consist of four ligands - nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). NTs are synthesized as proNTs and undergo cleavage to yield the mature form of the protein (Mowla et al., 2001). Both proNTs and NTs can act as ligands for p75NTR (Hempstead 2006). Mature NTs initiate signaling by binding to a complex of p75NTR and their cognate tropomyosin receptor kinase (Trk) receptors (Friedman & Greene, 1999). NGF typically binds TrkA,
BDNF and NT-4/5 bind TrkB and NT-3 binds TrkC (Chao, 2003; Friedman & Greene, 1999; Hempstead, 2006). In contrast to mature NTs, p75NTR binds proNTs with high affinity through an association with a co-receptor of the sortilin family of cell receptors (Chao, 2003). Signaling through the p75NTR/Trk receptor complexes promotes cell survival, proliferation, differentiation and increasing synaptic plasticity. Instead of survival and differentiation, upon injury, the p75NTR/sortilin complex initiate an apoptotic response to proNT binding (Nykjaer et al., 2004; Volosin et al., 2006).

5. **p75NTR expression in stem cells outside the CNS**

p75NTR is a well-established neural crest stem cell marker (Jiang et al., 2009). It has been used on its own or in combination with other markers to isolate stem cells from neural-crest derived tissues. Outside of the central nervous system (CNS), p75NTR is expressed actively in proliferating human oral mucosal epithelium (Nakamura et al., 2007), esophageal keratinocytes (Okumura et al., 2003), corneal keratinocytes and laryngeal squamous epithelial cells (Li et al., 2012). In human oral keratinocytes, p75NTR expression is mainly restricted to the basal cell layers where keratinocyte stem cells are thought to reside. p75NTR expressing keratinocyte stem cells are found to be Ki67-negative *in vivo* and potentially quiescent. *In vitro*, however, these cells produced clones suggesting a role for p75NTR in maintenance of the quiescent keratinocyte stem cell pool (Nakamura et al., 2007).

Mesenchymal cells also express p75NTR (or CD271). p75NTR expressing mesenchymal stromal cells (MSC) derived from the bone marrow of human donors were found to be multipotent and capable of generating adipogenic, osteogenic and chondrogenic cells (Kuçi et al., 2010). p75NTR expressing MSCs also had an increased proliferative potential *in
vitro compared to a p75NTR deficient MSC population obtained from the same bone marrow samples (Kuçi et al., 2010). Another independent study, however, showed differences in the proliferative ability of MSCs expressing p75NTR sourced from different tissues. Cells obtained from adipose tissue were found to be more proliferative than cells obtained from the bone marrow (Calabrese et al., 2015).

6. p75NTR expression in CNS progenitor cells

In the CNS, p75NTR has been detected in a population of mitotically active cells in the SVZ of rats (Calzà et al., 1998; Giuliani et al., 2004; van Strien et al., 2014) and humans (van Strien et al., 2014), as well as the dentate gyrus of the hippocampus (Catts et al., 2008); the two regions of the brain where neurogenesis persists into adulthood. In the dentate gyrus, absence of p75NTR produced fewer neuroblasts and new neurons as a result of an increase in neuroblast cell death. Further, behavioral experiments on the p75NTR null mice in the same study showed a mild difference in depressive-like behavior (Catts et al., 2008). In adult mice, p75NTR expression is absent in the SVZ (Galvão, RP, Garcea-Verdugo, JM, Alvarez-Buylla, 2008). It is, however, upregulated in migrating neuroblasts, where it interacts with BDNF secreted by blood vessels in the RMS to facilitate migration to the olfactory bulb (Grade et al., 2013). In P2 rats, p75NTR was shown to be involved in increasing the number of neuroblasts in the OB in vivo (Young, Merson, et al., 2007). Cerebellar granule cell precursors during development also express p75NTR robustly. The interaction between p75NTR and proNT-3 regulates withdrawal of the cerebellar granule progenitors from the cell cycle (Zanin et al., 2016).

45% of OLs isolated from the basal forebrain of P1 rats also express p75NTR (Du et al., 2003). These cells can be stimulated to generate MBP expressing mature OLs when treated
with NTs although the differentiation response is not attributed to signaling through p75NTR but through Trk receptors (Du et al., 2003). In disease conditions, in the adult SVZ, p75NTR expression gets upregulated in various populations of OPCs and OLs for remyelination (Dowling et al., 1999; Ladiwala et al., 1998; Petratos et al., 2004).

The studies mentioned so far demonstrate that p75NTR is expressed in numerous progenitor populations and plays a variety of roles in regulating proliferation and differentiation. Although the expression of p75NTR has been noticed in the SVZ of P2 rats (Young, Merson, et al., 2007), the role of p75NTR throughout postnatal developmental has not been thoroughly investigated. Given that neuronal and glial progenitors from the SVZ populate the entire brain during neonatal development (Suzuki & Goldman, 2003) and contribute towards the formation of intricate neuronal circuits, it is important to understand all the factors that may be regulating the development of these cells during brain maturation.
II. Research Aims

Specific Aim 1: To characterize the p75NTR-expressing cells in the postnatal SVZ of rats.

1. To determine the identity of p75NTR-expressing cells in the SVZ by assessing co-expression of established neuronal and glial progenitor cell markers.

2. To assess the mitotic activity of cells expressing p75NTR in the SVZ during postnatal development.

3. To determine the lineage of cells generated by the p75NTR-expressing cells of the SVZ.

Progenitors of the SVZ express multiple transcriptional or protein markers that can identify them into specific lineages (Levison & Goldman, 1997; Marshall et al., 2003; Suzuki & Goldman, 2003). SVZ progenitor cells can also express more than one marker at the same time (Zhang & Jiao, 2015) or change the expression of specific markers as they progress through a lineage (Englund et al., 2005; Kuhn et al., 2019). Further, based on the expression of these markers, SVZ progenitors can be categorized into type A migrating neuroblasts, type B NSCs or type C intermediate progenitors. In this aim I will determine the identity and lineage of the p75NTR expressing cells based on the expression of known SVZ progenitor markers.

Previous reports have shown p75NTR to be expressed in cycling cells in adult rat SVZ (Giuliani et al., 2004). I aim to assess if p75NTR regulates the proliferation of progenitors in the dSVZ during postnatal development.

Progenitors of the postnatal SVZ can generate both neurons and glia. In the human SVZ NSCs isolated based on their expression of p75NTR are found to be multipotent (van Strien et al., 2014). It has also been previously shown that p75NTR expressing cells can be
induced to generate neurons by the exogenous addition of BDNF in vitro (Young, Merson, et al., 2007). However, cells of the SVZ could have access to a combination of NTs and pro-NTs and might not be restricted to differentiating into mainly neurons, especially in postnatal stages. Therefore, I aim to determine the lineage commitment of the p75NTR expressing cells of the rat SVZ and analyze the spontaneous differentiation of the p75NTR expressing cells in the absence any specific, exogenous ligand.

**Specific Aim 2: To determine if the absence of p75NTR affects gliogenesis and myelination during postnatal development in vivo.**

1. To assess if p75NTR KO animals have an altered expression of oligodendrocyte lineage markers in vivo after the gliogenesis phase during postnatal development.

2. To assess myelination in the absence of p75NTR after gliogenesis in vivo.

During the characterization of p75NTR expression in the dSVZ, maximal expression of p75NTR was seen at ages P7 and P10, a period that coincides with gliogenesis in the postnatal rodent brain. Further, a subset of p75NTR expressing cells co-expressed Olig2 in vivo in the dSVZ. Upon differentiation, cells that lack p75NTR generated more Olig2 expressing cells in vitro. In this aim, I will determine if p75NTR influences the OPC population, by analyzing the expression of various OL lineage markers that define the maturation stage of OPCs, in the corpus callosum of postnatal p75NTR KO and WT rats in vivo.

Further, myelin formation in the corpus callosum of postnatal rats occurs between P10 and P14 (Downes & Mullins, 2014a). Changes in the number of OL progenitors in the absence of p75NTR could potentially affect proper myelination in the corpus callosum. Myelin basic protein (MBP) is expressed by mature myelinating OLs (Kuhn et al., 2019). I aim to
assess MBP expression in vivo to determine if there is a change in the terminal differentiation of OLs in the corpus callosum after the period of gliogenesis.

**Specific Aim 3: To investigate if p75NTR influences olfactory bulb neurogenesis in rats**

1. To show that p75NTR-expressing progenitor cells are present in the RMS during postnatal development in rats.
2. To detect differences in interneurons in the OB of postnatal and adult rats comparing WT and p75NTR KO animals.
3. To determine if the absence of p75NTR causes deficits in olfaction in rats.

It is well established that in adult rodents, neuronal progenitors of the SVZ become restricted to generating OB interneurons neurons via migration through the RMS (Bath & Lee, 2010). Pax6 is expressed by progenitor cells shown to give rise to interneurons in the OB (Kriegstein & Alvarez-buylla, 2011; Lim & Alvarez-buylla, 2016). The persistent expression of p75NTR in the SVZ along with expression of Pax6 in a subset of p75NTR expressing cells leads to the hypothesis that this population could be contributing to the interneuron population of the OB. A previous report in adult mice described the expression of p75NTR in DCX expressing migrating neuroblasts in the RMS (Grade et al., 2013). In this aim I will determine if p75NTR expressing cells of the rat SVZ are involved in generating OB interneurons. I also aim to determine if the absence of p75NTR alters the OB circuitry and therefore olfactory sensitivity in p75NTR KO rats.
III. Materials and Methods

1. Animals

p75NTR knock out (p75NTR KO) rats were obtained from SAGE/Horizon Laboratories and then bred in house. Wild type (WT) rats were obtained from Charles River Laboratories. For all animals the day of birth was considered postnatal day 0 (P0).

2. Subventricular zone microdissection and cell isolation

Acrylic brain matrices were used to cut 10mm thick coronal sections of brains dissected from P7 rat pups. 7-8 pups (14-16 SVZs) were used for each experiment. The sections were placed in ice cold PBS and the dorsolateral SVZ was carefully removed using forceps. The tissue was minced and incubated with 1:1 DMEM/F12 media containing 0.75% HEPES buffer and 0.25% trypsin-EDTA for 5 minutes at 37°C. Equal volume of 1:1 DMEM/F12 media containing 0.75% HEPES buffer and 10% FBS was used to stop the reaction. The cells were spun down at 800 rpm and washed with 3ml of DMEM/F12 HEPES media and triturated using a glass pipette. The cell suspension was passed through a 70µm cell filter to remove large clumps and bits of choroid plexus that may contaminate the culture. Cells were spun down at 800 rpm and resuspended in 1ml of appropriate media (proliferation/differentiation). Cells were then separated into p75NTR enriched (p75NTR+) and p75NTR deficient (p75NTR-) fractions using the EasySep™ Human CD271 Selection Kit (STEMCELL Technologies #17849).

3. Neurosphere assay

The p75NTR+, p75NTR- and p75NTR KO cells were grown in ultra-low attachment plates (VWR 29443-030) as suspension cultures for 7 days in proliferation medium. Proliferation medium consists of media 1:1 DMEM and F12 supplemented with glucose (6mg/ml),
putrescine (60µM), progesterone (20nM), transferrin (100µg/ml), selenium (30nM),
penicillin (0.5U/ml), and streptomycin (0.5µg/ml), HEPES (15mM), B27 (2%), epidermal
growth factor (20ng/ml) and fibroblast growth factor (10ng/ml). Cells were plated in a 6
well plate at a density of 5x10^5 cells per well in 1ml of media. Phase contrast images of the
neurospheres were taken using a Nikon Eclipse TE200 microscope at 1, 3, 5 and 7 DIV.
The diameter of neurospheres was determined using ImageJ. Clumps of neurospheres or
fused neurospheres were discarded from the analysis. Occasional neuroshperes fused on
one side but with a clear diameter were measured and included.

4. Differentiation assay

The p75NTR+, p75NTR- and p75NTR KO cells were plated onto 12mm glass coverslips
coated with poly-D-lysine (0.1 mg/ml) in 500µl of differentiation medium. Differentiation
medium consists of 1:1 DMEM and F12 supplemented with glucose (6mg/ml), putrescine
(60µM), progesterone (20nM), transferrin (100µg/ml), selenium (30nM), penicillin
(0.5U/ml), and streptomycin (0.5µg/ml), HEPES (15mM), B27 (2%). Cells were plated at
a density of 1x10^5 per well and allowed to grow for 1, 3 and 7DIV. At each time point the
cells were washed with PBS (1X) and fixed using ice cold 4% paraformaldehyde (PFA) in
PBS for 15 minutes followed by 3 washes with PBS (1X) for 10 minutes each.

5. Oligodendrocyte specific differentiation

The p75NTR+, p75NTR- and p75NTR KO cells were plated onto 12mm glass coverslips
coated with poly-D-lysine (0.1 mg/ml) in 500µl of differentiation medium supplemented
with Glutamine (6.6mM), thyroxine (0.05µM), triiodothyronine (0.08µg/ml). Cells were
plated at a density of 1x10^5 per well and allowed to grow for 1, 3 and 7DIV. At each time
point the cells were washed with PBS (1X) and fixed using ice cold 4% paraformaldehyde
(PFA) in PBS for 15 minutes followed by 3 washes with PBS (1X) for 10 minutes each.

6. Immunostaining

Immunohistochemistry

Animals were anesthetized with ketamine/xylazine and perfused transcardially with saline followed by 4% PFA/PBS at 1, 5, 7, 10, 14 and 21 days after birth. The brains were removed, post-fixed in 4% PFA/PBS overnight, cryopreserved in 30% sucrose for 2 days and subsequently frozen. 14μm coronal sections were cut through the lateral ventricle using a Leica cryostat. The sections were rehydrated using 1X PBS for 5 minutes and then permeabilized using 0.5% Triton-X/PBS for 15 minutes. The sections were blocked using 5% donkey serum in 0.1% BSA/PBS for 1 hour followed by incubation with the primary antibodies (Table 1) overnight at 4°C. Since Olig2 is a nuclear protein, an additional antigen retrieval step using 1N HCl for 20 minutes after permeabilization was conducted for the Olig2 mouse antibody. Additionally, all MBP antibodies require an extra fixation step after permeabilization with 100% Methanol at -20°C for 30 minutes. After methanol fixation the sections are washed 3 times with PBS and blocked for 1 hour at room temperature as described above. Sections were subsequently washed 3 times with PBS (1X), incubated with secondary antibodies (1:500 in 0.1% BSA/PBS) for 1 hour at room temperature. After the incubation period, sections were washed 3 times with PBS (1X) and mounted using Prolong Gold DAPI (Invitrogen P36931) or DAPI Fluoromount-G mounting media (SouthernBiotech #0100-20).

Immunocytochemistry

Cells were fixed in ice cold 4% PFA/PBS for 15 minutes at room temperature and subsequently washed with 1X PBS 3 times before permeabilization. Cells were
permeabilized with 0.5% Triton-X/PBS for 15 minutes for all primary antibodies with the exception of Olig2 antibodies where 0.25% Triton-X was used for permeabilization for 30 minutes. Coverslips were blocked using 5% donkey serum in 0.1% BSA in PBS for 1 hour followed by incubation with the primary antibodies (Table 1) overnight in 4°C. Coverslips were washed 3 times with PBS (1X), incubated with secondary antibodies (1:500 in 0.1%BSA in PBS) for 1 hour at room temperature, subsequently washed 3 times with PBS (1X) and mounted using Prolong Gold DAPI (Invitrogen P36931) or DAPI Fluoromount-G mounting media (SouthernBiotech #0100-20).

Table 1. Primary antibodies for immunostaining

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7. Western Blots

Tissue or cultured cells were washed with ice-cold PBS (1X) and homogenized using 1X RIPA lysis buffer. Proteins were quantified using the Bradford assay (Bio-Rad 500-0006), and equal amounts of protein (30 µg) were run on SDS gels and transferred to nitrocellulose membrane. For the detection of NTs, 80µg of total protein was loaded per sample. The blots were then rinsed and blocked in 5% nonfat dried skim milk in TBS-T for 2 hours at room temperature. Blots were incubated with primary antibodies (Table 2) diluted in 1%...
BSA/TBS overnight at 4°C. The blots were subsequently washed with TBS-T 3 times for 10 minutes each and incubated with Licor secondary antibodies for 1 hour at room temperature. All secondary antibodies were diluted 1:10,000. Membranes were washed 3 times for 10 min each in TBS-T and analyzed using Licor Odyssey infrared imaging system (LI-COR Biosciences). To confirm equal protein levels, blots were reprobed for actin. All analyses were performed at least three times in independent experiments. Bands were quantified using ImageJ.

Table 2. Primary antibodies for western blot

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8. **EdU/BrdU double labeling for cell cycle rate determination**

p75NTR+ cells, purified from WT dSVZ using magnetic beads, and p75NTR KO cells obtained from the dSVZ of p75NTR KO rats were dissected as described above and plated onto 12mm glass coverslips coated with poly-D-lysine in proliferation media. After the cells settled on the coverslips (2-3 hours after plating) cells were treated with 10mM of EdU for 1 hour to label the cells in the S-phase of the cell cycle. Following the 1-hour pulse, EdU was washed out by replacing the wells with fresh proliferation media. 12, 16, 20 and 24 hours after the initiation of the EdU pulse, the cells were treated with 20mM of BrdU for 2 hours and then fixed using ice-cold 4% PFA/PBS for 15 minutes. Subsequently, the cells were washed with PBS 2 times, and permeabilized with 0.5% Triton-X for 20 min. For BrdU detection, cells were treated with 2N HCl at room temperature for 30 min after permeabilization and then washed 3 times with PBS. EdU was developed following the manufacturer’s protocol (Invitrogen, C10337). For BrdU detection, after completing the EdU development, cells were washed 2X 10 min with PBS and blocked with 5% donkey serum in 1%BSA/PBS for 1 h at room temperature. Cells were incubated with BrdU primary antibody overnight at 4°C. Anti-BrdU (Millipore, BU-1, RRID: AB_11212826) was diluted 1:20 according to the manufacturer’s specification. The cells were washed with PBS 3X times for 10 mins and incubated with secondary antibodies diluted 1:500 for 1 h at room temperature. Cells were consequently washed 3 times for 10 mins in PBS. Coverslips were mounted using Prolong Gold DAPI (Invitrogen P36931) or DAPI Fluoromount-G mounting media (SouthernBiotech #0100-20). 15 images per coverslip
were taken with a Nikon Eclipse TE200 microscope. The number of EdU, BrdU, and EdU/BrdU-double positive cells was quantified using ImageJ.

9. **Retrieval of cerebrospinal fluid**

P7 rat pups were euthanized using CO2. The skin and muscle over the foramen magnum were cut away and the dura mater was punctured with a 22-gauge needle. CSF was collected using negative pressure. CSF from 4-5 rat pups was pooled together and depending on the total volume collected 1X protease and phosphatase inhibitor (Sigma-Aldrich, St. Louis, MO, USA) were added. The samples were then flash frozen and stored at -80°C until analysis.

10. **Flow cytometry**

P7 WT rat pups were euthanized using CO2. The dSVZ was microdissected out as previously described in ice cold PGM (1 mM MgCl₂ and 0.6% dextrose in PBS). SVZs from two animals (4 SVZs) were pooled together per sample. The tissue was minced and cells were incubated with 26 Wünsch units/mL of Liberase DH (Millipore Sigma, 540105400) and 1:100 dilution of DNase1 (Millipore Sigma, D5025-150KU) in cold PGM and then shaken at 24 rotations per minute (RPM) (Clay Adams Nutator, Marshall Scientific) at 37°C for 45 min. Enzymatic digestions were quenched with 8mL of PGB (PBS without Mg²⁺ and Ca²⁺ with 0.6% dextrose, 2 mg/mL fraction of bovine serum albumin (BSA)) (Fisher Scientific, BP1600-100), and 0.006% HEPES (Corning, 25-060-Cl) and the cells were centrifuged for 5min at 300g. The cells were dissociated by repeated trituration, collected by centrifugation, and counted using a ViCell XR Cell Counter (Beckman Coulter, Life Sciences; Indianapolis, IN). Cells were diluted to 1 million cells/mL in PGB.
For surface marker analysis, the cells were incubated in PGB for 25 min. with fluorescent-conjugated antibodies against: CD140-PDGFRa (Santa Cruz Biotechnology, sc-21789, 1:160) NG2 chondroitin-A700 sulfate proteoglycan (Millipore, AB5320, 1:50), GLAST-ASCA1 (MACs (Miltenyi), 130-118-984, 1:11), and O4-APC (MACs (Miltenyi), 130-119-897, 1:125). The cells were washed with PGB by centrifugation at 300g. Secondary antibody goat anti-rabbit IgG Alexa Fluor 700 was used to detect NG2 and APC 780 streptavidin to detect GLAST. The cells were incubated with secondary antibodies and Live/Dead blue in PGB for 25 min and then washed three times by centrifugation in PGB at 300 g. All sample data were collected on a BD Fortessa flow cytometer (BD Biosciences Immunocytometry Systems, San Jose, CA). Matching isotype controls for all antibodies were used and gates were set based on these isotype controls. Data were analyzed using FlowJo software (Tree Star, Inc., Ashland, OR). Gates were set according to a 98% confidence interval on the isotype controls.

11. Olfactory discrimination test

Test animals were habituated to a clean test cage for 30 minutes prior to carrying out the test. Rats were exposed to two non-social odors (almond and banana) and two social odors (adult female and adult male rats) using a cotton-tipped applicator. All odorant solutions were made fresh daily before the test. For the non-social odors 4 drops of almond extract or banana extract were mixed in 10ml of Milli-Q water. For the social odors bedding from cages housing two adult animals for at least one day was collected. The cotton-tipped applicator was dipped in water before being placed into the bedding prior to exposure to the test animal. Each odor was presented three times for two minutes each and the total time the rats spent exploring the scent which included licking or biting of the cotton swab
was determined from recorded videos.

12. Imaging

Images were obtained on a Zeiss LSM 510 META microscope with ZEN acquisition software or a Nikon Eclipse TE200 microscope using MetaMorph acquisition software.

13. Statistics

All sample sizes (n) are numbers of animals used. Student’s t-test was performed to determine statistical significance between genotypes assuming two-tailed distribution. Values represent mean ± SEM. Two-way ANOVA, followed by either Tukey’s or Sidak’s post-hoc test, was used to compare differences between genotypes and related factors including age or area of interest for in vivo experiments. One-way ANOVA was used to compare differences between genotypes and DIV for the in vitro experiments. Significance was assumed at *p < 0.05. Figure legends indicate the exact type of analysis used for each experiment.
IV. Results

Aim 1: To characterize the p75NTR-expressing cells in the postnatal SVZ of rats.

Introduction

Progenitors of the SVZ are a heterogeneous population of cells that express a combination of molecular markers which allows for their classification by function or lineage commitment. Resident NSCs of the postnatal and adult SVZ consist of slowly dividing type B1 cells which are further classified into a subset of glial fibrillary acidic protein (GFAP) expressing quiescent NSCs and another subset of active NSCs that express either Nestin, brain lipid-binding protein (BLBP) or epidermal growth factor receptor (EGFR) (Codega et al., 2014; Giachino et al., 2014). Activated type B1 cells give rise to rapidly dividing, transit amplifying or type C intermediate progenitor cells (Doetsch et al., 1999a) which express markers of neuronal and oligodendroglial lineages. Type C cells of the neuronal lineage can be identified by markers such as achaete-scute homolog 1 (Acsl1) or Mash1 (Parras et al., 2004), transcription factors Dlx1 and Dlx2 (Petryniak et al., 2007), paired box protein 6 (Pax6) (Brill et al., 2008; Kohwi et al., 2005), and T-box brain protein 2 (Tbr2) (Englund et al., 2005; Hevner, 2019). Type C cells of the OL lineage express oligodendrocyte transcription factor 2 (Olig2) (Menn et al., 2006). Ependymal, or type E, cells which line the lateral ventricles and interact with the circulating CSF express a glycosylphos-phatidylinositol-anchored membrane glycoprotein (mCD24), transcription factor SRY (sex determining region Y)-box 2 (Sox2), Nestin, and/or CD133 a membrane glycoprotein also called Prominin-1 (Beckervordersandforth et al., 2010; Merkle et al., 2004; Spassky et al., 2005; Tavazoie et al., 2008). It is often difficult to distinguish subependymal NSCs from ependymal cells in vivo because of their common radial glial
origin and expression of overlapping markers. Additionally, NSCs also generate type A or migrating neuroblast cells that differentiate into interneurons after reaching the OB (Fiorelli et al., 2015; Lois & Alvarez-Buylla, 1994) and express doublecortin or polysialylated neural cell adhesion molecule (PSA-NCAM) (Nacher et al., 2001).

Previous studies have reported the expression of p75NTR in the SVZ of adult rats and humans in proliferating cells (Calzà et al., 1998; Giuliani et al., 2004; van Strien et al., 2014). p75NTR/CD271 expressing NSCs of the human SVZ were also found to be multipotent upon differentiation (van Strien et al., 2014). Details about the classification and lineage commitment of the p75NTR expressing cells in the postnatal SVZ remain unknown.

In this chapter I aim to characterize the p75NTR expressing cells of the SVZ based on expression of specific well established NSC markers. In addition, I aim to determine if p75NTR plays a role in the proliferation of this dSVZ cell population or influences its lineage commitment.

1.1 p75NTR is persistently expressed throughout life in the dorsolateral SVZ of rats but not mice.

Six postnatal ages of rats, postnatal days 1 (P1), P5, P7, P10, P14 and P21, and two additional adult ages of 3 and 12-months were analyzed immunohistochemically for the expression of p75NTR in the SVZ (Figure 1A). Cells immunoreactive for p75NTR were expressed robustly in the dorsolateral SVZ (dSVZ) throughout postnatal development and at 3 and 12-months of age. Between the ages of P7 and P10, the period of peak oligodendrogenesis in rodents, p75NTR expression was maximal in the dSVZ and immunopositive cells were also observed along the ventral and medial walls of the lateral
ventricle. At older postnatal ages of P14 and P21, p75NTR expression was found in the dSVZ and no longer observed in the ventral and medial SVZ which was similar to the pattern of p75NTR expression observed in adult rats aged 3 and 12 months.

Cells of the SVZ are known to migrate anteriorly via the RMS and contribute to OB neurogenesis. The distribution of p75NTR expressing cells across the rostro-caudal extent of the SVZ was assessed at age P7 (Figure 1B). p75NTR expressing cells were found throughout the rostro-caudal extent of the SVZ. A higher concentration of p75NTR expressing cells was observed at the rostral end, consistent with anterior migration of cells into the RMS. In caudal regions, p75NTR expressing cells were limited to the dSVZ.

Analysis of the mouse SVZ showed that p75NTR was not expressed at any postnatal age in the dSVZ (Figure 1C). The basal forebrain and choroid plexus were used as a positive control for p75NTR staining in mice (Figure 1C; low magnification).

1.2 p75NTR expressing cells co-express markers of neuronal and oligodendroglial progenitor cells in the dSVZ

Coronal sections were double immunostained for p75NTR and the neural progenitor marker, nestin. During postnatal development from ages P1-P21 most of the p75NTR expressing cells of the dSVZ co-expressed nestin, suggesting p75NTR is expressed by multipotent progenitors in the SVZ (Figure 2A). There were some nestin expressing cells in the dSVZ that lacked the expression of p75NTR.

To determine the lineage of the p75NTR expressing cells, the co-expression of other known SVZ progenitor cell markers was analyzed. Coronal sections taken from WT P7 rat pups, where maximal expression of p75NTR was observed, were double immunostained for p75NTR and Pax6, a type-C neural progenitor marker; DCX, a marker for immature
neurons and migrating neuroblasts; Olig2, a marker of OL lineage cells; and GFAP, a marker of type-B NSCs and astrocytes. Subsets of p75NTR expressing cells co-expressed either Pax6 (Figure 2Ba) or Olig2 (Figure 2Bd) suggesting that p75NTR was being expressed by cells with different lineage commitments. The expression of Pax6 or Olig2 within the p75NTR expressing population was mutually exclusive (Figure 2C). Moreover, there was no overlap between the expression of p75NTR and DCX (Figure 2Bb) or GFAP (Figure 2Bc) suggesting that this was not a neuroblast or quiescent GFAP expressing, type B1, NSC population in postnatal stages.

1.3 **p75NTR expressing cells are mitotically active in the dSVZ throughout postnatal development**

Nestin is expressed by mitotically active progenitors. In addition, Pax6 and Olig2 function as markers of Type C intermediate progenitor cells of the neuronal and oligodendroglial lineage, which are also actively proliferating populations of cells. To confirm whether p75NTR was being expressed by proliferating cells, coronal sections taken from rats at different postnatal stages were analyzed for the co-expression of p75NTR and Ki67, a proliferation marker. Many of the p75NTR expressing cells co-expressed Ki67 at all the postnatal developmental ages analyzed (Figure 3A).

To understand if p75NTR was involved in regulating proliferation of the SVZ progenitors, cells were dissected from the SVZ of P7 WT animals, where an initial increase of p75-expressing cells was observed and separated based on their expression of p75NTR into a p75NTR+ and p75NTR- fraction of cells (Figure 3B). p75NTR can be detected in the p75NTR+ fraction after magnetic separation by western blot analysis while in the p75NTR- fraction p75NTR protein levels were very low (Figure 3B). Additionally, dSVZ cells were
obtained from age-matched p75NTR KO rats. All fractions of cells- p75NTR+, p75NTR- and p75NTR KO were cultured in proliferation media containing 20ng/ml of EGF and 10ng/ml of FGF for 7DIV to induce the formation of neurospheres.

All three cell types- p75NTR+, p75NTR- and p75NTR KO cells could amplify, self-renew and generate neuroospheres in vitro (Figure 3C, 3D). The neurospheres formed by p75NTR+ cells were significantly larger than neurospheres formed by p75NTR- or the p75NTR KO cells as early as 3DIV (Figure 3E). At the end of 7DIV the average diameter of neurospheres formed by p75NTR+ cells was 207.50 ± 12.00µm (mean ± SE) compared to p75NTR- fraction of cells that generated neurospheres with an average diameter of 154.54 ± 13.00µm (mean ± SE). Neurospheres generated by the p75NTR KO cells had an average diameter of 143.23 ± 8.94µm (mean ± SE) and were significantly smaller than neurospheres of the p75NTR+ fraction. There were no significant differences in neurosphere sizes of the two p75NTR deficient cell populations, p75NTR- and p75NTR KO at any time point in culture. This suggests that progenitor cells that lack p75NTR proliferate less or at a slower rate compared to cells expressing p75NTR.

1.4 p75NTR+ cells cycle at the same rate as p75NTR KO cells in the SVZ.

To determine if the higher proliferative potential of p75NTR+ cells compared to p75NTR KO cells was because of a difference in the duration of the cell cycle, a double-labelling experiment using thymidine analogues EdU and BrdU was conducted. p75NTR+ cells purified from the WT dSVZ using magnetic beads and dSVZ cells of p75NTR KO rats were plated onto poly-D lysine coated coverslips in proliferation medium and allowed to settle for a minimum of two hours. Both cell populations were then pulsed with 10µM of EdU for 1 hour to label the cells in the S-phase of the cell cycle. This was considered the
0-hour time point. Subsequently, cells were labelled with a 2-hour BrdU pulse at 12, 16, 20 and 24 hours after 0-hour initiation of the EdU pulse to determine the time point at which there were maximal EdU-BrdU double labelled cells, indicating that they completed a cell cycle and re-entered the S phase. (Figure 4A). No clear differences were observed in the length of the cell cycle between the p75NTR+ and p75NTR KO cells (Figure 4B). The data suggests that the increase in size of the p75NTR+ neurospheres is not because of a shorter cell cycle.

1.5 Absence of p75NTR promotes differentiation along the oligodendroglial lineage in vitro.

To understand if the expression of p75NTR affected the differentiation of the two cell lineages it was being expressed by, p75NTR+, p75NTR- and p75NTR KO cells were induced to differentiate in media lacking EGF and FGF for 7DIV. Each cell population was analyzed at 1, 3 and 7 DIV for Tuj1 expressing neurons, GFAP expressing astrocytes and Olig2 expressing oligodendroglial cells. All three cell sources generated Tuj1+, GFAP+ and Olig2 + cells in vitro. There was no overlap among the three markers at any stage of the differentiation process. While no differences were found in the total number of Tuj1+ neurons (Figure 5A, 5B) or GFAP+ cells (Figure 5C, FD), Olig2 expressing cells of the OL lineage were affected by the absence of p75NTR. p75NTR- and p75NTR KO cells produced a significantly higher number of OL lineage cells compared to p75NTR+ cells as early as 3DIV which remained consistent for the p75NTR- cells at 7DIV (Figure 5E, 5F). The majority of p75NTR KO cells failed to survive until 7DIV to observe significant differences at the latest time point, as shown by the loss of DAPI-positive cells at 3 and 7 DIV (Figure 5G).
The SVZ cells that lack p75NTR proliferated less and differentiated more than their WT counterparts. These data suggested that there may be a depletion of progenitor cells in the SVZ as development proceeds, and that there may be an insufficient pool of oligodendrocyte progenitors for potential remyelination if the need were to arise later in the adult or aged animal. I investigated the number of proliferating progenitors remaining in the SVZ in 12-month old rats and found that there were fewer cells expressing Ki67 in the p75NTR KO (Figure 6A, 6B).

1.6 p75NTR expressing SVZ cells do not express tropomyosin receptor kinase (Trk) receptors but may have access to various pro and mature neurotrophins.

To understand if the p75NTR expressing progenitor cells co-express other cognate receptors, required to mediate pro-NT or NT signaling, the expression of Trk receptors and sortilin was analyzed in the dSVZ. Coronal sections taken from a P7 rat brain were double immunostained for p75NTR and the three Trk receptors- TrkA, TrkB and TrkC (Figure 7A). p75NTR+ cells of the SVZ did not co-express any Trk receptors. Several ependymal cells expressed TrkB however there were very few cells expressing TrkA or TrkC in the dSVZ. Western blot analysis of samples collected from SVZ, CSF and choroid plexus as the source of CSF, showed the presence of NGF, pro-BDNF, NT-3 and proNT-3 in the choroid plexus, proBDNF and NT-3 in the CSF and proNGF and proNT-3 in the SVZ suggesting that these ligands could be accessible to cells of the dSVZ expressing p75NTR (Figure 7B).

WB analysis revealed that p75NTR+ cells isolated from WT dSVZ co-expressed sortilin while p75NTR- cells expressed very low levels of the co-receptor (Figure 7C). These data suggest that effects observed on the p75NTR expressing population are not being mediated
through a p75NTR/Trk receptor complex however the pro-NTs could potentially be involved in signaling through the p75NTR/sortilin complex.
Discussion Aim 1

Expression of p75NTR in the rat SVZ

Although p75NTR has previously been reported to be expressed in the SVZ at specific neonatal and adult ages there have been no studies that have analyzed the expression of p75NTR over the course of postnatal development and determined what its function might be. Our immunostaining data shows that p75NTR is persistently expressed throughout life in the SVZ of rats, with maximal expression during the period of gliogenesis in rodents, between the ages of P7 and P10. I also showed co-expression of p75NTR with nestin, a multipotent NSC marker throughout postnatal development. Additionally, I found p75NTR was expressed in two mutually exclusive subpopulations of progenitors in the dSVZ with two different lineage commitments- Pax6 expressing cells of the neuronal lineage, and Olig2 expressing cells of the oligodendroglial lineage. These transcription factors, Pax6 and Olig2, have been previously reported to be sufficient for defining a specific lineage (Jang & Goldman, 2011; Klempin et al., 2012).

Analysis of the mouse postnatal SVZ showed the absence of p75NTR in this niche, which is consistent with reports from a previous study (Galvão, RP, Garcea-Verdugo, JM, Alvarez-Buylla, 2008). In contrast, expression of p75NTR has been reported in the human SVZ, where it has been used to isolate proliferating NSCs with multipotentiality (van Strien et al., 2014). Our data, combined with these previous reports, suggests the possibility of a potential role for p75NTR in animals with a larger brain size where there may be a need for producing more neurons or glia during postnatal maturation and enlargement of brain volume.
Role of p75NTR in dSVZ progenitor cell proliferation

I found that most of the p75NTR expressing cells co-expressed Ki67, indicating that they were mitotically active progenitors throughout postnatal development. Furthermore, the neurosphere analysis demonstrated that dSVZ cells lacking p75NTR, both the p75NTR- and p75NTR KO cells, produced significantly smaller neurospheres in culture when compared to p75NTR+ cells, suggesting that p75NTR could be restricting progenitor cells from exiting the cell cycle.

Analysis of the length of the cell cycle in p75NTR+ or p75NTR deficient cells through the EdU-BrdU double labeling experiments showed no significant differences in the onset of a second S-phase after the initial EdU pulse within a 24-hour period in any cell type. This suggests that the proliferating cells that lack p75NTR do not exit the cell cycle earlier than p75NTR+ cells within a 24-hour window. This also suggests that the increase in size of the p75NTR+ neurospheres was not because of a shorter rate of their cell cycle. The SVZ is a heterogenous progenitor niche where cells have varying durations of cell cycle (Smith & Luskin, 1998). Exposure to mitogens in the culture medium in vitro could further trigger the activation of some cells which may have been a quiescent or a slowly dividing population in vivo. A hypothesis that emerges from these data is that p75NTR acts as a brake on the differentiation of progenitors, therefore reserving a pool of progenitors in the SVZ throughout life. In cerebellar granule cells, a progenitor population that exists only until postnatal development is complete and expresses p75NTR, this receptor needs to be downregulated for cells to exit the cell cycle and initiate migration (Zanin et al., 2019).

I have shown the presence of various NTs and pro-NTs potentially accessible to the p75NTR cells of the dSVZ, however this population of progenitors lacks any Trk receptors
that would facilitate signaling of mature NTs together with p75NTR. I also analyzed the expression of sortilin, another co-receptor for p75NTR. I found that p75NTR+ cells expressed sortilin more robustly than p75NTR- cells isolated from the dSVZ. This indicates the possibility that p75NTR could be signaling in a complex with sortilin family of cell receptors and the complementary ligands. p75NTR is also known to be capable of signaling in the absence of a ligand. The intracellular domain of p75NTR can constitutively activate RhoA which is a downstream effector for multiple signaling pathways, including the sonic hedgehog (Shh) pathway, and promote proliferation (Yamashita & Tohyama, 2003). Shh pathway has been reported to facilitate the proliferation of OPCs in postnatal mice (Sanchez & Armstrong, 2018) and influence neuronal progenitors of the SVZ in postnatal and adult mice (Álvarez-Buylla & Ihrie, 2014; Ihrie et al., 2011). It remains to be determined if p75NTR could be maintaining proliferation of the rat dSVZ progenitors through the constitutive activation of RhoA.

**Effects of p75NTR on SVZ progenitor cell differentiation**

Given the expression of both Pax6 and Olig2 in p75NTR expressing cells, I investigated if p75NTR was playing a role in the differentiation of two different lineages from the dSVZ. Previously it has been shown that exogenous treatment of p75NTR expressing cells from the SVZ with BDNF induces the cells to generate neurons (Young, Merson, et al., 2007). In the WB analysis of NTs, I did not detect any mature BDNF in samples of SVZ, CSF, or choroid plexus. While there was abundant proBDNF possibly accessible to p75NTR+ cells which could get cleaved to produce mature BDNF, I also did not detect any TrkB in the p75NTR+ cell population to facilitate neurogenesis through BDNF **in vivo**. When induced to differentiate **in vitro** in the absence of any exogenous NTs, the total numbers of neurons
or astrocytes generated was not affected, and the absence of p75NTR promoted the
differentiation of p75NTR- and p75NTR KO only towards the oligodendroglial lineage. A
dramatic increase was observed in the number of Olig2 expressing cells generated by the
cells lacking p75NTR. These data support the idea that in the absence of p75NTR cells
differentiate predominantly along the oligodendroglial lineage.

While p75NTR expression was minimal over the course of differentiation in the p75NTR-
fraction of cells it was not completely absent in the p75NTR+ fraction of cells as detected
by WB analysis. It is possible that this continued expression of p75NTR is maintained by
progenitors present in the cultures responding to potential autocrine signals required for
proliferation.
Figures and figure legends Aim 1

Figure 1.
Figure 1. p75NTR is expressed in the dorsolateral SVZ of rats. (A) Coronal sections taken through the lateral ventricles of rats at the indicated postnatal and adult ages immunostained for p75NTR (green). p75NTR expression is mainly observed in the dorsolateral SVZ at all indicated stages of development and is maximal at ages P7 and P10 where a few cells are also found along the medial aspect of the SVZ. Robust expression of p75NTR is seen in the choroid plexus within the ventricle. (B) Coronal sections taken at age P7 show the expression of p75NTR throughout the rostro-caudal extent of the SVZ. p75NTR expressing cells were more densely concentrated in the rostral SVZ. (C) Scale bar represents 100µm. (C) p75NTR is not expressed in the SVZ of mice during postnatal development. Low magnification image taken at P7 shows positive immunostaining for p75NTR in the basal forebrain (BF) and in the choroid plexus within the lateral ventricle. Scale bar represents 100µm. High magnification images show absence of p75NTR in the dSVZ between ages P5 and P21 but positive staining in the choroid plexus. Scale bar represents 50µm.
Figure 2.
**Figure 2. Characterization of p75NTR based on SVZ progenitor markers.** (A) Coronal sections taken through the lateral ventricles at the indicated stages of development show colocalization of p75NTR (red) with nestin (green) at all ages of postnatal development in the dSVZ. Scale bar represents 50µm. (B) Immunostaining analysis of SVZ markers at developmental age P7 shows co-expression of p75NTR and Pax6 (a). White arrows show cell colocalization of p75NTR and Pax6 in the zoomed image. p75NTR expression did not overlap with DCX (b), or GFAP (c). Yellow arrows indicate DCX or GFAP expressing cells while white arrows indicate p75NTR expressing non-overlapping cells in the zoomed images. A subset of p75NTR expressing cells co-express Olig2 (d). White arrows show cell colocalization of p75NTR and Olig2 in the zoomed image. (C) p75NTR/Pax6 double positive cells (yellow arrows) and p75NTR/Olig2 (yellow arrowheads) double positive cells are two sub-populations of the p75NTR expressing progenitors.
Figure 3. p75NTR expressing progenitors are mitotically active in the postnatal dSVZ. (A) Coronal sections taken through the lateral ventricles at the indicated ages show cell colocalization of p75NTR (green) and Ki67 (red) in many of the p75NTR expressing cells (inset zoomed images). Scale bar represents 20µm. (B) Schematic diagram explaining the separation of p75NTR+ cells from the dSVZ of WT rats using magnetic beads linked to a p75NTR antibody. Western blot shows the efficiency of cell separation based on p75NTR expression. P7 cerebellum tissue sample serves as a positive control for p75NTR detection. (C,D) Representative images show a consistent growth in size of WT neurospheres from 1DIV to 7DIV (C: top panel). p75NTR-depleted cells (C: middle panel) and SVZ cells from age matched p75NTR KO (C: bottom panel). Scale bar represents 100µm. There is no difference in the size of neurospheres at 1DIV however p75NTR+ cells are significantly larger than p75NTR- or p75NTR KO cells at 3, 5 and 7DIV (E). Data were analyzed using one-way ANOVA with Tukey’s post-hoc analysis. Error bars indicate mean ± SEM.
Figure 4. Expression of p75NTR does not alter the duration of cell cycle. (A) Schematic elucidating the experimental paradigm. Edu was added for 1 hour and washed away, subsequently BrdU was added for 2 hours at 12, 16, 20 and 24 hours after the EdU pulse. EdU and BrdU double positive cells represent cells that have undergone a second round of S phase at the indicated timepoints after the EdU pulse. (B) The proliferating cells are represented as a ratio of double-labeled cells over the total EdU-positive cells. There is no time point in which either population shows an increase in EdU- and BrdU-double positive cells indicating that there is no difference in the rate of the cell cycle between p75NTR+ and p75NTR KO cells. Data are representative of n=3 and were analyzed using two-way ANOVA followed by Sidak’s post-hoc test.
Figure 5.
C) Images show the expression of p75NTR+ and p75NTR- at 1DIV, 3DIV, and 7DIV. The p75NTR KO group is also included for comparison. 

D) Graph illustrates the number of GFAP cells/DAPI for p75NTR+, p75NTR-, and p75NTR KO at 1DIV, 3DIV, and 7DIV. Error bars indicate standard deviation.
Figure 5. The absence of p75NTR promotes differentiation of SVZ cells into cells of the oligodendroglial lineage. p75NTR+ and p75NTR- cells were induced to differentiate in media lacking EGF and FGF for 7DIV. (A, B) No significant differences were observed in the total number of Tuj1+ or (C,D) GFAP+ cells generated by the p75NTR+, p75NTR- and p75NTR KO fractions at 1, 3 and 7DIV. (E,F) p75NTR- and p75NTR KO cells produced a significantly higher number Olig2 expressing cells as early as 3DIV when compared to p75NTR+ cells. Data are representative of n=4 experiments for p75NTR+, p75NTR- and p75NTR KO cells and were analyzed using a two-way ANOVA with Sidak’s post-hoc analysis. (E) Quantification of total DAPI at 1, 3 and 7 DIV shows loss of cells p75NTR KO cells at 7DIV. Scale bar represents 50µm.
Figure 6. Absence of p75NTR leads to a reduction in the proliferating progenitor pool of the dSVZ in aged rats. Coronal sections taken through the lateral ventricle of 12-month old WT and p75NTR KO rats were immunostained for p75NTR (green) and Ki67 (red). (A) p75NTR expressing cells of the dSVZ continue to express Ki67 in the WT rats. The dSVZ of p75NTR KO rats has considerably fewer Ki67 expressing cells. Scale bar represent 50μm (B) quantification of the Ki67 expressing cells over the total number in the dSVZ shows a clear trend towards fewer proliferating cells present in the aged p75NTR KO rats. Data are representative of n=2 for each genotype. Error bars indicate mean ± SEM.
Figure 7.
**Figure 7. Expression of cognate receptors and ligands of p75NTR.** (A) Coronal sections taken through the lateral ventricles of P7 WT rats were double immunostained for p75NTR (green) and TrkA, TrkB and TrkC (red). p75NTR+ cells do show colocalization with Trk receptors in the dSVZ. TrkB expression, however, is abundant in cells lining the ventricle. In contrast, while some cells express TrkC, TrkA expression is minimally observed in the rat dSVZ. Data are representative of n=3. (B) Western blot analysis of samples collected from dSVZ, cerebrospinal fluid (CSF) and choroid plexus at P7 shows presence of proNGF and proNT-3 in the SVZ; NGF, pro-BDNF, pro-NT3 and NT-3 in the choroid plexus and proBDNF and NT-3 in the CSF. Data are representative of n=3. (C) WB analysis shows abundant expression of sortilin in p75NTR expressing cells of the dSVZ after magnetic separation. Limited expression of sortilin is detected in the p75NTR- fraction.
Aim 2: To determine if the absence of p75NTR in the SVZ affects gliogenesis and myelination during postnatal development in vivo.

Introduction

OLs are generated in three waves sequentially in the forebrain. The medial ganglionic eminence and anterior entopeduncular area generate the first wave of OLs around embryonic day E11.5. Progenitors from the lateral ganglionic eminence serve as the source of the second wave of OLs at E15. The third and final wave is generated postnatally by the SVZ (Kessaris et al., 2006). Postnatally, OPCs migrate out from the SVZ and colonize the developing corpus callosum, striatum and cortex where they differentiate into fully mature, myelinating OLs. Myelin production, the final step in OL maturation, is initiated in the rat brain around postnatal day P10 and is maximal at P20 (Downes & Mullins, 2014b). Accumulation of myelin continues into adult stages at slower rates (Doretto et al. 2011). Since myelination during development is an essential process required to stabilize axon structure and neural networks, several factors tightly regulate the total numbers, survival, and differentiation of OPCs to fully mature, myelinating OLs. These factors include several transcriptional regulators, such as Olig1, Olig2 and SoxE proteins (Emery & Lu, 2015), axonal signals (Bozzali & Wrabetz, 2004) and cytoskeletal proteins (Nawaz et al., 2015). Growth factors also influence the differentiation and maturation of OPCs, and these include platelet derived growth factor (PDGFRα) (Fruttiger et al., 1999), epidermal growth factor (EGFR) (Gonzalez-Perez et al., 2009; Yang et al., 2017), fibroblast growth factor (FGF) (Fortin et al., 2005), and neurotrophins (Barres, Raff, et al., 1994; Du et al., 2003).

Under normal conditions, in adult animals, p75NTR expression is observed in OLs of the basal forebrain (Du et al., 2003, 2006). In certain pathological conditions, however,
p75NTR expression gets upregulated in various populations of OPCs and OLs and is suggested to be involved in remyelination (Dowling et al., 1999; Ladiwala et al., 1998; Petratos et al., 2004). In developmental stages, a role for p75NTR in regulation of survival and migration has been reported in Schwann cells, the primary myelinating cells of the peripheral nervous system (Anton et al., 1994; Bentley & Lee, 2000; Lemke & Chao, 1988). It remains unclear if p75NTR influences OL development in the CNS specifically in postnatal stages.

Analysis of p75NTR expression in the rat SVZ revealed an increase in this population during the period of gliogenesis (P7 and P10). In addition, a subset of p75NTR expressing cells co-expressed Olig2 which labels all cells of the OL lineage. Furthermore, upon differentiation of the p75NTR expressing and p75NTR deficient cells, it was observed that absence of p75NTR promoted more cells to differentiate along the OL lineage. These data lead to the hypothesis that p75NTR is involved in the regulation of oligodendroglial cells of the dSVZ during postnatal development.

In this chapter, I investigate if p75NTR is involved in the differentiation of OPCs both \textit{in vitro} and in the corpus callosum \textit{in vivo} in postnatal stages, and if the absence of p75NTR affects myelin development \textit{in vivo}.

\section*{2.1 \textbf{p75NTR expressing cells express markers of cells of the} \textbf{oligodendrocyte lineage} \textit{in vivo}.}

The expression of three markers for cells at different stages of OL differentiation, PDGFR\textalpha for OPCs, O4 for pre-myelinating OLs and NG2 a marker for a subset of OLs was analyzed in the p75NTR expressing cells in the SVZ \textit{in vivo}. p75NTR expression colocalized with O4 (Figure 8, top panel) and PDGFR\textalpha (Figure 8, bottom panel) in the dSVZ. Limited
colocalization was observed with the NG2 expressing OLs in the SVZ (Figure 8, middle panel). These data confirmed that a subset of the cells expressing p75NTR in the SVZ were committed to the OL lineage in vivo.

2.2 p75NTR restricts differentiation along the oligodendroglial lineage in vitro.

Thyroid hormones, thyroxine and triiodothyronine, were used to induce maturation of OPCs in p75NTR+, p75NTR- magnetically sorted WT dSVZ cells and dSVZ cells obtained from p75NTR KO rats at P7 for 1, 3 and 7 DIV. OPCs express specific markers at each stage of their development that can be used to identify the progression of the cells along the OL lineage. PDGFRα was used to identify OPCs and O4 was used as the marker for more mature, pre-myelinating OLs. Additionally, Olig2 was used to identify all cells of the oligodendroglial lineage. After 1DIV, p75NTR+, p75NTR- and p75NTR KO cells generated comparable numbers of PDGFRα+/Olig2+ OPCs (Figure 9A, top panel). At 3DIV, however, the proportion of PDGFRα+ OPCs in the p75NTR- and the p75NTR KO fractions of cells were significantly less compared to the proportion of OPCs generated by p75NTR+ fraction (0.1048 ± 0.03733 and 0.1452 ± 0.03690 compared to 0.3879 ± 0.02177 (means ± SE)) (Figure 9A, bottom panel, 9B). In contrast, by 3DIV, Olig2 expressing cells of the p75NTR- fraction had generated a ratio of 0.6489 ± 0.03871 (mean ± SE) O4+/Olig2+ pre-myelinating OLs and the p75NTR KO fraction generated 0.7224 ± 0.01535 (mean ± SE) of O4+/Olig2+ cells which were significantly higher than the ratio of O4+/Olig2+ cells generated by the p75NTR+ fraction, 0.4132 ± 0.03237 (mean ± SE) (Figure 9A, bottom panel, 9C). These data suggest that the expression of p75NTR restricts the differentiation of OPCs and stalls them at the PDGFRα expressing, OPC stage. In the absence of p75NTR, OPCs progress rapidly through the OL lineage to generate O4
expressing pre-myelinating OLs.

2.3 p75NTR KO rats show accelerated oligodendrogenesis in the corpus callosum in vivo.

OPCs migrate from the SVZ to populate different white matter regions of the forebrain, including the corpus callosum, and subsequently begin differentiating into mature, myelinating OLs. To assess if the absence of p75NTR altered the oligodendroglial population in the white matter in vivo, the expression of Olig2, a marker expressed at all stages of OL development, was analyzed in the corpus callosum of postnatal rats after gliogenesis. Coronal sections taken from WT and p75NTR KO rats at P10, P14 and P21 were immunostained for Olig2. The number of Olig2 cells was quantified as a ratio of the total number of cells, identified by nuclear staining of DAPI, from three areas of the corpus callosum- the medial corpus callosum (mCC), the cingulum, and the external capsule. At P10, the number of oligodendroglial cells present in the corpus callosum were comparable between p75NTR KO and WT rats (Figure 10A, 10B). WT rats generated a ratio of 0.3770 ± 0.02724, while p75NTR KO rats generated 0.3175 ± 0.01989 Olig2+ cells in the corpus callosum (means ± SE for WT and KO). By P14, however, p75NTR KO rats showed a significantly greater proportion of Olig2+ cells in the corpus callosum, 0.6346 ± 0.02571, compared to WT rats which generated a ratio of 0.5181 ± 0.009562 Olig2+ cells (means ± SE for WT and KO) (figure 10A, 10C). In older postnatal rats aged P21, the increase in Olig2 expressing cells seen at P14 in the KO rats did not persist (figure 10A, 10C). P21 WT rats generated a ratio of 0.4297 ± 0.04665 while p75NTR KO rats generated a comparable ratio of 0.5022 ± 0.08812 (means ± SE for WT and KO) Olig2+ cells in vivo. These data suggested that, as seen in vitro, the absence of p75NTR accelerates
oligodendrogenesis *in vivo* during postnatal development.

2.4 *p75NTR KO rats have more OPCs in the corpus callosum in vivo at P14.*

To understand how oligodendrogenesis was being affected in the *p75NTR KO* rats, first the number of OPCs present in the corpus callosum at P10, P14 and P21 was analyzed. PDGFRα was used as the marker for OPCs that had initiated the process of progressing through the stages of OL maturation *in vivo*. Coronal sections taken from rats at ages P10, P14 and P21 were double immunostained for PDGFRα and Olig2. The number of PDGFRα+/Olig2+ OPCs was quantified relative to the total number of Olig2 expressing cells in the corpus callosum. There were no significant differences observed in the numbers of OPCs in the corpus callosum at P10 between WT and KO rats (Figure 11A, 11B). At P14, however, *p75NTR KO* rats generated significantly more OPCs, 0.3701 ± 0.01257, compared to WT rats, 0.2565 ± 0.01512 (means ± SE) (Figure 11A, 11C). There was no difference in number of OPCs present in the corpus callosum at age P21 in WT or *p75NTR KO* animals (Figure 11A, 11D). These data demonstrate that, although not detectable immediately post gliogenesis (at age P10), in the absence of *p75NTR*, OPCs show an accelerated maturation process *in vivo* seen by P14, which appears to normalize by P21.

2.5 *p75NTR KO rats have aberrant distribution of pre-myelinating oligodendrocytes in the corpus callosum in postnatal stages.*

To determine if there were more cells expressing a mature OL marker, suggesting accelerated maturation of OPCs in *p75NTR KO* rats, the number of O4 immunopositive pre-myelinating OLs were analyzed at ages P14 and P21. Coronal sections from P14 and P21 WT and KO rats were immunostained for O4. Dense, punctate staining of O4 was observed at both ages and in both genotypes, WT and *p75NTR KO*, throughout the corpus
callosum (Figure 12A). Quantification of percentage area of corpus callosum covered by O4 staining revealed no significant differences between WT or KO rats at any age (Figure 12B, 12C) however, at P14 there appeared to be some differences in the expression pattern of O4 staining in the cingulum and mCC (Figure 12A). O4 expression in the cingulum was concentrated towards the dorsal side along the cortical fibers and less staining was observed just above the dorsal wall of the ventricle in the colossal fibers. There was also decreased O4 staining observed in the mCC in the p75NTR KO rats at P14. At P21, however, O4 expression was similar throughout the corpus callosum in WT and p75NTR KO rats.

2.6 p75NTR KO rats show accelerated maturation of oligodendrocytes \textit{in vivo}.

Brain Enriched Myelin Associated Protein 1 (BCAS1) has been identified as a marker for mature OLs initiating myelination in the corpus callosum in developmental and adult stages (Ishimoto et al., 2017). This protein was used as a late-stage oligodendrocyte development marker to determine if OL maturation was accelerated in postnatal stages in the p75NTR KO rats as suggested by the \textit{in vitro} experiments and the increased Olig2 expression in the corpus callosum. Coronal sections from P10, P14 and P21 WT and KO rats were immunostained for BCAS1 and Olig2. The number of BCAS1+/Olig2+ cells were quantified as a ratio of the total number of oligodendroglial cells in the corpus callosum detected by nuclear staining of Olig2. While no differences were observed between WT and p75NTR KO rats in the expression of BCAS1+/Olig2+ cells in the corpus callosum at age P10 (Figure 13A, 13B). An increase in total BCAS1+/Olig2+ expressing cells was observed at P14 in the p75NTR KO rats which generated a proportion of \(0.4640 \pm 0.02060\) cells compared to WT rats which generated \(0.3476 \pm 0.03605\) BCAS1+/Olig2+ per total Olig2 cells \textit{in vivo} (means \(\pm\) SE, n=2 for WT and KO) (Figure 13A, 13C). Furthermore,
most of the BCAS1 expression in the P14 p75NTR KO rats was found limited to the dorsal part of the cingulum in the cortical fibers while the colossal fibers lying just above the ventricle had fewer BCAS1+/Olig2+ cells (Figure 13A, middle panel). The BCAS1+/Olig2+ cells in P14 WT rats were more evenly distributed. At the older postnatal stage of P21, there were no significant differences in BCAS1+/Olig2+ cells in vivo (Figure 13A, 13D). This suggested that terminal differentiation of OPCs is accelerated in p75NTR KO rats postnatally and there is aberrant distribution of these cells at P14 which normalizes in older postnatal rats.

2.7 Myelination is affected in the absence of p75NTR post gliogenesis.

To determine if the accelerated maturation of OPCs in the corpus callosum in p75NTR KO rats alters the numbers of terminally differentiated, MBP expressing OLs in vivo, the expression of MBP was analyzed in the corpus callosum at P10, P14 and P21. At P10, western blot analysis showed a significant increase in the amount MBP being expressed in the corpus callosum of p75NTR KO rats (Figure 14A). Immunostaining analysis also showed a dramatic increase in the amount of MBP expressed in the external capsule and cingulum by p75NTR KO rats by immunostaining in vivo. Some myelin segments were also observed in the mCC of the p75NTR KO rat which were not seen in the WT at this stage of development (Figure 14B). Quantification of the area of corpus callosum expressing MBP as well as intensity of MBP showed significant increase in p75NTR KOs (Figure 14C, 14D).

At P14, western blot analysis continued to detect a significant increase in the amount of MBP being expressed by p75NTR KO rats in the corpus callosum in vivo (Figure 15A). In addition, immunostaining analysis revealed an aberrant, aggregated, and clumped MBP
phenotype in the corpus callosum of p75NTR KO rats compared to a diffuse pattern of expression observed in WTs (Figure 15B). P14 p75NTR KO rats had significantly less area of corpus callosum expressing MBP (Figure 15C), however, a comparison of the intensity of fluorescence in the corpus callosum showed a significant increase in MBP expression in the p75NTR KOs, reflecting the MBP aggregates.

The aberrant MBP phenotype present at P14 in p75NTR KOs normalized by P21 as seen by both by western blot analysis (Figure 16A) and immunostaining (Figure 16B). At P21, levels of MBP were comparable between WT and p75NTR KO rats (Figure 16A). The clumps of MBP observed in the external capsule and cingulum at P14 were no longer observed in the P21 p75NTR KO rats (Figure 16B). A diffuse MBP expression pattern was observed in both WTs and KOs. Quantification of the area of corpus callosum expressing MBP and overall intensity of MBP staining in the corpus callosum also showed no significant differences between P21 WT and p75NTR KO rats (Figure 16C, 16D).

These data suggest that in the absence of p75NTR, OPCs undergo accelerated differentiation, reaching the MBP expressing mature state prematurely as observed at P10. This leads to improper distribution of MBP at P14 in the corpus callosum which normalizes by P21.
**Discussion Aim 2**

**p75NTR restricts differentiation along the oligodendroglial lineage *in vitro***.

To determine if p75NTR was regulating the oligodendroglial population of the SVZ, OPCs present in p75NTR+, p75NTR- and p75NTR KO fractions of cells were induced to mature into OLs using thyroid hormones. OPCs of the p75NTR- and p75NTR KO fractions generated more pre-myelinating OLs marked by the expression of O4 and absence of PDGFRα, while most of the Olig2 expressing cells in the p75NTR+ continued to express PDGFRα. These data suggest that in the absence of p75NTR, OPCs mature more rapidly along the OL lineage. Another likely explanation could be that p75NTR stalls OPCs at the progenitor stage, acting as a ‘break’ to prevent their premature differentiation. It has been previously reported that in Schwann cells, the myelinating cells of the PNS, expression of p75NTR is mainly observed in precursor or non-myelinating cell stages. In several other stem cell populations including cerebellar granule cells (Zanin et al., 2016), mesenchymal stem cells (Mikami et al., 2011), limbal stem cells (Kolli et al., 2019), myogenic cells (Erck et al., 1998), the expression of p75NTR is associated with a proliferative progenitor state while the downregulation of p75NTR has been associated with promoting differentiation both *in vivo* and *in vitro*.

During the maturation of OPCs, under the influence of thyroid hormones *in vitro*, there was a dramatic loss of cells in the p75NTR- and p75NTR KO by 7DIV. Absence of appropriate axonal cues is known to trigger apoptosis in pre-myelinating OLs (Trapp et al., 1997). It is possible that the accelerated maturation of OPCs of the p75NTR- and p75NTR KO fractions, coupled with the absence of appropriate axons to myelinate in the culture, results in their diminished survival. It has been shown that PDGF can maintain OPCs in
the cell cycle through upregulation of PDGFRα (McKinnon et al., 1990) and in the absence of PDGF OPCs prematurely differentiate into OLs (Temple & Raff, 1986). Since most of the Olig2 expressing cells of the p75NTR+ fraction continue to express PDGFRα and remain in the progenitor stage, signals from other cells in the culture acting through PDGFRα could be playing a role in their enhanced survival.

**p75NTR KO rats show an increase in the number of oligodendroglial cells and OPCs at P14 in the corpus callosum in vivo.**

OPCs undergo proliferation during gliogenesis and migrate from the SVZ to the overlying corpus callosum where they differentiate sequentially to pre-myelinating and then fully myelinating OLs. To understand if the absence of p75NTR promotes premature differentiation of OLs in vivo, the number of oligodendroglial cells, identified by Olig2 expression, were analyzed by immunostaining in the corpus callosum following gliogenesis at postnatal ages P10, P14 and P21. No detectable differences in the number of Olig2 expressing cells were observed at P10 however, at P14 I found significantly more Olig2 expressing cells in the corpus callosum in p75NTR KO rats. By age P21 the differences in Olig2 numbers had normalized in the KO rats. In addition to the increase in Olig2 expressing cells at P14, an increase in PDGFRα+/Olig2+ OPCs was also observed. While no differences were detected in total OPC numbers at P10 between WT and p75NTR KO rats, p75NTR KO rats showed an increase in MBP expressing fully mature OLs in the external capsule and cingulum suggesting that several OPCs had already reached a terminally differentiated stage in those areas of the corpus callosum prematurely. It is possible that the increase in OPC numbers seen at P14 may compensate for the lack of appropriately differentiated OLs present to myelinate developing axons. By age P21, the
OPC numbers in the corpus callosum normalized in the p75NTR KO rats. A previous study conducted in mice reported that after P21, once myelination is complete, PDGFRα expression declines in oligodendroglial cells and begins to be expressed by neuronal populations instead (Oumesmar et al., 1997). It is likely that the appropriate axons have developed by P21, and this helps the OPC numbers normalize. The p75NTR KO rats used in this study have a global elimination of p75NTR expression. It remains to be determined if that affects specific neuronal populations, the axons of which would require myelination in the corpus callosum or cingulum. Further analysis of the timing of development of various neuronal populations in p75NTR KO rats could help elucidate the myelin phenotype observed.

These data also support the hypothesis that p75NTR may potentially be regulating the differentiation of progenitors in vivo by keeping them in an immature state and preventing premature differentiation.

Expression of O4 and BCAS1 in the corpus callosum in postnatal stages

I analyzed the expression of O4 in the corpus callosum at ages P14 and P21 and found that dense, punctate staining of O4 was observed at both ages throughout the corpus callosum. At P14 in the p75NTR KO rats there appeared to be some differences in the distribution in the cingulum and medial corpus callosum. O4 expression in the cingulum was concentrated towards the dorsal side and less staining was observed just above the dorsal wall of the ventricle along the colossal fibers running between the left and right hemispheres. There was also less staining observed in the medial corpus callosum. At P21, however, O4 expression was similar in all three parts of the corpus callosum. The pattern of O4 expression made it complicated to quantify the total numbers of pre-myelinating OLs in
the corpus callosum but the staining along the cingulum suggested that there were differences in the distribution of these cells at age P14. Assessment of BCAS1 as an additional marker of late-stage OLs also began to reveal that there might be an increase in the total number of differentiated oligodendroglial cells at P14 but not at P10 or P21. Interestingly, the pattern of BCAS1 staining resembled the O4 staining in P14 p75NTR KO rats where majority of the BCAS1 expressing cells were present in association with the cortical fibers of the cingulum (separated from the colossal fibers by white horizontal dotted lines, Figure 13A, middle panel). Very few BCAS1+/Olig2+ cells were observed in the colossal fibers, however PDGFRα expressing OPCs appeared to densely colonize the colossal fibers at P14 in p75NTR KO rats. This difference in distribution of OL lineage cells was not observed in WT rats suggesting aberrant development of this lineage in the p75NTR KOs.

Myelination is altered in the absence of p75NTR at early stages after gliogenesis

Myelin formation in the corpus callosum of postnatal rats is initiated between P10 and P14 (Downes & Mullins, 2014a). In order to myelinate axons, terminally differentiated MBP expressing OLs extend their plasma membrane making contact with the axon and then wrapping around it while extending laterally along the length to generate the paranodal loops. This process is accompanied by compaction of the concentric layers of myelin formed. Further, cytoplasmic channels appear in nascent myelin membranes which aid in communication from the outside to the inside of the developing myelin sheath. These channels have been reported to rapidly decline between the ages of P10 and P14 (Bercury & Macklin, 2015).

It is possible that the accelerated differentiation of OPCs leads to premature MBP
expression, even before the development of the appropriate axons required to facilitate the myelination process. The increased MBP intensity observed at P10 introduces the possibility that OPCs begin to express MBP before the axons that require myelination have been extended in the brain, leading potentially to newly formed myelin membrane wrapping around itself causing the clumped aggregated appearance noticed later at age P14. The O4 staining mimics the MBP staining in the P14 p75NTR KO rats. Dense staining was observed in the anterior cingulum where the majority of the clumped MBP staining was localized, and this likely represents where most of the mature OLs are present.

Since myelin is such an important structure for proper neural circuit formation it is likely that there are several molecular fail-safes that regulate its development allowing normalization of the clumped myelin by P21. Considering the timeline of development, by P21, it is possible that cues from the developing axons in the corpus callosum facilitate proper myelination by other resident OPCs of the corpus callosum. Fully differentiated OLs that fail to myelinate axons are known to be eliminated by apoptosis (Barres et al., 1992; Caprariello et al., 2015; Trapp et al., 1997). The prematurely differentiated OLs as well as other OPCs further along the maturation stages in p75NTR KO rats could have a similar fate and potentially be eliminated by apoptosis by age P21 allowing for myelin formation to normalize in the corpus callosum.
Figures and Figure legends Aim 2

Figure 8. p75NTR expressing cells of the dSVZ express markers of oligodendrocyte lineage cells in vivo. (A) Flow cytometry analysis of the p75NTR expressing cells isolated from the dSVZ of P7 WT rats determines the co-expression of p75NTR with markers for cells at different stages of OL differentiation, PDGFRα and O4, an additional OL subset
marker, NG2, and the astrocyte marker GLAST. O4 and PDGFRα were found to be the predominant markers of the p75NTR expressing cells. 42% of the p75NTR expressing cells co-expressed PDGFRα, 58% were immunopositive for O4. Minimum co-expression was observed with NG2 and GLAST. NG2 and GLAST label 12.72% and 9.57% of the p75NTR population respectively. Data were collected and analyzed in collaboration with Dr. Michelle J. Frondelli and Dr. Steven W. Levison. Error bars indicate mean ± SEM. (B) Immunostaining of coronal sections from P7 WT rats confirms colocalization between p75NTR, O4 (top panel) and PDGFRα (bottom panel) and minimal colocalization between p75NTR and NG2 (middle panel). Scale bar represents 50µm.
Figure 9. p75NTR restricts differentiation along the oligodendroglial lineage in vitro.

OL lineage cells of the p75NTR+, p75NTR- and p75NTR KO dSVZ cells were induced to differentiate in the presence of thyroid hormones. Olig2 (green) was used as a marker to identify cells of the OL lineage. (A) PDGFRα+/Olig2+ expressing OPCs were observed in p75NTR+, p75NTR- and p75NTR KO cells fractions at 1DIV (top panel). At 3DIV (bottom panel), however, fewer PDGFRα expressing cells were observed in the p75NTR- and p75NTR KO fraction of cells. In contrast, at 1DIV very few Olig2 expressing cells co-express the pre-myelinating OL marker O4 (white), in the p75NTR+, p75NTR- and
p75NTR KO fractions of cells, however by 3DIV majority of the Olig2 expressing cells in
the p75NTR- and p75NTR KO cells begin to express O4 indicative of an increase in the
number of pre-myelinating OLs in cells that lack p75NTR. (B) Quantification of
PDGFRα+/Olig2+ OPCs over total number of OL lineage cells shows significantly fewer
OPCs in the p75NTR- and p75NTR KO fractions at 3DIV. (C) Quantification of
O4+/Olig2+ cells over total number of Olig2 expressing cells at 3DIV shows a significant
increase in in pre-myelinating oligodendrocytes in the p75NTR- and p75NTR KO fractions
of cells. Analysis of PDGFRα+/Olig2+ cells is n=3 independent experiments while
analysis of O4+/Olig2+ cells is an n=5 independent experiments for the p75NTR+ and
p75NTR- fractions of cells and n=4 for p75NTR KO fraction of cells. Error bars indicate
mean ± SEM. Data were analyzed using one-way ANOVA with Tukey’s post-hoc analysis.
Figure 10. p75NTR KO rats generate more oligodendroglial cells in the corpus callosum in vivo. Number of Olig2-expressing cells (red) over the total number of cells indicated by DAPI (blue) were quantified in three parts of the corpus callosum in vivo (indicated by white dotted lines): the medial corpus callosum (mCC), the cingulum, and
the external capsule at P10, P14 and P21. (A,B) the number of Olig2 expressing cells were not significantly different between WT and KO rats at P10 (B) by age P14 a significantly greater number of Olig2 expressing cells were detected in the corpus callosum of p75NTR KO rats (C) at P21 the number of Olig2 expressing cells were comparable in WT and KO rats. Number of animals analyzed per genotype at each age are indicated on the graphs. Data were analyzed using unpaired student’s t-test. Error bars indicate mean ± SEM.
Figure 11.
**Figure 11.** p75NTR KO rats generate more OPCs in the corpus callosum *in vivo.*

Number of PDGFRα (green) Olig2 (red) double positive cells, or OPCs, indicated by white arrows, were quantified over the total number of Olig2 expressing cells at P10 (top panels), P14 (middle panels) and P21 (bottom panels) in three parts of the corpus callosum *in vivo* (indicated by white dotted lines): the medial corpus callosum (mCC), the cingulum, and the external capsule. (A,B) The number of PDGFRα+/Olig2+ cells were not significantly different between WT and KO rats at P10. (A,C) By age P14, a significantly greater number of OPCs were detected in the p75NTR KO rats in the corpus callosum *in vivo.* Majority of the PDGFRα-Olig2 double positive cells were observed along the colossal fibers (labeled cf) in the p75NTR KO rats. Area of colossal fibers is separated from the cortical fibers by white dotted lines in the cingulum of WT and p75NTR KO rats at age P14 (middle panels). (A,D) At P21 the number of PDGFRα+/Olig2+ OPCs were not significantly different in WT and KO rats. Number of animals analyzed per genotype at each age are indicated on the graphs. Data were analyzed using unpaired student’s t-test. Error bars indicate mean ± SEM.
Figure 12. Differences in the distribution of pre-myelinating oligodendrocytes in the corpus callosum of p75NTR KO rats. (A) At P14 p75NTR KO rats have reduced O4 staining in the mCC and punctate dense staining limited to the dorsal part of the cingulum when compared to WT rats. AT P21 there is no difference in the pattern of O4 staining between WT and KO rats. (B) No significant differences between the overall area of corpus callosum expressing O4 at P14. (C) No significant differences between the overall area of corpus callosum expressing O4 at P21. Data are representative of n=4 WT and p75NTR KO at P14 and n=2 WT and p75NTR KO at P21. Data were analyzed using unpaired student’s t-test. Error bars indicate mean ± SEM.
Figure 13. p75NTR KO rats display accelerated differentiation of oligodendrocytes in vivo. (A) Representative images from the cingulum of the corpus callosum at P10 (top panel), P14 (middle panel) and P21 (bottom panel) show the colocalization and distribution of BCAS1 expressing cells (green) and Olig2 expressing cells (red). White arrows indicate the BCAS1/Olig2 double positive cells quantified. At P14 most of the BCAS1 staining is limited to the dorsal part of the cingulum and fewer cells are observed along the colossal fibers (labeled cf). This aberrant distribution is not observed at P10 or P21. BCAS1+ cells
were quantified as a ratio of the total number of OL lineage cells in the corpus callosum determined by the nuclear expression of Olig2. (B) the number of BCAS1+/Olig2+ cells were not different between WT and KO rats at P10 (C) by age P14 there appears to be an increase in BCAS1+/Olig2+ cells in the p75NTR KO rats (D) at P21 the number of BCAS1+/Olig2+ expressing cells was comparable in WT and KO rats and no statistical significance was observed. Data are representative of n=2 for WT and p75NTR KO at P10 and P14 and n=3 for WT and KO at P21. Data for P21 rats were analyzed using unpaired student’s t-test. Error bars indicate mean ± SEM.
Figure 14. p75NTR KO rats display increased MBP expression in the corpus callosum at P10 *in vivo*. (A) Western blot analysis and quantification show a significant increase in the amount of MBP expressed by p75NTR KO rats at P10. (B) Representative images show increased MBP expression (green) in the corpus callosum of P10 KO rats (bottom panel).
compared to WT (top panel). (C) P10 p75NTR KO rats show an increase in the distribution of MBP over area of corpus callosum and (D) an overall increase in the intensity of MBP. Data were analyzed using unpaired student’s t-test. Error bars indicate mean ± SEM.
Figure 15. p75NTR KO rats display aberrant MBP expression in the corpus callosum at P14 in vivo. (A) Western blot analysis and quantification show a significant increase in the amount of MBP expressed by P14 p75NTR KO rats. (B) Representative images show
aggregated MBP expression (green) in the corpus callosum of P14 KO rats (bottom panel) compared to WT (top panel). (C) In the p75NTR KO rats, at P14, the distribution of MBP staining over the area of corpus callosum was reduced, however (D) an increase in intensity of MBP was observed as a result of the aggregated phenotype of the myelin. Data were analyzed using unpaired student’s t-test. Error bars indicate mean ± SEM.
Figure 16. MBP expression normalizes at P21 in p75NTR KO rats in vivo. (A) Western blot analysis and quantification show no significant difference in the amount of MBP expressed by P21 WT and p75NTR KO rats. (B) At P21, p75NTR KO rats show a diffused
pattern of MBP expression similar to that observed in WT rats. (C) In P21 p75NTR KO rats the myelin is comparable to WT in distribution and (D) intensity. Data were analyzed using unpaired student’s t-test. Error bars indicate mean ± SEM.
Aim 3: To investigate if p75NTR influences olfactory bulb neurogenesis in rats

Introduction

The SVZ is the main source of adult born olfactory bulb (OB) interneurons. Resident NSCs of the SVZ generate type A migrating neuroblasts which express doublecortin (DCX) or polysialylated neural cell adhesion molecule (PSA-NCAM) and migrate from the anterior SVZ towards the OB via the RMS. This tangential migration of type A cells occurs in chains along the blood vessels within the RMS (Lois & Alvarez-Buylla, 1994; Peretto et al., 2005). After the neuroblasts reach the OB, they leave the RMS and migrate out radially from the core of the OB to the different layers to differentiate. The rodent OB is composed of the outermost granule layer followed by the external plexiform layer (EPL), the mitral cell layer (MCL), the internal plexiform layer (IPL) and the innermost granule cell layer (GCL) (Figure 19). The two populations of interneurons most abundantly produced by the migrating neuroblasts in the adult olfactory bulb include the granule cells (GC) of the GCL or periglomerular cells (PGC) of the GL. Post embryonically generated PGCs are categorized based on their expression of tyrosine hydroxylase (TH), glutamic acid decarboxylase (GAD) or the calcium binding proteins calbindin and calretinin (Lledo et al., 2008).

Characterization of the postnatal dSVZ showed that the progenitor population included a subset of p75NTR expressing cells that co-express Pax6. In adult animals, Pax6 expressing neuroblasts are known to generate dopaminergic PGCs in the OB (Kohwi et al., 2005). The interneurons produced in the OB are temporally regulated, CB expressing PG interneurons are produced in early postnatal life while TH expressing cells are generated in adult stages (de Marchis et al., 2007).

p75NTR expressing cells were found to be densely concentrated in the anterior SVZ. Cells
of the anterior SVZ have been suggested previously to be a predominantly neurogenic population (Luskin, 1993). This leads us to postulate that p75NTR is involved in regulating olfactory bulb neurogenesis in postnatal animals. In this chapter, I attempt to determine if p75NTR regulates the development of a specific interneuron population in the OB. Inhibitory interneurons have been reported to outnumber mitral cells in the OB, suggesting that odor processing is regulated by local inhibition (Shepherd, 2004) therefore I will also determine if there is any effect of p75NTR deletion on olfactory function. Proper OB function in rodents is essential for foraging for food, mating and evading predators. In humans, OB dysfunction has been observed in cases of various pathological conditions such as autism spectrum disorder (Ashwin et al., 2014), attention-deficit/hyperactivity disorder (Romanos et al., 2008), in cases of COVID-19 (Aziz et al., 2021) and in age related neurodegenerative diseases such as Parkinson’s disease (Huisman et al., 2004). Therefore, I also aim to determine if there is an impact of the loss of p75NTR in OB neurogenesis as a result of ageing.

3.1 **p75NTR is expressed by proliferating cells in the Rostral Migratory Stream of postnatal rats**

To determine if p75NTR is expressed in the RMS, sagittal sections taken from P7 WT rats were immunostained for p75NTR. p75NTR expressing cells were observed distributed throughout the RMS emerging from the anterior SVZ (Figure 17). To determine if the p75NTR expressing cells of the RMS were migrating neuroblasts, as previously reported in adult mice (Grade et al., 2013), sagittal sections taken through a P7 rat pup were double immunostained for p75NTR and DCX. Interestingly, no colocalization was observed between p75NTR and DCX in the RMS (Figure 18, top panel). The DCX expressing cells
of the RMS had a filamentous morphology while p75NTR expressing cells appeared more rounded reminiscent of a proliferating cell (Figure 18, inset top panel). Double immunolabelling for p75NTR and Ki67, a proliferation marker, showed that most of the p75NTR expressing cells in the RMS co-expressed Ki67 (Figure 18, bottom panel), similar to what I observed in the SVZ (Figure 18 middle panel), which indicates that p75NTR expressing cells continue to be mitotically active in the RMS. This suggests that unlike what has been reported in adult mice (Grade et al., 2013), p75NTR expressing cells in the postnatal rat RMS are a proliferating cell population.

3.2 Absence of p75NTR affects OB neurogenesis in aged rats

In juvenile and adult animals, neurogenesis in the OB leads to the generation of interneurons in the GL and GCL throughout life. The different populations of OB interneurons can be distinguished based on their expression of markers such as tyrosine hydroxylase (TH), a marker for dopaminergic/GABAergic interneurons in the GL, calbindin, a calcium binding protein expressed in inhibitory interneurons of the GL and parvalbumin (PV), expressed by GABAergic interneurons of the EPL. Coronal sections taken through the OB at P21 were analyzed by immunostaining for TH, calbindin and PV expressing cells. The total number of cells expressing these markers was quantified as a ratio over the area of GL or EPL. There were no differences observed in the total numbers of PV expressing interneurons (Figure 20A top panel, 20B), CB expressing interneurons (Figure 20A middle panel, 20C) or TH expressing cells (Figure 20A bottom panel, 20D) between WT and p75NTR KO rats at P21. These data suggested that the development of the OB interneurons, until age P21, was similar in WT and p75NTR KO rats. Several pathological conditions and neuropsychiatric disorders, such as Parkinson’s
disease, Autism Spectrum Disorders as well as ADHD report altered olfactory sensitivities. In Parkinson’s disease patients, numbers of TH expressing cells are found to be twice that of normal patients of the same age (Huisman et al., 2004). To understand if the absence of p75NTR altered OB neurogenesis over time, I assessed TH, CB and PV expressing neurons in 12-month old WT and p75NTR KO rats. Coronal sections taken through the OB of 12-month old rats were immunostained for TH, PV and CB. The total number of cells expressing either TH, PV or CB was quantified as a ratio over the area of GL or EPL. At 12-months of age, p75NTR KO rats had a ratio of 0.0007701 ± 0.0005769 (mean ± SEM, n=2) TH+ cells per area of GL which is greater than the proportion generated by WT rats, 0.0004588 ± 0.0003112 (mean ± SEM, n=2) (Figure 21A bottom panel, 21B). No differences were observed in total numbers of CB (Figure 21A middle panel, 21C) or PV (Figure 21A top panel, 21D) expressing interneurons between WT and p75NTR KO 12-month old rats.

It has previously been shown, in adult mice, that a lack of p75NTR results in the decrease of weight of the OB in aged mice. To determine if there were overall differences in the weight of the OBs of WT and p75NTR KO rats, OBs were dissected out carefully from brains of 12-month old rats and weighed. No significant differences were observed in the total OB weight (Figure 22).

3.3 Absence of p75NTR affects olfaction in ages rats

To understand if overall olfactory behavior could be affected by a lack of p75NTR, an olfactory discrimination test was carried out comparing WT and p75NTR KO rats aged P14, P21, 1-month and 12-months. All animals were exposed to two non-social odors (almond and banana) and two social odors (an unknown adult female and an unknown adult
male). Each odor was presented three times for two minutes each and the total time spent by the animals exploring the odor, which included licking or biting the cotton swab, was counted. P14 and P21 rat pups moved around in the cage and explored their environment but showed no interest in any odor. In some instances, P21 pups of both genotypes recognized one of the two social odors presented however they did not show any interest in any other odors. Bedding from the cage the pups were housed in with their mother was used as an additional ‘familiar’ odor in the experimental paradigm for the P21 pups. There was no response to the familiar odor by WT or p75NTR KO rats.

In contrast, 1-month old WT and p75NTR KO rats demonstrated interest in both social and non-social odors however there was no significant difference in the cumulative time spent sniffing the odors between the two genotypes for any odor tested (Figure 23A). Further, sniffing is expected to decline over the course of three presentations as the novelty of the odor wears off and the animal habituates to the odor. No significant differences between genotypes were observed to the habituation trend as determined by a two-way ANOVA suggesting that at 1-month of age both WT and p75NTR KO habituated as expected to both non-social (Figure 23B) and social odors (Figure 23C). I observed that the p75NTR KO rats showed significantly decreased exploratory time between the 1st and the 3rd presentation for the social odors but not for the non-social odors. In contrast, the WT rats showed significant decrease in exploration time between the 1st and 3rd presentation of the non-social odors but not for the social odors. This suggests that although overall both genotypes habituate to the presented odors, WT and KO rats have could have varied responses to specific odorants.

Furthermore, dishabituation from the previous odor was observed upon introduction of a
new odor especially when exposed to a social odor after the non-social odors in both WT and p75NTR KO rats (Figure 23D) but this interaction was not significantly different between genotypes.

Since ageing affects OB neurogenesis and a trend for an increase in the number of TH expressing interneuron population was observed in the p75NTR KO 12-month old rats, their response to olfactory stimulus was also tested. It was found that, in general, aged p75NTR KO rats spent more time sniffing and exploring both social and non-social odors compared to WT rats (Figure 24A). The p75NTR KOs spent a significantly longer amount of time sniffing a social odor when compared to WT rats (Figure 24A). Both the WT and p75NTR KO rats showed a similar trend of habituation to non-social as well as social odors over the course of three presentations (Figure 24B, 24C). The p75NTR KO rats demonstrate a significant decrease in the time spent exploring social or non-social odors between the 1st and the 3rd presentations of the odor which was not observed for the WT rats suggesting that the p75NTR KO rats habituate more to a specific odor at this age. Consistent with the increased sniffing observed for each odor, dishabituation was also significantly enhanced in the p75NTR KO rats when exposed to a social odor after being presented with non-social odors. Although dishabituation was also noticed in WT rats it was not significantly different (Figure 24D).
Discussion Aim 3

p75NTR is expressed by proliferating cells in the Rostral Migratory Stream of postnatal rats

In adult rodents, neural progenitors from SVZ progenitors generate OB granule and periglomerular neurons via migration through the Rostral Migratory Stream (RMS) (Bath & Lee, 2010). Consistent with the observation that p75NTR was expressed in adult neuronal progenitors, I found the p75NTR expressing progenitors to be clustered more densely in the rostral SVZ in postnatal rats aged P7, extending into the RMS. A previous study conducted in adult mice reported that p75NTR expression was upregulated in doublecortin (DCX) expressing migrating neuroblasts in the RMS, and that p75NTR was required to facilitate the migration process of these cells (Snapyan et al., 2009). In rats, I found that the p75NTR expressing cells co-express Ki67 in the RMS and not DCX suggesting that p75NTR is expressed by proliferating cells in the RMS. Given that p75NTR is not expressed in mouse SVZ (Galvão, RP, Garcea-Verdugo, JM, Alvarez-Buylla, 2008) but is then upregulated in the neuroblast population within the RMS (Snapyan et al., 2009), it is likely that the role of p75NTR in mouse OB neurogenesis might be different from its role in the rat. I found in Aims 1 and 2 of this study that expression of p75NTR was associated with increased proliferation and its absence promoted a rapid differentiation of oligodendroglial cells. It has previously been reported that some migrating cells can re-enter the cell cycle in the RMS, proliferate, then exit the cell cycle and continue to migrate to the OB (Coskun & Luskin, 2002). It could be hypothesized that in the rat RMS, progenitors upregulate p75NTR to proliferate and then need to downregulate it in order to migrate or differentiate.
Absence of p75NTR affects OB neurogenesis and olfactory behavior in aged rats

Pax6 is expressed by progenitor cells that generate tyrosine hydroxylase expressing interneurons in glomerular layer of the OB (Kriegstein & Alvarez-buylla, 2011; Lim & Alvarez-buylalla, 2016). Co-expression of Pax6 with p75NTR in the SVZ along with the expression of p75NTR in the RMS suggested that a subpopulation of p75NTR cells could be destined for the OB and potentially differentiate into interneurons. Several studies report that p75NTR needs to be downregulated by progenitors in order to promote differentiation. In addition, I have observed that absence of p75NTR promotes differentiation in oligodendroglial cells leading us to hypothesize that p75NTR might be playing a restrictive role in the differentiation of interneurons of the OB. Analysis of the interneuron populations revealed no significant differences in p75NTR KO rats when compared to WT rats at younger ages. However, OB neurogenesis is a process that continues throughout life and abnormal olfactory function is linked to several pathological and neuropsychiatric conditions (Ashwin et al., 2014; Huisman et al., 2004; Romanos et al., 2008). A study of the OB of Parkinson’s patients revealed that they had double the number of tyrosine hydroxylase expressing cells when compared to healthy subjects (Huisman et al., 2004). Parkinson’s disease patients also often report anosmia or reduced odor sensitivity which precedes other symptoms of the disorder. Interestingly, our data also reveals a trend towards an increase in the number of TH expressing interneurons in 12-month old p75NTR KO rats. However, our olfactory discrimination experiments showed that aged p75NTR KO rats spend a longer time sniffing non-social as well as social odors when compared to WT rats. While TH expressing neurons are known to inhibit olfactory transmission to the glomeruli, they produce both dopamine as well as GABA neurotransmitters (Borisovska et
Further analysis of the TH expressing cells in p75NTR KO rats would be required to determine if release of specific neurotransmitters is altered in the absence of p75NTR or if interaction of TH expressing cells with other cell types in the glomeruli is affecting the olfactory behavior differences between WT and p75NTR KO aged rats.

p75NTR KO rats at 1-month of age showed no differences in olfactory behavior overall and did not display the higher exploration time for non-social and social odors seen in the aged animals. Further, I also found that early postnatal animals had no response to olfactory discrimination tests. It is possible that since postnatal animals are housed with their mother and do not have to forage for food, or they have not reached the appropriate age for mating their responses to olfactory cues are not as acute as older rats.
Figures and figure legends Aim 3

Figure 17. p75NTR expressing cells are found throughout the rostral migratory stream. Montage of images of a sagittal section taken from a P7 WT rat immunostained for p75NTR (green), doublecortin (red) and DAPI (blue) show p75NTR expressing cells throughout the RMS extending from the anterior SVZ to the OB suggesting a role for p75NTR in OB neurogenesis.
Figure 18. **p75NTR is expressed by proliferating cells in the RMS.** (A) Confocal images of the anterior arm of the RMS show no colocalization between p75NTR (green) and DCX (red). Inset shows linear, filamentous DCX positive cells along the RMS while the p75NTR expressing cells have a rounded morphology. (B) p75NTR expressing cells (green) of the RMS colocalize with Ki67 (red) in the SVZ and (C) in the RMS. Inset shows colocalization of the two markers. Scale bar represents 50µm.
Figure 19. Organization of the layers of the olfactory bulb. Coronal section of the olfactory bulb shows outermost granule layer (GL) followed by the external plexiform layer (EPL), the mitral cell layer (MCL) and the innermost granule cell layer (GCL) surrounding the core of the main olfactory bulb.
Figure 20. Absence of p75NTR does not alter the numbers of parvalbumin, calbindin or tyrosine hydroxylase expressing cells in the olfactory bulb at P21. (A) coronal sections of the OB show expression of PV interneurons in the EPL (top panel), CB expressing interneurons in the GL (middle panel) and TH expressing interneurons in the GL (bottom panel) of WT and p75NTR KO rats at P21. (B) Quantification of number of PV+ cells over area of EPL determines fewer PV+ cells present in the p75NTR KO rats. Data are representative of n=3 for WT rats and n=2 for p75NTR KO rats. (C) Quantification of number of CB+ cells over area of GL shows no differences in the number of CB+ cells present in the p75NTR KO and WT rats. Data are representative of n=2 for WT rats and n=3 for p75NTR KO rats. (D) Quantification of number of TH+ cells over
area of GL shows fewer TH+ cells present in the p75NTR KO rats which is not statistically significant from WT rats as determined by unpaired student’s t-test. Data are representative of n=3. Error bars indicate mean ± SEM. Scale bar represents 50µm.
Figure 21. p75NTR KO rats show differences in the number of tyrosine hydroxylase expressing interneurons but not calbindin or parvalbumin expressing cells at 12-months of age. (A) Coronal sections of the OB show expression of PV interneurons in the EPL (top panel), CB expressing interneurons in the GL (middle panel) and TH expressing interneurons in the GL (bottom panel) of WT and p75NTR KO rats aged 12-months. (B) Quantification of number of PV+ cells over area of EPL show no difference in the number of PV+ cells present in the p75NTR KO rats. (C) Quantification of number of CB+ cells over area of glomerular layer show no differences between p75NTR KO and WT rats. (D) Quantification of number of TH+ cells over area of glomerular layer determines an increase
in the number of TH+ cells present in the p75NTR KO rats. Data are representative of n=2 animals per genotype. Error bars indicate mean ± SEM.

Figure 22.

Figure 22. Absence of p75NTR does not lead to a decrease in the weight of the OB. Quantification of weight of olfactory bulb as a ratio of the weight of the brain of 12-month old rats. There was no significant difference in the weight of the olfactory bulbs between the two genotypes as determined by unpaired student’s t-test. Data are representative of n=5 WT and n=6 p75NTR KO rats. Error bars indicate mean ± SEM.
Figure 23. Odor discrimination test comparing 1 month old WT and p75NTR KO rats. (A) 1-month old WT and p75NTR KO rats have an enhanced response to social odors compared to non-social odors however there is no significant difference between the time spent investigating any odor by the two genotypes. (B,C) Both WT and KO rats show no significant differences in habituation to non-social or social odors over three presentations.
of the odors. (D) The dishabituation of rats from non-social to social odors show the same trend for WT and KO rats and was not significantly different. Data are representative of n=15 WT and n=13 p75NTR KO rats and analyzed using a two-way ANOVA. Error bars indicate mean ± SEM.
Figure 24. Odor discrimination test comparing 12-month old WT and p75NTR KO rats. (A) 12-month old p75NTR KO rats have an increased olfactory response to all non-social and social odors. The response of p75NTR KO rats to social odors of the opposite sex is significantly greater than WT rats. (B,C) Both WT and KO rats show habituation to the non-social and social odors over the three presentations. No significant differences
between the habituation trend was observed between genotypes however KO rats spend a longer time exploring the odor at each presentation. (D) The dishabituation of rats from non-social to social odors shows the same trend for WT and KO rats however, KO rats spend a significantly longer time exploring social odors compared to WT rats. Data are representative of n=8 WT and n=14 p75NTR KO rats. Data were analyzed using a two-way ANOVA. Error bars indicate mean ± SEM.
V. Conclusions and Future Directions

This thesis aimed to understand the role of the p75 neurotrophin receptor (p75NTR) in the germinal subventricular zone (SVZ). While the expression of p75NTR has been previously reported in the rat and human SVZ (Galvão, RP, Garcea-Verdugo, JM, Alvarez-Buylla, 2008; Giuliani et al., 2004; van Strien et al., 2014), no specific implications for its role have yet been described. Additionally, only some neonatal and adult ages have been analyzed for the expression of p75NTR in the SVZ. There have been no comprehensive studies that encapsulate the role of p75NTR in the SVZ, which acts as a source for various lineage committed cells during brain maturation/development at different ages.

In this study, I analyzed the expression of p75NTR throughout postnatal development and determined two diverse roles for p75NTR: in oligodendrogenesis and neurogenesis. I found that p75NTR was expressed in a multipotent, nestin expressing, progenitor population in the dorsolateral SVZ (dSVZ) postnatally. Expression of p75NTR has been previously described in postnatal day 1 (P1) or P2 rat pups (Giuliani et al., 2004; Young et al., 2007). I showed that p75NTR was expressed robustly in the dSVZ between P1-P21, with maximal expression observed between the ages of P7 and P10, the period of rodent gliogenesis. The p75NTR population consisted of two distinct subsets of cells- a Pax6 expressing neuronal population and an Olig2 expressing oligodendroglial population. I found no overlap between p75NTR and the migrating neuroblast marker DCX, or the inactive NSC, ependymal cell or astrocyte marker, GFAP. These data suggested that p75NTR expression was restricted to neuronal and oligodendroglial cells in the postnatal dSVZ.

A previous study had reported that p75NTR expression was observed in cycling cells in adult rats (Giuliani et al., 2004). I demonstrated, through the Ki67 immunostaining
analysis, that p75NTR was expressed in mitotically active cells throughout the postnatal period of rats. I also report that p75NTR expressing cells continued to proliferate for a longer period in culture than cells that lack the receptor. Further analyses will required to determine the mechanism through which p75NTR could be regulating proliferation in the dSVZ progenitors, and if both the Pax6 expressing and the Olig2 expressing subsets are subject to proliferation regulation through p75NTR.

This work showed that several mature and pro-NTs that could potentially be accessed by the dSVZ cells. The lack of Trk receptors in the p75NTR expressing cells of the dSVZ raised the question of mature NTs being involved in activating downstream pathways for the p75NTR cells in the dSVZ. Western blot analysis determined the expression of sortilin, another co-receptor for p75NTR, in the magnetically sorted p75NTR+ cells with reduced expression levels observed in the p75NTR- fraction which offers the possibility that immature or pro-NTs could be involved in the regulation of proliferation of the p75NTR expressing cells. Alternatively, p75NTR could be functioning autonomously to activate proliferation enhancing signaling pathways in the absence of a ligand potentially through constitutional activation of RhoA (Yamashita & Tohyama, 2003).

Interestingly, upon differentiation of p75NTR+, p75NTR- and p75NTR KO cells obtained at age P7, I found that the absence of p75NTR promoted differentiation along the oligodendroglial lineage. Furthermore, absence of p75NTR accelerated the maturation of OL progenitors into pre-myelinating OLs. The majority of the Olig2 expressing cells of the p75NTR+ cell fraction continued to co-express PDGFRα throughout the differentiation period in vitro and appeared to be halted in the progenitor stage without progressing to pre-myelinating OLs. These in vitro differentiation studies, and OPC maturation experiments,
clearly demonstrate that one role for p75NTR is the regulation of OL lineage cells by restricting their differentiation during postnatal development.

This was also observed in vivo in p75NTR KO rats, where absence of p75NTR generated an increase in the number of oligodendroglial lineage cells in the corpus callosum. Interestingly, this was an age dependent phenomenon. Aspects of oligodendrogenesis were observed to be enhanced as early as P10 in the white matter, and then found to be aberrant at P14 but normalized by P21. Olig2 expression did not show differences at P10, however, terminally differentiated cells expressing MBP were already found to be increased in the external capsule and cingulum regions of the corpus callosum. No differences were observed in the overall numbers of OPCs or late-stage myelinating cells, as determined by analysis of the BCAS1-Olig2 cells at P10. However, at P14 Olig2 expressing cells were found in significantly higher numbers in the corpus callosum of p75NTR KO rats, and MBP expression was found to be aberrant and aggregated. The clumped MBP aggregates were observed in the external capsule as well as in the dorsal cingulum where cortical fibers project. The colossal fibers lying just above the lateral ventricle appeared devoid of MBP in the p75NTR KO rats. Numbers of PDGFRα-Olig2 expressing OPCs were also found to be increased at P14 in the corpus callosum and interestingly they were concentrated in the colossal fibers where MBP expression had not been very abundant. Further, analysis of BCAS1 expressing mature OLs showed that a greater number of BCAS1+/Olig2+ expressing cells were distributed along the cortical fibers in the p75NTR KO mimicking the aberrant MBP expression pattern at this age. By the older postnatal age of P21 these phenotypes had normalized in the p75NTR KO rats. Numbers of Olig2 expressing cells, PDGFRα expressing OPCs, and BCAS1 expressing mature OLs were comparable in WT
and p75NTR KO rats by this age. The MBP expression was also found to be diffuse in the KO rats at P21 and was now observed in the colossal fibers as well. MBP traditionally has been shown to be important for the compaction of myelin membranes and is translated locally at the site of myelin synthesis (Müller et al., 2013). MBP protein translation is repressed during intracellular transport to prevent its ectopic localization (Staugaitis et al., 1990). Future studies can be aimed at determining if intracellular transport is being affected in the absence of p75NTR or if translation repression of MBP is aberrant in the p75NTR KO rats leading to compaction of intracellular membranes and the “clumping” phenotype observed at P14. Further the mechanism behind the normalization of the “clumped” MBP phenotype by age P21 will have to be investigated to understand if the MBP aggregates observed at P14 are cleared either through apoptosis of the prematurely developed OLs, or by phagocytic clearing of the aggregated clumps through microglial activation. Additionally, in future studies investigations into the mechanism of myelin formation in p75NTR KO rats including electron microscopy studies could be conducted to determine if the structure or wrapping of myelin is affected in the absence of p75NTR.

Overall, these results indicate that in the postnatal SVZ, p75NTR facilitates the development of oligodendroglial progenitors, in vitro and in vivo, by regulating their numbers through proliferation and consequently restricting their premature differentiation during the period of gliogenesis and early myelination until P21. To determine any genetic factors that could potentially account for -1) the accelerated differentiation in the p75NTR KO cell population and 2) the heterogeneity of the progenitor pool within the SVZ between genotypes, genetic differences between p75NTR KO and WT OL dSVZ progenitors using techniques like single cell RNA sequencing will have to be analyzed.
Moreover, p75NTR expression has been reported to be upregulated in MS plaques where it is not associated with cell death but has been implicated in remyelination (Chang et al., 2000; Dowling et al., 1999). Given that the proportions of OL lineage cells are increased in the p75NTR KO it can be postulated that the OPC pool in the p75NTR KO SVZ may get exhausted over time, which could impact the progenitor pool available for remyelination. Furthermore, a subpopulation of OLs, NG2 expressing cells, have been shown to co-express p75NTR in the CNS in cases of remyelination (Chang et al., 2000). In the FACS analysis conducted to look at the expression of p75NTR with other SVZ glial subtypes, I found limited co-expression of p75NTR and NG2 expressing cells in the dSVZ. It is possible that in the global p75NTR KO rats the NG2+ oligodendroglial subtype could be more abundant in comparison to other oligodendroglial cells. Additional investigations into the NG2+ population of cells in the p75NTR KO rats will be necessary to determine if the absence of p75NTR alters this population from developmental stages having negative consequences in pathological conditions later in life. Future studies aimed at understanding the impact of the absence of p75NTR in remyelination could prove to be extremely relevant in developing strategies for promoting remyelination therapeutically.

Since p75NTR was co-expressed in a subset of Pax6 expressing progenitors, suggesting a role for p75NTR in OB neurogenesis, I analyzed the p75NTR expression in the RMS. Previous studies in mice have reported that DCX expressing, migrating neuroblasts upregulate p75NTR in the RMS (Grade et al., 2013). I showed that DCX was not expressed in the p75NTR expressing cells of the RMS in rats. Interestingly, in the rat RMS, p75NTR cells expressed Ki67, a marker of proliferation. It has been shown that some cells continue to proliferate in the RMS and cells that divide in the RMS generate more periglomerular
cells as opposed to cells of the anterior SVZ that generate granule cells preferentially in the OB (Smith & Luskin, 1998). It remains to be determined if the expression of p75NTR is necessary to maintain the appropriate numbers of cells that finally reach the OB. Further investigations will also be required to determine if some cells in the RMS require p75NTR to facilitate the process of proliferation and then subsequently need to downregulate p75NTR to enable migration in a kind of ‘stop-divide-migrate’ pattern. Our data show that p75NTR expression did not alter the numbers of interneurons being produced at younger ages, however in aged, 12-month old, rats there was an increase in the tyrosine hydroxylase (TH) expressing, dopaminergic, periglomerular interneurons. TH expressing interneurons for the OB are generated by Pax6 expressing progenitors of the SVZ. I have shown that cells that lack p75NTR generally proliferate less than cells expressing p75NTR. It is possible that changes in the rate of proliferation of cells in the p75NTR KOs could lead to an excess of differentiated cells in the OB over time however, further analysis is required to prove this hypothesis. The olfactory sensory perception is dependent on the local inhibitory interneuron population. While our study shows that in the absence of p75NTR an excess of TH expressing inhibitory interneurons are produced, future studies could be aimed at understanding if the excess, adult-born, TH expressing, PG cells, alter the sensory cells they synapse onto in the OB glomeruli.

In the absence of p75NTR the aged KO rats displayed enhanced olfactory sniffing behavior and this difference was not seen in younger, 1-month old rats. Further analysis of intermediate ages of rats would be required to determine if the enhancement in olfactory behavior is an incremental phenotype as a result of more cells being incorporated into the OB circuit in p75NTR KO rats over time. It also remains to be determined if behaviors
such as learning and memory formation are affected in the absence of p75NTR, in other regions of the brain, that could indirectly alter olfactory behavior.

Taken together, the data in this thesis have demonstrated that p75NTR has an important role in regulating oligodendroglial progenitors in postnatal stages and neuronal progenitors over time in adults. The precise timing of NSC development and the correct balance of proliferation and differentiation is crucial to generating a functional brain. During development, in the CNS, neurogenesis and gliogenesis are mainly non-overlapping processes, spatially and temporally controlled by a variety of factors. While neurons are generated first, glial cells are generated second from RG derived NSCs that undergo a fate-switch in late embryonic stages. This majorly separate timing of cell genesis allows for the neuronal circuitry to be established before appropriate numbers of glial cells are matched to it. I have shown that p75NTR is expressed in the SVZ starting from postnatal stages, likely following the RG NSC fate-switch. This leads us to speculate that p75NTR could be involved in the coordinated population level control of cells of varying lineages and therefore in regulating their timing of development. In case of oligodendrogenesis, which is a process that is predominantly postnatal in its timing, this study has determined that expression of p75NTR prevents premature development of OPCs and their incorporation in the white matter, adding a level of temporal control on these progenitors during brain development. In neurogenesis, p75NTR appears to regulate OB interneuron numbers in adult stages. These roles for p75NTR are distinctly different from the cell death role most widely described for this receptor.
VI. References


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